THE CRAYFISH PLAGUE IN THE CZECH REPUBLIC – REVIEW OF RECENT SUSPECT CASES AND A PILOT DETECTION STUDY

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ABSTRACT

There are only very limited reports about the occurrence of the crayfish plague in Czechia. In recent years, mass mortalities of *Astacus* spp. with symptoms of possible crayfish plague were noticed in three streams in the country – two in Central Bohemia (1998-9) and one in Silesia (2004). Three dead individuals from the last outbreak were examined for the presence of the crayfish plague pathogen, *Aphanomyces astaci*, by observation of the presence of hyphae in their cuticle and by a PCR-based diagnostic method. In all three cases the detection was positive. Although causes of mass mortalities from two other localities lack such a direct confirmation, the indirect evidence supports the same conclusion. The main potential vector of *A. astaci* in Czechia is the American spiny-cheeck crayfish *Orconectes limosus*, widespread in large rivers of the western part of the country and in various isolated standing waters. Using the same molecular method, we investigated the presence of *A. astaci* in living *O. limosus* individuals from six localities (three running and three standing waters). The analysis indicated the presence of the pathogen in animals from five out of six investigated *Orconectes* populations. One of them is present in the stream where two European *Astacus* species had gone extinct in 1998-9. Our results suggest that the crayfish plague is still present in Czechia, and that populations of *O. limosus* represent a reservoir for the crayfish plague pathogen, which directly endangers populations of the native crayfish.

Key-words: crayfish plague, *Aphanomyces astaci*, oomycete, mass mortality, Czech Republic, molecular diagnosis, PCR, *Orconectes limosus*, *Astacus astacus.*

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LA PESTE DES ÉCREVISSES EN RÉPUBLIQUE TCHÈQUE – EXAMEN DES CAS SUSPECTS RÉCENTS ET UNE ÉTUDE PILOTE DE DÉTECTION

RÉSUMÉ

Il n'y a que peu de comptes rendus sur la présence de la peste des écrevisses en République Tchèque. Ces dernières années, la mortalité d'*Astacus* spp. avec des symptômes de la peste des écrevisses ont été relevés dans trois cours d'eau en République tchèque – deux en Bohème centrale (1998-9) et une en Silésie (2004). On a examiné la présence d'*Aphanomcyes astaci*, un parasite oomycète causant cette peste, dans trois individus morts lors de la dernière épidémie, utilisant des observations directes du mycélium de l'agent pathogène dans la cuticule, ainsi qu'une méthode de diagnostic à base de PCR. À chaque occasion, les résultats furent positifs. Bien que les causes de mortalité dans deux autres localités manquent de confirmations directes, des évidences indirectes supportent la même conclusion. La peste des écrevisses est traditionnellement transmise par *Orconectes limosus,* une écrevisse nord américaine. Cette espèce est largement répandue dans les grands fleuves à l'ouest du pays et dans les eaux stagnantes. Par la même méthode moléculaire, nous avons étudié la présence d'*A. astaci* chez des individus vivants d'*O. limosus* de six localités (trois en eaux courantes et trois en eaux stagnantes). La présence de l'agent pathogène était confirmée chez les animaux dans cinq de ces populations d'*O. limosus*, dont une présente dans un cours d'eau où deux espèces d'*Astacus* s'était éteintes en 1998-9. Nos résultats indiquent que la peste reste présente en République tchèque, et les populations d'*O. limosus* sont un réservoir pour son agent pathogène qui menace directement les populations d'écrevisses natives.

Mots-clés : peste des écrevisses, *Aphanomyces astaci*, oomycète, mortalité de masse, République tchèque, diagnostic moléculaire, PCR, *Orconectes limosus*, *Astacus astacus.*

INTRODUCTION

Crayfish plague is the best-known and probably the most serious disease of freshwater crayfish (CERENIUS *et al.,* 1988; EDGERTON *et al.,* 2004), caused by the parasitic oomycete *Aphanomyces astaci* of North American origin. For European and Australasian species of crayfish, the infection by the plague pathogen is fatal (ALDERMAN *et al.,* 1987). However, crayfish species native to North America are much more resistant to the acute disease (UNESTAM, 1969; PERSSON and SÖDERHÄLL, 1983; DIÉGUEZ-URIBEONDO and SÖDERHÄLL, 1993). They act as carriers of *A. astaci*, and thus represent one of the most significant threats for other crayfish species (CERENIUS *et al.,* 1988).

Two crayfish species are native to the Czech Republic, the noble crayfish *Astacus astacus* and the stone crayfish *Austropotamobius torrentium* (KOZÁK *et al.,* 2002). Another indigenous European species, the narrow-clawed crayfish *Astacus leptodactylus*, was introduced to the country from Eastern Europe at the end of the 19th century (ŠTĚPÁN, 1932-33; ĎURIŠ and HORKÁ, 2001). It became naturalised and is now considered as an integral part of the Czech fauna. Additionally, two non-indigenous species of crayfish of North American origin are present in Czechia: the spiny-cheek crayfish *Orconectes limosus* and the signal crayfish *Pacifastacus leniusculus* (HOLDICH, 2003). *P. leniusculus* was introduced in 1980 and is still known only from a few localities, whereas *O. limosus* had spread to the country already in the 1960s and is now common, especially in large rivers of the Elbe watershed and in a number of isolated standing waters (PETRUSEK *et al., 2006*). Both of these American crayfish are known carriers of crayfish plague (VEY *et al.,* 1983; ALDERMAN *et al.,* 1990).

Knowledge of the prevalence of the crayfish plague pathogen is necessary for efficient protection of indigenous crayfish species and management of potential reintroduction attempts. Unfortunately, a reliable diagnosis of *A. astaci* is generally tedious and difficult, requiring cultivation of the pathogen with subsequent infection experiments on susceptible crayfish (CERENIUS *et al.,* 1988; OIDTMANN *et al.,* 1999). Recently, however, molecular tools were developed in order to speed up the identification of *A. astaci* from cultures and clinical samples (OIDTMANN *et al.,* 2002, 2004), and also to test for the presence of the pathogen in crayfish carriers (OIDTMANN *et al.,* 2005). In the present study, we used the PCR-based detection method to investigate the cause of a mass mortality of a native crayfish species, and to test for the presence of latent infection in American crayfish from Czech localities. Due to the lack of proper diagnostic methods, the pathogen could not be reliably confirmed in Czechia before, therefore our study presents the first actual evidence of the contemporary occurrence of *A. astaci* in the territory of the Czech Republic. We also review several cases of mass mortalities in the 20^{th} century, probably caused by the crayfish plague.

MATERIAL AND METHODS

For the molecular detection of the presence of *A. astaci*, we collected dead *A. astacus* from a mass mortality with suspicion of a crayfish plague outbreak in the Křivec brook in the town Třinec (49°40’ N, 18°40’ E) on September 15, 2004, and living *O. limosus* from six populations in the Czech Republic, including both running and standing waters (Table I). The position of all localities is shown in Figure 1.

Table I

Origin of *Orconectes limosus* populations tested for the presence of *Aphanomyces astaci.*

Tableau I

Origine des populations d’*Orconectes limosus* testées pour la présence d’*Aphanomyces astaci.*

Locality	Locality character	Closest settlement	Latitude	Longitude	Date of sampling
Stará pískovna	sandpit	Provodín	50° 37' N	14° 36' E	13.9.2004
Pšovka	stream	Lhotka	50° 23' N	14° 33' E	13.9.2004
Elbe	river	Mělník	50° 21' N	14° 28' E	13.9.2004
Kojetice - quarry	quarry	Kojetice	50° 14' N	14° 31' E	15.9.2004
Jickovický potok	stream	Jickovice	49° 26' N	14° 13' E	15.9.2004
Orlík reservoir	reservoir	Strouhy	49° 20' N	14° 17' E	9.7.2003

Living *O. limosus* were hand collected and transported to the laboratory in boxes, containing wet grass as a substrate. Animals from the reservoir Orlík were preserved in 70% ethanol. Animals from other localities were kept alive before analysis (up to two weeks for some populations), those from the same population sharing a common aquarium. Aquaria were aerated, with unidirectional water flow allowing frequent exchange of the aquarium volume, and contained sufficient amount of shelters for the animals. The number of individuals from each locality was variable, depending on the availability of material. All available specimens were tested for the presence of *A. astaci* in the abdominal cuticle.

Freshly dead *A. astacus* from the Křivec brook were kept on ice after the collection. Three randomly selected individuals were used for the diagnosis. Before the DNA extraction, the soft abdominal cuticle of the dead crayfish was inspected microscopically for the presence of hyphae.

Living *O. limosus* were killed using chloroform vapours. The details of cuticle dissection and extraction protocol are given in OIDTMANN *et al.* (2004). Dissection tools and the workspace were disinfected after processing each individual. The total genomic DNA from the crayfish cuticle was extracted by the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions.

The protocol used for detection of *A. astaci* genetic material (OIDTMANN *et al.,* 2005) is a further improvement of the method presented by OIDTMANN *et al.* (2004). The protocol applied here introduces another outer PCR round before using the primers presented in OIDTMANN *et al.* (2004). This approach increases the specificity of the assay due to the use of an additional species-specific primer. The detection is considered positive if the second PCR step yields a product of identical length as that from the positive control (DNA isolate from a pure *A. astaci* culture). The details of the protocol are described in a separate publication (OIDTMANN *et al.*, submitted).

RESULTS

Crayfish mass mortalities in Czechia: case studies

The area of Czechia was hit by crayfish plague during the first wave of the plague spread on European continent, at the end of the 19th century. Between the years 1883 and 1906 many crayfish populations, which had been dense and continuous until that time, were decimated (KRUPAUER, 1968; LOHNISKÝ, 1983). The infection had spread to the country probably from two directions (Figure 1), from southeastern Germany (Bavaria) to Bohemia (western part of the Czech Republic) and from southern Poland (Silesia) to Moravia (eastern part of the country) (KRUPAUER, 1968).

Later during the 20th century, only a few records of isolated mass mortalities of crayfish have been reported in Czechia with sufficient details to allow speculations about crayfish plague as the cause of mortality. There have very likely been more isolated plague outbreaks in the territory but these mostly stayed either undetected or unreported, or the disease was not identified as the cause. VOTRUBEC (1931) stated that isolated crayfish plague outbreaks occurred infrequently in the country; however, he did not list any particular localities. The locations of the mortalities described below are shown in Figure 1.

In 1924, crayfish (most likely *A. astacus*) were wiped out in the river Jemčina near the town Jindřichův Hradec and in the watershed of the river Volyňka near the town Volyně, both in southern Bohemia (VOLF, 1926). The case of the Volyňka was described in more details. Large numbers of dead crayfish were observed and the infection quickly spread upstream. The mortality was restricted to crayfish only. These symptoms rule out pollution as the cause of the mortality and point to a probable outbreak of crayfish plague.

To our knowledge, the next likely crayfish plague outbreaks were not reported until the late 1990s (apart from the general note of VOTRUBEC in 1931). In 1998-1999, a mass mortality of *A. astacus* and *A. leptodactylus* occurred in the Pšovka brook, a tributary of the Elbe, in central Bohemia (north of Prague). This case is interesting because of the presence of three crayfish species in a single watercourse. BERAN (1995) described the situation in the locality in 1994: *A. astacus* was present in the upper and middle part of the brook, and in ponds connected with the watercourse where it coexisted with

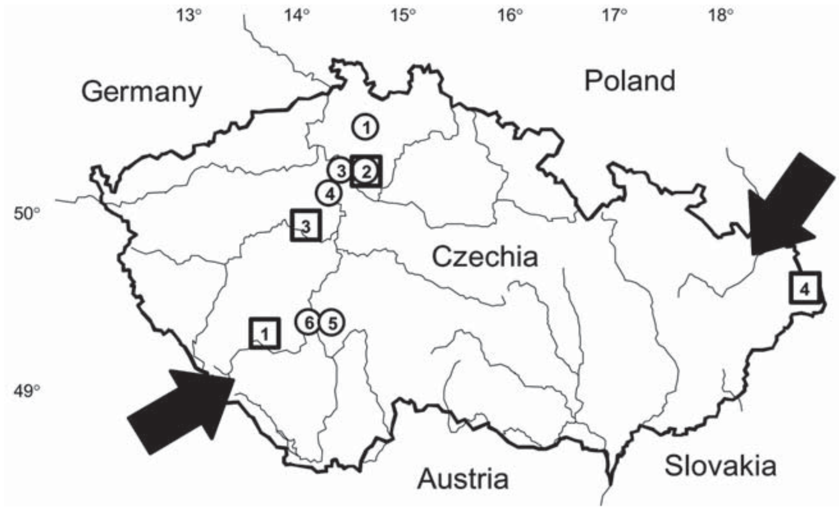

Figure 1

Location of the presumed crayfish plague outbreaks in Czechia, discussed in the text (squares), populations of *Orconectes limosus* sampled for the study (circles), and directions of the crayfish plague spread into the country at the end of 19th century (arrows).

Figure 1

Localisation des apparitions présumées de la peste des écrevisses en République tchèque, discutées dans le texte (carrés), populations *d'Orconectes limosus* échantillonnées pour l'étude (cercles), et dissémination directe de la peste des écrevisses dans le pays à la fin du XIXe siècle (flèches).

Mass mortalities (squares):
Mortalités de masse (carrés) :
1 – river Volyňka, 1924; 2 – brook Pšovka, 1998-9; 3 – brook Loděnice, 1999; 4 – brook Křivec, 2004.

Sampled localities (circles):
Localités échantillonnées (cercles) :
1 – sandpit Stará pískovna, 2 – brook Pšovka, 3 – river Elbe (Labe), 4 – quarry Kojetice, 5 – brook Jickovický potok, 6 – reservoir Orlík.

A. leptodactylus. In the lower part of the brook *O. limosus* was observed, in one place co-occurring with infrequent individuals of *A. leptodactylus.* According to a local fisherman, the spiny-cheek crayfish were intentionally stocked by humans to a pond on the brook, following an undocumented mass mortality of the noble crayfish at the end of the 1980s. A new mass mortality of *Astacus* species in the Pšovka started in May 1998 and spread upstream during the summer and the following year. Thousands of moribund or dead crayfish, mostly *A. astacus*, could be found in the stream. The infection did not affect *Orconectes* individuals or other organisms, and the chemical analysis of the stream water did not show any abnormal contamination (KOZÁK *et al.,* 2000a).

In 1999, a mass mortality of *A. astacus* spreading upstream was observed in the Loděnice brook in central Bohemia (west of Prague). Similarly to the previous case, the chemical analysis did not show any changes of water quality and no other organisms were affected, which again points to a possible crayfish plague outbreak (KOZÁK *et al.,* 2000b).

The most recent analysed plague outbreak in the Czech Republic was recorded in the brook Křivec in Třinec (Silesia, north-east part of the country). Mass mortality of *A. astacus* was observed in this watercourse in September 2004. No other crayfish species were present at the locality. On the collection date, most noble crayfish were dead or dying out of the shelters, often with missing claws or legs. In the lower part of the affected region of the stream, all crayfish were dead in various stages of decomposition; recently dead as well as dying crayfish were found in the upper part. However, some crayfish were alive at the time of the sampling, with no visible symptoms of a disease. Fish and other aquatic macroinvertebrates in the stream were not affected, excluding poisoning or oxygen depletion as the cause of the crayfish mass mortality.

Detection of *Aphanomyces astaci* in Czech crayfish

Microscopic examination of the soft abdominal cuticle of dead *A. astacus* from the mass mortality in the Křivec brook revealed presence of a dense growth of branching, aseptated hyphae with round tips growing within the cuticle, typical of *A. astaci*. This observation was in agreement with the PCR-based detection – all three individuals tested positive (Figure 2A).

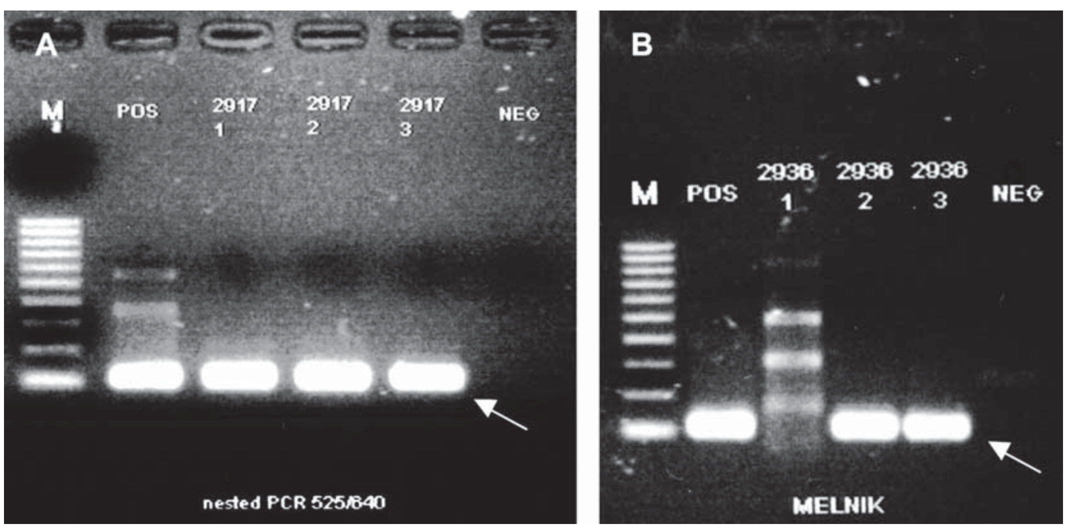

Figure 2

Example of agarose gels with results of the molecular detection of *Aphanomyces astaci.* A: analysis of three dead noble crayfish from the 2004 mass mortality in the Křivec brook, B: analysis of three living spiny-cheek crayfish from the river Elbe near Mělník. Bright band at 115 bp (arrow) indicates positive detection. M – 100 bp size marker; POS – positive control (DNA isolated from the *A. astaci* culture); NEG – negative control; 1,2,3 – DNA isolates from the crayfish cuticle.

Figure 2

Gels d'agarose montrant la présence d'*Aphanomyces astaci* à base de méthodes moléculaires. A : analyse de trois spécimens d'*Astacus* morts, ramassés lors de la mortalité massive dans le ruisseau Křivec. B : analyse de trois spécimens d'*O. limosus* provenant de l'Elbe près de Mělník. Les résultats positifs sont repérés par une bande claire de 115 pb (flèche). M : marqueur de tailles de fragments de 100 pb; POS : contrôle positif (ADN provenant de culture d'*A. astaci*) ; NEG : contrôle négatif ; 1, 2, 3 – isolats de cuticule d'écrevisse.

A summary of the results of detection of *A. astaci* in *O. limosus* is shown in Table II. Latent infection was detected in the spiny-cheek crayfish from five out of six populations (example of one population is shown in Figure 2B). In three populations, over 50% of animals tested positive. In one locality (the brook Jickovický potok, a right-side tributary of the river Vltava in the reservoir Orlík), all tested specimens were positive. The only locality of *O. limosus* where the presence of *A. astaci* was not detected in the analysed samples was the flooded sandpit Stará pískovna, which is isolated from other populations of crayfish.

Table II

Results of the molecular detection of *Aphanomyces astaci* in *Orconectes limosus*.

Tableau II

Résultats de la détection moléculaire d'*Aphanomyces astaci* chez *Orconectes limosus*.

Locality	Animals tested	Positive for *A. astaci*	Percentage of infected animals
Stará pískovna	9	0	0 %
Pšovka	10	6	60 %
Elbe	3	2	67 %
Kojetice - quarry	16	2	13 %
Jickovický brook	15	15	100 %
Orlík reservoir	20	5	25 %

DISCUSSION

The diagnosis of *A. astaci* in the dead *A. astacus* from the Křivec brook (molecular evidence corroborated by the microscopic examination) is the first confirmation of this pathogen causing mass mortalities of native crayfish species in Czechia. The source of the infection of this plague outbreak is not known. There are no vectors of *A. astaci* present in the same locality or in its vicinity. The nearest population of *O. limosus* was present at least up to 1992 in the Goczałkowice reservoir, southern Poland (KRZYŹANEK and KASZA, 1998). This locality, however, belongs to a different watershed than the Křivec brook, and the current status of *Orconectes* presence there is unclear. As the outbreak was located in the outskirts of the town Třinec, an anthropogenic source of infection is the most likely explanation. It is possible that *A. astaci* was spread to this locality by fishermen. Another possible way of spread of the infection could be American crayfish released by aquarists.

The source of infection in the brook Loděnice where the crayfish population died out in 1999 is also unknown. In this case, however, a potential vector may have been at least present in the same watershed, in the river Berounka. The place where the crayfish mass mortality was first recorded was over 20 km upstream from the confluence of the Loděnice and the Berounka (but only about 9 km from the nearest stretch of the latter). *O. limosus* had been reported from the Berounka in 1992 (HAJER, 1994) but was not confirmed there at the time of the crayfish mortality (KOZÁK *et al.*, 2000b) or later (PETRUSEK *et al.,* 2006).

Positive results of the molecular detection of *A. astaci* for individuals of *O. limosus* from the Pšovka brook (indicating a latent infection by the pathogen in the local spiny-cheek crayfish population) suggest that this species may have been the source of infection causing mass mortalities of the noble and narrow-clawed crayfish in this brook in 1998-1999.

We should, however, consider potential limitations of the molecular technique used. There remains a small likelihood that a sensitive PCR-based method may give false positive results. (Apart from contamination, a positive reaction to previously untested species or strains of non-pathogenic *Aphanomyces* cannot be completely excluded.) Whenever possible, especially in case of mass mortalities, it would therefore be beneficial to assess various lines of evidence, and corroborate results of one method with another (e.g. molecular detection together with microscopic examination of the animals affected by the disease, or with cultivation techniques).

The lack of records of crayfish plague outbreaks in Czechia during the 20th century is probably due to various reasons. It is unlikely that many crayfish plague outbreaks occurred in the mid-20th century, since native crayfish populations had declined and North American crayfish, which could have served as carriers of the pathogen, were not present in the country until the 1960s (PETRUSEK *et al.*, 2006). Although latent infection by the plague pathogen within native crayfish populations could have been present, the substrate for the plague pathogen was in general rare, and its spreading ability limited. Some later plague outbreaks may have not been identified, as declines in crayfish populations had been usually attributed to industrial, agricultural, and sewage pollution. Since the 1990s, however, the water quality of surface waters has improved and this, combined with restocking programs, is likely to have led to increased numbers of native crayfish populations. At the same time, non-native crayfish (especially *O. limosus*) have spread, often directly due to human-mediated translocations. Since more crayfish are present in Czech rivers and standing waters nowadays, they come into contact more often and the frequency of transmissions of the pathogen may increase.

Positive results of the molecular detection of *A. astaci* in most studied populations of the spiny-cheek crayfish suggest that the causal agent of the crayfish plague may be widespread in the country. Although in case of the disease carriers the molecular method cannot be easily confirmed with other approaches (microscopic examination is not sufficient; presence or absence of melanized spots is not always linked to the presence or absence of the latent infection; and the cultivation from carriers has a very low yield), already the indication that the pathogen may be present in a number of populations of the spiny-cheek crayfish in Czechia is alarming.

The locality Stará pískovna, where none of nine tested spiny-cheek crayfish specimens showed positive results, is a flooded sandpit with no tributaries or outflows, supplied by seepage water. Crayfish colonising such standing waters are very likely introduced by humans (PETRUSEK *et al., 2006*). It may be speculated that the population of *O. limosus* in Stará pískovna developed from a small number of non-infected animals. However, the number of animals tested is too low to draw conclusions on the carrier status within the entire population. Some reports document the coexistence of European and American crayfish species in a single locality (NYLUND and WESTMAN, 2000) or close vicinity (PÖCKL and PEKNY, 2002), which might be explained by the absence of *A. astaci* in the North American crayfish in those particular places.

CONCLUSIONS

This study shows that some of the recent mass mortalities of European crayfish species in Czechia were likely caused by the crayfish plague. In the last documented case, the presence of the plague pathogen was confirmed by two independent methods – a microscopic examination and molecular diagnosis. Our results also suggest that

Aphanomyces astaci seems to be widespread within populations of the non-indigenous spiny-cheek crayfish *Orconectes limosus*. This confirms that the spread of North American crayfish within the country represents a significant threat to native crayfish populations. The same situation is present in other European countries (e.g. Germany, Great Britain, Finland, Spain, and Sweden), where crayfish plague outbreaks are presently not rare, and are related to the spread of non-native crayfish (mostly the signal crayfish *Pacifastacus leniusculus*) (HOLDICH, 2003; BOHMAN *et al.*, *2006*; DIÉGUEZ-URIBEONDO, 2006).

More detailed investigation of the presence of *A. astaci* in its carriers in Czechia, including *P. leniusculus,* monitoring of crayfish plague outbreaks and of non-indigenous crayfish spread in the country, and education of the wider public to prevent the translocation of plague carriers, are necessary for suitable protection management of endangered native crayfish.

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REFERENCES

ALDERMAN D.J., HOLDICH D., REEVE I., 1990. Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture, 86(1),* 3-6.

ALDERMAN D.J., POLGLASE J.L., FRAYLING M., 1987. *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *Journal of Fish Diseases*, *10(5),* 385-393.

BERAN L., 1995. Raci v CHKO Kokořínsko, část 1. [Crayfish in the Landscape Protected Area Kokořínsko, part 1.] *Ochrana přírody, 50,* 114-115 (in Czech).

BOHMAN P., NORDWALL F., EDSMAN L., 2006. The effect of large-scale introduction of signal crayfish on the spread of crayfish plague in Sweden. *Bull. Fr. Pêche Piscic., 380-381,* 1291-1302.

CERENIUS L., SÖDERHÄLL K., PERSSON M., AXAJON R., 1988. The crayfish plague fungus *Aphanomyces astaci* diagnosis, isolation, and pathobiology. *Freshwater Crayfish, 7,* 131-144.

DIÉGUEZ-URIBEONDO J., 2006. The dispersion of the *Aphanomyces astaci*-carrier, *Pacifastacus leniusculus* by humans represents the main cause of disappearance of the indigenous crayfish *Austropotamobius pallipes* in Navarra. *Bull. Fr. Pêche Piscic., 380-381,*1303-1312.

DIÉGUEZ-URIBEONDO J., SÖDERHÄLL K., 1993. *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture and Fisheries Management*, *24*, 761-765.

ĎURIŠ Z., HORKÁ I., 2001. K výskytu raka bahenního *Pontastacus (=Astacus) leptodactylus* Eschscholtz na Karvinsku. [Occurrence of narrow-clawed crayfish *Pontastacus (=Astacus) leptodactylus* Eschscholtz in vicinity of Karviná.] *Časopis Slezského zemského muzea z Opavě (A*), *50 (supl.),* 78-84. (Czech).

EDGERTON B.F., HENTTONEN P., JUSSILA J., MANNONEN A., PAASONEN P., TAUGBØL T., EDSMAN L., SOUTY-GROSSET C., 2004. Understanding the causes of disease in European freshwater crayfish. *Conservation Biology, 18(6),* 1466-1474.

HAJER J., 1994. Expanze raků druhu *Cambarus affinis* Say ve vodách České republiky. [Expansion of the crayfish Cambarus affinis Say in surface waters of the Czech Republic.] *Fauna Bohemiae Septentrionalis, 19,* 123-128. (in Czech).

HOLDICH D.M., 2003. Crayfish in Europe – an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. *In:* Management & Conservation of Crayfish, Proceedings of a conference held on 7th November 2002 at the Nottingham Forest Football Club, Nottingham, UK. HOLDICH D.M., SIBLEY P.J. (Eds), 15-34. Environment Agency, Bristol, UK.

KOZÁK P., ADÁMEK Z., ŘEHULKA J., 2000a. Úhyn raků v potoce Pšovka v roce 1998. [Mortality of crayfish in the brook Pšovka in 1998]. Study no. 17. *In:* Ekotoxikologie, praktická cvičení, část 2. Diagnostika havarijních úhynů ryb a dalších vodních organizmů. [Ecotoxicology, practical course, Part 2. Diagnosis of mass mortalities of fish and other aquatic organisms.] SVOBODOVÁ Z., MÁCHOVÁ J. (Eds), 109-112. Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Brno, Czech Republic (in Czech).

KOZÁK P., ČERVINKA S., VLADÍK P., 2000b. Úhyn raků na potoce Loděnický (Kačák) v roce 1999. [Mortality of crayfish in the brook Loděnický (Kačák) in 1999]. Study no. 18. *In:* Ekotoxikologie, praktická cvičení, část 2. Diagnostika havarijních úhynů ryb a dalších vodních organizmů. [Ecotoxicology, practical course, Part 2. Diagnosis of mass mortalities of fish and other aquatic organisms.] SVOBODOVÁ Z., MÁCHOVÁ J. (Eds), 113-116. Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Brno, Czech Republic (in Czech).

KOZÁK P., ĎURIŠ Z., POLICAR T., 2002. The stone crayfish *Austropotamobius torrentium* (Schrank) in the Czech Republic. *Bull. Fr. Pêche Piscic., 367,* 707-713.

KRUPAUER V., 1968. Zlatý rak. [The golden crayfish.] Nakladatelství České Budějovice, Czechoslovakia. 109 p. (in Czech).

KRZYŹANEK E., KASZA H., 1998. Raki zbiornika Goczałkowickiego [Crayfish of the Goczałkowice reservoir]. *Przyroda Górnego Śląska, 13,* 13 (in Polish).

LOHNISKÝ K., 1983. Raci v našich vodách. [Crayfish in our waters.] *Rybářství, 6,* 128-129 (in Czech).

NYLUND V., WESTMAN K., 2000. The prevalence of crayfish plague (*Aphanomyces astaci*) in two signal crayfish (*Pacifastacus leniusculus*) populations in Finland. *Journal of Crustacean Biology*, *20*, 777-785.

OIDTMANN B., BAUSEWEIN S., HÖLZLE L., HOFFMANN R., WITTENBRINK M., 2002. Identification of the crayfish plague fungus *Aphanomyces astaci* by polymerase chain reaction and restriction enzyme analysis. *Veterinary Microbiology, 85(2)*, 183-194.

OIDTMANN B., CERENIUS L., SCHMID I., HOFFMANN R., SÖDERHÄLL K., 1999. Crayfish plague epizootics in Germany – classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. *Diseases of Aquatic Organisms, 35(3),* 235-238.

OIDTMANN B., CULAS A., GEIGER S., HOFFMANN R.W., 2005. A molecular method for the detection of *Aphanomyces astaci* in American crayfish (detection crayfish plague carriers). 12th International Conference “Diseases of Fish and Shellfish”, 11-16 September 2005, Copenhagen, Denmark; book of abstracts O-135.

OIDTMANN B., SCHAEFERS N., CERENIUS L., SÖDERHÄLL K., HOFFMANN R. W., 2004. Detection of genomic DNA of the crayfish plague fungus *Aphanomyces astaci* (Oomycete) in clinical samples by PCR. *Veterinary Microbiology, 100(3-4),* 269-282.

PERSSON M., SÖDERHÄLL K., 1983. *Pacifastacus leniusculus* Dana and its resistance to the parasitic fungus *Aphanomyces astaci* Schikora. *Freshwater Crayfish, 5*, 292-298.

PETRUSEK A., FILIPOVÁ L., ĎURIŠ Z., HORKÁ I., KOZÁK P., POLICAR T., ŠTAMBERGOVÁ M., KUČERA Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present. *Bull. Fr. Pêche Piscic., 380-381,* 903-918.

PÖCKL M., PEKNY R., 2002. Interaction between native and alien species of crayfish in Austria: Case studies. *Bull. Fr. Pêche Piscic., 367,* 763-776.

ŠTĚPÁN V.J., 1932-33. Soudobý stav rakařství v Čechách. [Current state of the crayfish fishery in Bohemia]. Separatum, ex: *Československý rybář*, 1932-33. Vodňany, 20 p. (in Czech).

UNESTAM T., 1969. Resistance to the crayfish plague in some American, Japanese and European crayfishes. *Report of the Institute of the Freshwater Research Drottningholm, 49,* 202-209.

VEY A., SÖDERHÄLL K., AJAXON R., 1983. Susceptibility of *Orconectes limosus* Raff. to the crayfish plague, *Aphanomyces astaci* Schikora. *Freshwater Crayfish, 5,* 284-291.

VOLF F., 1926. Račí mor a hynutí raků v řece Volyňce. [The crayfish plague and the mortalities of crayfish in the river Volyňka.] *Rybářský věstník, (7-9),* 98-100, 116-118, 131-133 (in Czech).

VOTRUBEC J., 1931. Chov raků a velevruba perlonosného. [Culture of crayfish and the pearl mussel.] Textbook of the State school of fishery in Vodňany, vol. 9. Praha, 94 p. (in Czech).