Aquatic Invasions (2014) Volume 9, Issue 2: 203–209
doi: http://dx.doi.org/10.3391/ai.2014.9.2.09

Open Access

Research Article

Invasive Chinese mitten crab (*Eriocheir sinensis*) transmits crayfish plague pathogen (*Aphanomyces astaci*)

Anne Schrimpf*, Thomas Schmidt and Ralf Schulz

Institute for Environmental Sciences, University Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

E-mail: schrimpf@uni-landau.de (AS), schmidt-th@uni-landau.de (TS), schulz@uni-landau.de (RS)

**Corresponding author*

Received: 25 February 2014 / Accepted: 20 May 2014 / Published online: 9 June 2014

Handling editor: Vadim Panov

Abstract

The Chinese mitten crab (*Eriocheir sinensis)* is a vector of the fatal crayfish plague pathogen (*Aphanomyces astaci*). Both species are listed among the world´s 100 worst invasive species. From its origin in China the Chinese mitten crab was introduced to Europe, presumably via ballast water from ships. *A. astaci* originated in North America and is assumed to have been firstly introduced to Europe through human-mediated transportation of live specimens of North American freshwater crayfish species. While the North American crayfish species are resistant to the pathogen, it causes a fatal disease to native European crayfish species. It was generally assumed that the crayfish plague could only infect freshwater crayfish species. Until recently, the Chinese mitten crab had not been recognised as a host of the pathogen. And no study has yet been carried out to investigate if they also serve as a vector. In this study, transmission experiments are carried out in combination with subsequent species-specific molecular analysis. The results establish that the Chinese mitten crab carry the crayfish plague pathogen and can transmit it to the European noble crayfish (*Astacus astacus*). The implications of these findings are ecologically alarming, considering the high invasive potential and the catadromous life cycle of Chinese mitten crabs, facilitating the widespread distribution of the pathogen.

Key words: crayfish plague, Brachyura, Decapoda, transmission experiment, real-time PCR

Introduction

The Chinese mitten crab *Eriocheir sinensis* (Milne-Edwards, 1853) is listed among the 100 worst invasive species worldwide (Lowe et al. 2004). Originating from China, it is currently an invasive species in many countries, including Europe and North America (Rudnick et al. 2000; Herborg 2003). In Europe, the first specimens were found in 1912 in the River Aller, in Northern Germany where they were presumably introduced via ballast water from ships (Gollasch 2011). As a catadromous species, the Chinese mitten crab migrates after a marine larval stage from brackish river estuaries several hundreds of kilometres upstream to reach maturity and return to the estuary to reproduce (Hymanson 1999). Besides the life cycle that allows Chinese mitten crabs to travel over large distances, the extensive network of river systems and often interconnected man-made channels in Europe additionally facilitate the spread of invasive macroinvertebrate species (Bij de Vaate et al. 2002). These crabs are opportunistic omnivores (Hymanson 1999) and consequently compete for resources with local freshwater fish and other large invertebrates like crayfish.

Another species nominated among the 100 worst invasive species worldwide (Lowe et al. 2004) is the crayfish plague pathogen *Aphanomyces astaci* (Schikora, 1906). Originating from North America, this species was introduced to Europe through human-mediated transportation of live specimens of North American freshwater crayfish species in the nineteenth century and all freshwater crayfish species of the infraorder Astacidea (Decapoda), are assumed to serve as a potential host and vector of the disease (Oidtmann 2012). This means that *A. astaci* can reproduce in representatives of all these crayfish species through the discharge of sporangia and the release of swimming zoospores outside the crayfish tissue (Olson et

al. 1984). While North American crayfish species are assumed to have evolved a resistance during a co-evolution with the pathogen, and serve as a host, European crayfish species on the other hand, are highly susceptible and usually die after an infection (Alderman 1996). One pathogen spore may be sufficient to start a new infectious wave, which ultimately can lead to a 100% mortality of the European crayfish present in the waterbody or large parts thereof (Oidtmann 2012). Moreover, American crayfish species can also die from an infection with *A. astaci* when their immune system is weakened due to other factors (Persson et al. 1987).

The original hosts of *A. astaci*, namely North American crayfish species, as well as Chinese mitten crabs belong to the suborder Pleocyemata within the order Decapoda. But within the Pleocyemata the crayfish species belong to the infraorder Astacidea, while the Chinese mitten crab is a member of the infraorder Brachyura. Over 70 years ago, the susceptibility of Chinese mitten crabs to the crayfish plague was investigated; high mortalities were observed in the crabs after an artificial infection with assumedly *A. astaci* (Benisch 1940). However, at that time, it was neither possible to identify *A. astaci* as the pathogen responsible for the mortalities nor was it possible to quantify the pathogen. The precise identification of *A. astaci* based on morphological characteristics is impossible since there is no morphological characteristic that distinguishes *A. astaci* from other species of the genus *Aphanomyces* (Royo et al. 2004). Therefore, the identification of *A. astaci* in that previous experiment could not be fully proven. Since that time molecular technology has advanced and a transmission experiment followed by molecular genetic analysis for a species-specific verification represents more reliable proof of species identification.

For a long time *A. astaci* has been considered a crayfish-specific pathogen. However, recently molecular detection methods were applied to show that a Chinese mitten crab population from Sweden was infected with *A. astaci* (Svoboda et al. 2014). Although Svoboda et al. (2014) have shown that the pathogen grew in *E. sinensis* and *Potamon potamios* (Olivier, 1804) captured in two different localities, they did not assess if the crabs served as a transmitter of the pathogen. This research examines if the Chinese mitten crabs from the River Rhine are infected with *A. staci,* and if they can transfer the pathogen to susceptible European crayfish species.

Methods

In a pilot study in February 2012, prior to the transmission experiment, we received 12 Chinese mitten crabs from the River Rhine (close to Cologne) that were stored at -20°C until they were tested for the presence of *A. astaci* with molecular analyses.

Subsequently, we received 25 Chinese mitten crabs that were collected using crab traps by a commercial fisherman from the River Rhine close to Kalkar, Germany, where they coexist with spiny-cheek crayfish (*Orconectes limosus* Rafinesque, 1817). Of these, 14 adult specimens were stored at -20°C for subsequent genetic analysis. The remaining specimens were kept alive and used for transmission experiments. Additionally, noble crayfish (*Astacus astacus* Linnaeus, 1758) susceptible to *A. astaci* were obtained from the "First Bavarian Crayfish Hatchery" in Augsburg. These crayfish were known to be uninfected with *A. astaci* as no plague outbreaks had been observed in the farm during the previous 20 years (Schrimpf et al. 2013a).

Noble crayfish were kept in an outdoor tank (1 m^2 base; 760 L) outside the laboratory for two months (May and June 2012) for acclimatization. Chinese mitten crabs were also kept outdoors in a comparable tank, under similar conditions for more than one week in June. Regular tap water was added and a simple pond filter was used to keep the water in movement and to aerate the water. Water temperature (9–23°C) was monitored over the experimental period and was found to be within the physiological tolerance range of both crustaceans and the pathogen *A. astaci*. Specimens were monitored and fed daily with fish pellets. Dead individuals were removed from tanks. Each tank had separate handling tools and separate water inlets and outlets to eliminate any cross-infection. Extreme care was taken to avoid any transfer of water between tanks. Gloves were changed regularly and after any contact with potentially infected material. Used material was disinfected with peracetic acid (PAA) as recommended by Jussila et al. (2011).

Transmission experiments

In the transmission experiment European noble crayfish [mean carapace length (CL) 48 mm, range 36–62 mm] and potentially infected Chinese mitten crabs (mean CL 44 mm, range 39–53 mm) were kept together under controlled conditions.

Table 1. Result of the qPCR analysis of Chinese mitten crabs from the pilot study (Cologne) and Chinese mitten crabs from Kalkar that were killed before the start of the experiment. Agent level A2 to A5 are considered positive (according to Vrålstad et al. 2009). N = number of individuals.

Sampling site	N	Agent level[a]						Infected	
		A0	A1	A2	A3	A4	A5	N	%
Cologne	12	3		5		3	1	9	75.0
Kalkar	14	11		2		1		3	21.4

[a] Agent levels refer to semi-quantitative categories based on the numbers of observed PFUs (PFUobs) from the *A. astaci*−specific real-time PCR. Agent level A0: no detection; A1: below the limit of detection ($PFU_{obs} < 5$); A2: $5 \leq PFU_{obs} < 50$; A3: $50 \leq PFU_{obs} < 10^3$; A4: $10^3 \leq PFU_{obs} < 10^4$; A5: $10^4 \leq PFU_{obs} < 10^5$.

Eleven replicate aquaria, each with a bottom area of 0.25 m^2 (70 L) were shared by one infected Chinese mitten crab from the River Rhine and one noble crayfish. They were separated by a water-permeable mesh of 10 mm. A further nine smaller aquaria (0.125 m^2 bottom area; 35 L) each contained one, non-infected noble crayfish (mean CL 50 mm, range 42–58 mm) as a control. Regular tap water was used with air-stones to aerate the water (two for the aquaria with both species and one in the control). The water was not changed during the study period (145 days) but separate airlift water filters including filter floss and a thin layer of activated carbon were used to keep the water clean. Water temperature (14.8–24.0 °C) was continually monitored and was in the physiological tolerance range of both crustacean species and the pathogen *A. astaci*. Fine gravel was used as a substratum in all aquaria. A piece of plastic roof gutter (width 12.5 cm, length ca. 15 cm) was used as a shelter. Both species were fed commercial fish pellets daily. The behaviour was monitored daily.

Behavioural changes in the noble crayfish and ultimate mortality, was an indication of a positive carrier of the pathogen. If one specimen in the transmission experiment aquaria died, the other one was killed. The transmission experiment was terminated after 145 days and any remaining crustaceans were killed. All dead individuals were frozen and kept for subsequent verification of *A. astaci* DNA by molecular analyses. All materials were cleaned with PAA after the experiment.

Molecular analysis

Presence or absence of the crayfish plague pathogen was tested in all crustaceans applying molecular analysis. To evaluate the *A. astaci* carrier status we used the TaqMan® minor groove binder (MGB) real-time PCR (qPCR) according to Vrålstad et al. (2009). This method demonstrated very high sensitivity and specificity for the crayfish plague pathogen (Tuffs and Oidtmann 2011). Three tissue types of crayfish were assessed; the inner joint of a walking leg, uropods and soft abdominal cuticle, based on Vrålstad et al. (2011). For the Chinese mitten crab analyses, corresponding pieces of the exoskeleton were examined, where the cuticle is thin and soft, since this is where *A. astaci* usually penetrates (Nyhlén and Unestam 1975). Therefore the inner joint of a walking leg, the eyestalks, and soft abdominal cuticle, which is usually entirely hidden under the thorax in crabs, were examined. DNA extraction and qPCR reactions were carried out according to Vrålstad et al. (2009) with some modifications (Schrimpf et al. 2013a). Agent levels and assessment of infection status were defined again according to Vrålstad et al. (2009). Samples with agent levels A0 (no detection) and A1 are considered free of *A. astaci* DNA while agent levels A2 and higher are considered an evidence of *A. astaci* DNA presence (Table 1).

Additionally species identification of *A. astaci* was confirmed by sequencing a 630 bp long ITS fragment (including partial ITS1 as well as ITS2) with primers 42 and 640 according to Oidtmann et al. (2006). The sequence was aligned to *A. astaci* sequences from the GenBank.

Results

Infection status of Chinese mitten crabs from the Rhine

Nine out of 12 (75%) Chinese mitten crabs from the River Rhine, close to Cologne tested positive for crayfish plague infection (Table 1). Agent levels of infected individuals ranged between A2 (N=5), A4 (N=3) and A5 (N=1). Of the 14 Chinese mitten crabs from the River Rhine close to Kalkar (that were frozen before the start of the experiment), three tested positive (21.4%) with agent levels of A2 (N=2) and A4 (N=1).

Table 2. Result of the qPCR analysis of Chinese mitten crabs from the transmission experiment. For agent levels see footnote of Table 1. Number of days from the start of the experiment until moulting are presented, also the species that died during the experiment and the number of days until its death. The experiment was terminated after 145 days. The asterisk marks the samples that were sequenced.

	Agent level		No. of days until moulting		Species that	Days
	A. astacus	*E. sinensis*	*A. astacus*	*E. sinensis*	died	until death
Aquarium 1	5*	2	22	42	-	-
Aquarium 2	2	0	-	-	*E. sinensis*	5
Aquarium 3	3	0	41	39	-	-
Aquarium 4	1	0	24	18	*A. astacus*	30
Aquarium 5	2	3	-	52	-	-
Aquarium 6	5	0	7	-	*E. sinensis*	62
Aquarium 7	1	2	-	45	-	-
Aquarium 8	0	0	31	63	*E. sinensis*	107
Aquarium 9	3	2	-	52	-	-
Aquarium 10	6*	2	-	4	*A. astacus*	33
Aquarium 11	2	2	-	-	-	-

Transmission experiments

After day 30 and 33 respectively, the European noble crayfish from aquaria 4 and 10 died (Table 2). The Chinese mitten crab from aquaria 2, 6 and 8 died after 5, 62 and 107 days, respectively. In all other aquaria including the controls, no obvious behavioural changes or mortalities were observed until the experiment was terminated. Five noble crayfish and eight Chinese mitten crabs moulted during the experiment (see table 2 for the number of days until moulting).

In six of the Chinese mitten crabs from the transmission experiments, the pathogen was positively identified, in aquaria 1 (A 2), 5 (A 3), 7 (A 2), 9 (A 2), 10 (A 2) and 11 (A 2) (agent levels are given in brackets, see also Table 2). The pathogen was also detected in eight noble crayfish from the transmission experiment, in aquaria 1 (A 5), 2 (A 2), 3 (A 3), 5 (A 2), 6 (A 5), 9 (A 3), 10 (A 6), 11 (A 2). The crayfish plague pathogen was not detected in any of the noble crayfish control aquaria.

The sequence analysis from the DNA extract from the noble crayfish from aquarium 1 and 10 confirmed the results from the qPCR. The ITS sequence fragment was 100% identical to sequences of *A. astaci* from the GenBank (e.g. accession numbers: FM999257 or GU320214).

Discussion

Transmission experiments and subsequent molecular diagnostics have proven that the Chinese mitten crab from the River Rhine is a) carrier of the crayfish plague pathogen and is b) capable of transmitting the pathogen to the noble crayfish. This study has identified a crustacean of the infraorder Brachyura as a confirmed transmitter of the crayfish plague pathogen, thereby refuting the assumption that only freshwater crayfish, order Decapoda can be infected with *A. astaci*. This research supports the results from Benisch (1940) and the more recent findings of Svoboda et al. (2014). While these researchers show that the Chinese mitten crab can be infected with the crayfish plague pathogen (penetrating the crab´s exoskeleton) this study has shown that the Chinese mitten crab can also transmit this fatal pathogen to native crayfish, thereby posing a high risk to these vulnerable species.

Despite positive verification of the pathogen in eight of the eleven noble crayfish from the transmission experiments, the pathogen could only be verified in five of the crabs from the same aquaria (aquaria 1, 5, 9, 10 and 11). However, the fact that the pathogen could not be detected in five individuals does not necessarily mean they were not infected. It is known that the verification of the crayfish plague can fail in infected crayfish specimens (Vrålstad et al. 2011). The failure in the detection of the pathogen in infected crayfish specimens can be attributed to the uneven distribution of the pathogen over the host body (Vrålstad et al. 2009), especially in resistant species that are able to prevent a further spread of the pathogen throughout their body. A resistance of Chinese mitten crabs to *A. astaci* is supported by the fact that no mass mortalities are known of Chinese mitten crabs from the River Rhine or other rivers where American crayfish and the crayfish plague are also present. The

chance of false negative detection is increased by the large body size of the crabs compared to the small tissue material tested for *A. astaci*. The percentage of infected crabs in these experiments and in the wild may therefore be underestimated. On overestimation of infected individuals is possible in cases of attached spores to the carapace that did not penetrate into the crustacean. This should most significantly affect the Chinese mitten crabs that were tested prior to the experiment, but the number of positive verifications in these tests was relatively small (Table 1). Also, in the case of spores attached to the surface of the crab or crayfish the probability of detecting *A. astaci* DNA in a sample should be reduced in individuals that have moulted. However, we did not observe a correlation between the non-moulted individuals and a positive verification of *A. astaci* DNA in the experiment (Table 2). Though, in order to finally verify the growth of *A. astaci* hyphae in the Chinese mitten crabs and to prove that the *A. astaci* DNA was not only attached to the surface of the crabs, individual samples of presumably infected crab need to be checked microscopically for hyphal growth as carried out by Svoboda et al. (2014).

The transmission experiment was stopped after 145 days, although we did not observe high mortality rates in the noble crayfish, as would be assumed for infected species (Oidtmann 2012). A longer duration of the experiment may have led to a higher mortality rate in the noble crayfish. The unexpected long survival of the noble crayfish may be attributed to the good experimental conditions for the noble crayfish in the aquaria in this research. In comparable transmission experiments by other researchers, crayfish were exposed to higher stress conditions, e.g. crayfish density: 4 crayfish in 8 L tank (Makkonen et al. 2012) or 2 crayfish in 5 L tank (Svoboda et al. 2013) compared to one crayfish and one crab in a 70 L tank in this study. Reduced diet may also have induced stress levels e.g. carrots once a week (Svoboda et al. 2013) or peeled peas every other day (Makkonen et al. 2013) compared to fish pellets daily in this research. Furthermore, other factors, such as the temperature, influence the mortality rate of infected European crayfish. A decrease in temperature results in an increase in host survival (Alderman 1987). Also the filters may have decreased the spore density in the water since activated carbon is also used to adsorb organic material. This would also lead to a reduced mortality in the noble crayfish. Additionally, it is assumed that different North American crayfish species are carriers of different strains of *A. astaci* (Huang et al. 1994; Kozubíková et al. 2011) and these strains differ in their virulence (Makkonen et al. 2012). Today several latent infected noble crayfish populations are known, that do not show clinical signs of a crayfish plague infection (e.g. Viljamaa-Dirks et al. 2011; Schrimpf et al. 2012; Svoboda et al. 2012; Kušar et al. 2013). Since the specific strain of *A. astaci* in this study was not identified and its virulence is unknown, it is possible that some of the infected noble crayfish did not die due to the low strain virulence of *A. astaci*.

In the River Rhine, Chinese mitten crabs coexist with spiny-cheek crayfish and calico crayfish (*Orconectes immunis* Hagen, 1870). An earlier study showed that 60% of these two American crayfish species are infected with *A. astaci* (Schrimpf et al. 2013b). It is therefore most likely that the crabs in the River Rhine were infected from the spiny-cheek crayfish or calico crayfish. While the strain of spiny-cheek crayfish from a Czech population was identified (Kozubíková et al. 2011), the strain of calico crayfish and its virulence is still unknown. With the identification of the specific *A. astaci* strains from the two crayfish species in the Rhine and the Chinese mitten crabs, this could be further examined.

Because the transmission was observed under laboratory conditions, it is very likely that the transmission of *A. astaci* from infected Chinese mitten crabs to native crayfish will also occur in the wild. These results are alarming if one considers how fast and how far Chinese mitten crabs migrate along large European rivers, like the River Rhine or the Elbe. Travel over dry land (Brockerhoff and McLay 2011) further increases the invasive potential of Chinese mitten crabs and associated organisms. They can migrate 400 km downstream within a 3-month migration period (Herborg et al. 2003). Adult specimens have been found more than 600 km upstream of the estuary, in the River Rhine (Löb, personal communication). During the life cycle of a crab, it can potentially carry the pathogen vast distances. The pathogen can be transmitted from sites where infected American crayfish species are present, to distant sites where European species occur. This may also explain why in some areas the plague is present, despite the absence of American crayfish species. In the Danube delta for instance, narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1832) were found to be infected with *A. astaci* (Schrimpf et al. 2012), although infected American crayfish were only present 900 km upstream, in

the Danube (Pârvulescu et al. 2012). Chinese mitten crabs could have infected the narrow-clawed crayfish population in the Danube delta. Although it is not confirmed that the Chinese mitten crab is established in the Danube, the species has been reported several times from the Danube (e.g. Paunovic et al. 2004; Puky et al. 2005) and one migrating individual might be enough to spread *A. astaci* over a large distance. However, other origins of the pathogen in the Danube delta are also possible (Schrimpf et al. 2012).

Bentley (2011) assumes that many more river systems around the globe will become host to the Chinese mitten crab. Until now, management actions to remove established Chinese mitten crabs populations have been unsuccessful (Gollasch 2011). However, precautionary action should be applied in areas where the crab is not yet present, to prevent the further spread of the crab and associated pathogens, like the crayfish plague pathogen. This applies to the control of ballast water and human-mediated dispersal, e.g. the sale of living crabs.

Acknowledgements

We would like to thank Dr. Max Keller for providing noble crayfish and Rudi Hell and Dr. Georg Becker for providing Chinese mitten crabs. Thanks also to Patrick Baudy, Bogdan Dahelean, Marco Konschak and Dr. Tim Schikora for help during the transmission experiments, Revina-Rosa Resch and Doreen Roblick for their help in the laboratory. We acknowledge the inspiring discussions with Dr. René Gergs. We further thank Gertrud Klumpp from the Landesuntersuchungsamt Rheinland-Pfalz for advice on animal welfare practice during the transmission experiment. Also, we thank Jiří Svoboda and two anonymous reviewers for constructive comments on the manuscript.

References

Alderman DJ (1996) Geographical spread of bacterial and fungal diseases of crustaceans. *Revue Scientifique et Technique – Office International des Epizooties* 15: 603–632

Alderman DJ, Polglase JL, Frayling M (1987) *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *Journal of Fish Diseases* 10: 385–393, http://dx.doi.org/10.1111/j.1365-2761.1987.tb01086.x

Benisch J (1940) Kuenstlich hervorgerufener *Aphanomyces* Befall bei Wollhandkrabben. *Zeitschrift für Fischerei* 38**:** 71–80

Bentley MG (2011) The global spread of the Chinese mitten crab *Eriocheir sinensis*. In: Galil BS, Clark PF, Carlton JT (eds), In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts. Invading Nature – Springer Series in Invasion Ecology, Volume 6. Springer, Dordrecht, The Netherlands, pp 107–127, http://dx.doi.org/10.1007/978-94-007-0591-3_3

Brockerhoff A, McLay C (2011) Human-Mediated Spread of Alien Crabs. In: Galil BS, Clark PF, Carlton JT (eds), In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts. Invading Nature - Springer Series in Invasion Ecology 6, pp 27–106

Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1159–117, http://dx.doi.org/10.1139/f02-098

Gollasch S (2011) NOBANIS – Invasive Alien Species Fact Sheet – *Eriocheir sinensis*. Online Database of the European Network on Invasive Alien Species – NOBANIS. http://www.nobanis.org (Accessed 2 October 2013)

Herborg LM, Rushton SP, Bentley, Clare AS, Bentley MG (2003) Spread of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards) in Continental Europe: analysis of a historical data set. *Hydrobiologia* 503: 21–28, http://dx.doi.org/10.1023/B:HYDR.0000008483.63314.3c

Huang TS, Cerenius L, Söderhäll K (1994) Analysis of genetic diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA. Aquaculture 126: 1–10, http://dx.doi.org/10.1016/0044-8486(94)90243-7

Hymanson Z, Wang J, Sasaki T (1999) Lessons from the home of the Chinese mitten crab. *IEP Newsletter* 12(3): 25–32

Jussila J, Makkonen J, Kokko H (2011) Peracetic acid (PAA) treatment is an effective disinfectant against crayfish plague (*Aphanomyces astaci*) spores in aquaculture. *Aquaculture* 320: 37–42, http://dx.doi.org/10.1016/j.aquaculture.2011.08.008

Kozubíková E, Viljamaa-Dirks S, Heinikainen S, Petrusek A (2011) Spiny-cheek crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague agent *Aphanomyces astaci*. *Journal of Invertebrate Pathology* 108: 214–216, http://dx.doi.org/10.1016/j.jip.2011.08.002

Kušar D, Vrezec A, Ocepek M, Jencic V (2013) *Aphanomyces astaci* in wild crayfish populations in Slovenia: first report of persistent infection in a stone crayfish *Austropotamobius torrentium* population. *Diseases of Aquatic Organisms* 103: 157–169, http://dx.doi.org/10.3354/dao02567

Lowe S, Browne M, Boudjelas S, De Poorter M (2004) 100 of the world's worst invasive alien species. A selection from the global invasive species database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN)

Makkonen J, Jussila, J, Kortet R, Vainikka A, Kokko H (2012) Differing virulence of *Aphanomyces astaci* isolates and elevated resistance of noble crayfish *Astacus astacus* against crayfish plague. *Diseases of Aquatic Organisms* 102: 129–136, http://dx.doi.org/10.3354/dao02547

Makkonen J, Strand DA, Kokko H, Vrålstad T, Jussila J (2013) Timing and quantifying *Aphanomyces astaci* sporulation from the noble crayfish suffering from the crayfish plague. *Veterinary Microbiology* 162: 750–755

Nyhlén L, Unestam T (1975) Ultrastructure of the penetration of the crayfish integument by the fungal parasite, *Aphanomyces astaci*, Oomycetes. *Journal of Invertebrate Pathology* 26(3): 353–366

Oidtmann B, Geiger S, Steinbauer P, Culas A, Hoffmann RW (2006) Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. *Diseases of Aquatic Organisms* 72: 53–64, http://dx.doi.org/10.3354/dao072053

Oidtmann B (2012) Crayfish plague (*Aphanomyces astaci*). Chapter 2.2.1. In: Manual of Diagnostic Tests for Aquatic Animals 2012. Office international des épizooties, Paris, pp 101–118, http://www.oie.int/international-standard-setting/aquatic-manual/access-online (Accessed 2 October 2013)

Olson LW, Cerenius L, Lange L, Söderhäll K (1984) The primary and secondary spore cyst of *Aphanomyces* (Oomycetes, Saprolegniales). *Nordic Journal of Botany* 4: 681–696, http://dx.doi.org/10.1111/j.1756-1051.1984.tb01994.x

Pârvulescu L, Schrimpf A, Kozubíková E, Resino SC, Vrålstad T, Petrusek A, Schulz R (2012) Invasive crayfish and crayfish plague on the move: first detection of the plague agent

Aphanomyces astaci in the Romanian Danube. *Diseases of Aquatic Organisms* 98: 85–94, http://dx.doi.org/10.3354/dao02432

Persson M, Cerenius L, Söderhäll K (1987) The influence of haemocyte number on the resistance of the freshwater crayfish, *Pacifastacus leniusculus* Dana, to the parasitic fungus *Aphanomyces astaci*. *Journal of Fish Diseases* 10: 471–477, http://dx.doi.org/10.1111/j.1365-2761.1987.tb01098.x

Paunovic M, Cakic P, Hegedis A, Kolarevic J, Lenhardt M (2004) A report of *Eriocheir sinensis* (H. Milne Edwards, 1854) [Custacea: Brachyura: Grapsidae] from the Serbian part of the Danube River. *Hydrobiologia* 529: 275–277, http://dx.doi.org/10.1007/s10750-004-5493-8

Puky M, Reynolds JD, Schad P (2005) Native and alien Decapoda species in Hungary: distribution, status, conservation importance. *Bulletin Français de la Pêche et de la Pisciculture* 376–377: 553–568, http://dx.doi.org/10.1051/kmae:2005015

Royo F, Andersson G, Bangyeekhun E, Muzquiz JL, Söderhäll K, Cerenius L (2004) Physiological and genetic characterisation of some new *Aphanomyces strains* isolated from freshwater crayfish. *Veterinary Microbiology* 104: 103–112, http://dx.doi.org/10.1016/j.vetmic.2004.09.012

Rudnick DA, Halat KM, Resh VH (2000) Distribution, ecology and potential impacts of the Chinese mitten crab (*Eriocheir sinensis*) in San Francisco Bay. Technical Completion Report, University of California, Berkeley, Water Resources Center, Contribution 26

Schrimpf A, Pârvulescu L, Copila-Ciocianu D, Petrusek A, Schulz R (2012) Crayfish plague pathogen detected in the Danube Delta – a potential threat to freshwater biodiversity in southeastern Europe. *Aquatic Invasions* 7(4): 503–510, http://dx.doi.org/10.3391/ai.2012.7.4.007

Schrimpf A, Chucholl C, Schmidt T, Schulz R (2013b) Crayfish plague agent detected in populations of the invasive North American crayfish *Orconectes immunis* (Hagen, 1870) in the Rhine River, Germany. *Aquatic Invasions* 8(1): 103–109, http://dx.doi.org/10.3391/ai.2013.8.1.12

Schrimpf A, Maiwald T, Vrålstad T, Schulz HK, Smietána P, Schulz R (2013a) Absence of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of European and American crayfish in central Europe. *Freshwater Biology* 58: 1116–1125, http://dx.doi.org/10.1111/fwb.12112

Svoboda J, Kozubíková E, Kozák P, Kouba A, Bahadir Koca S, Diler Ö, Diler I, Policar T, Petrusek A (2012) PCR detection of the crayfish plague pathogen in narrow-clawed crayfish inhabiting Lake Eğirdir in Turkey. *Diseases of Aquatic Organisms* 98: 255–259, http://dx.doi.org/10.3354/dao02445

Svoboda J, Kozubíková-Balcarová E, Kouba A, Buřič M, Kozák P, Diéguez-Uribeondo J, Petrusek A (2013) Temporal dynamics of spore release of the crayfish plague pathogen from its natural host, American spiny-cheek crayfish (*Orconectes limosus*), evaluated by transmission experiments. *Parasitology* 140: 792–801

Svoboda J, Strand DA, Vrålstad T, Grandjean F, Edsman L, Kozák P, Kouba A, Fristad RF, Bahadir Koca S, Petrusek A (2014) The crayfish plague pathogen can infect freshwater-inhabiting crabs. *Freshwater Biology* 59: 918–929, http://dx.doi.org/10.1111/fwb.12315

Tuffs S, Oidtmann B (2011) A comparative study of molecular diagnostic methods designed to detect the crayfish plague pathogen, *Aphanomyces astaci*. *Veterinary Microbiology* 153: 343–353, http://dx.doi.org/10.1016/j.vetmic.2011.06.012

Viljamaa-Dirks S, Heinikainen S, Nieminen M, Vennerström P, Pelkonen S (2011) Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population – a case report. *Bulletin of the European Association of Fish Pathologists* 31(5): 182–188

Vrålstad T, Knutsen AK, Tengs T, Holst-Jensen A (2009) A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Veterinary Microbiology* 137: 146–155, http://dx.doi.org/10.1016/j.vetmic.2008.12.022

Vrålstad T, Johnsen S, Fristad R, Edsman L, Strand D (2011) Potent infection reservoir of crayfish plague now permanently established in Norway. *Diseases of Aquatic Organisms* 97: 75–83, http://dx.doi.org/10.3354/dao02386