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Observations of crayfish plague infections in commercially important narrow-clawed crayfish populations in Turkey

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Abstract – We studied the presence of possible *Aphanomyces astaci* infections in eight Turkish narrow-clawed crayfish (*Astacus leptodactylus*) populations by analyzing the prevalence and genotypes of the disease agent *A. astaci*. The qPCR analyses revealed *A. astaci* infection in seven of the studied eight populations, with the agent level A2 or higher. The agent levels among the infected populations varied from A0 to A5, *i.e.,* from negative to high level of infection, based on qPCR ranking. Based on the sequencing of the chitinase gene and the mitochondrial ribosomal rnnS and rnnL subunits, we detected both A (As) and B (PsI) haplogroups of *A. astaci* in our samples, with each of the studied populations being carriers of only one haplotype. The results confirm previous detections of *A. astaci* in Turkish narrow-clawed crayfish populations and reveal, that both A and B haplogroup *A. astaci* carriers exist widely in *A. leptodactylus* populations of Turkey.

Keywords: *Astacus leptodactylus* / *Aphanomyces astaci* / Turkey

Résumé – Observations sur l'infection de la peste de l'écrevisse dans des populations d'écrevisses à pattes grêles d'importance commerciale en Turquie. Nous avons étudié la présence possible d'*Aphanomyces astaci* dans huit populations turques d'écrevisses à pattes grêles (*Astacus leptodactylus*) en analysant la prévalence et les génotypes de l'agent pathogène *A. astaci*. Les analyses qPCR ont révélé une infection à *A. astaci* dans sept des huit populations étudiées, avec le niveau d'agent A2 ou plus. Les taux d'agents chez les populations infectées variaient de A0 à A5, c'est-à-dire du niveau négatif au niveau élevé d'infection, selon le classement qPCR. En se basant sur le séquençage du gène de la chitinase et des sous-unités rnnS et rnnL du ribosome mitochondrial, nous avons détecté les haplogroupes A (As) et B (PsI) d' *A. astaci* dans nos échantillons, chacune des populations étudiées n'étant porteuse que d'un seul haplotype. Les résultats confirment les détections précédentes d' *A. astaci* dans les populations d'écrevisses à pattes grêles et révèlent que les porteurs des deux haplogroupes d'*A. astaci* existent largement dans les populations d' *A. leptodactylus* de Turquie.

Mots-clés : Astacus leptodactylus / Aphanomyces astaci / Turquie

The narrow-clawed crayfish, *Astacus leptodactylus*, is the only native freshwater crayfish species in Turkey. In addition to its natural distribution, it has also been widely introduced into lakes, reservoirs and rivers in many parts of the country because of its economic importance and restoration of the crayfish stocks previously devastated by *Aphanomyces astaci* infections (Harlıoglu, 2008). The narrow-clawed crayfish has been reported either to have some resistance against *A. astaci* (Unestam, 1969) or being very susceptible to *A. astaci* infection (Schikora, 1906; Alderman *et al.*, 1987).

The Turkish narrow clawed crayfish populations got infected with *A. astaci* in early 1980's (Rahe and Soylu, 1989; Timur, 1990; Alderman, 1996; Kokko *et al.*, 2012; Svoboda *et al.*, 2012)

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Table 1. *Aphanomyces astaci* in Turkish narrow-clawed crayfish (*A. leptodactylus*) populations from eight lakes. Agent level A0 and A1 (>5 PFU's) indicates negative samples, A2 (5–50 PFU's) very low level infection, A3 (50–1000 PFU's) low level infection, agent level A4 (10^3–10^4 PFU's) moderate infection and agent level A5 (10^5–10^6 PFU's) a high level infection. Abbreviations As = As genotype *A. astaci,* commensurate to mitochondrial haplogroup A; PsI = PsI-genotype *A. astaci,* commensurate to mitochondrial haplogroup B.

Lake (City)	Gross symptoms (%)[2]	Crayfish (*n*)	*A. astaci* agent level in qPCR (*n*)						Chitinase (*n*)	Mt haplogroup	
			A0	A1	A2	A3	A4	A5		rnnS (*n*)	rnnS (*n*)
Lake Iznik (Bursa)	10–15	20[3]		1	5	10	2	2	As (3)		
Lake Porsuk Dam (Kütahya)	1.5–10	5			2	2	1			A (1)	
Lake Çıldır (Ardahan)	0.8–5	5	1	1	1	1	1			A (1)	A (1)
Lake Sarımsaklı Dam (Kayseri)	1–5	5		1	2		2			A (1)	
Lake Yenikarpuzlu Dam (Edirne)	3.5–12.5	4	1	1			1	1	PsI (2)	B (1)	B (1)
Lake Hirfanlı Dam (Kırsehir)	4.5–15	20[3]	1		9	9	1		PsI (2)		
Lake Hirfanlı Dam (Kırsehir)	4.5–15	5			2	2	1			B (2)	B (1)
Lake Egirdir (Isparta)[1]	0.8–12	5		1	4						
Lake Keban Dam (Elazıg)	0	6	4	2							

[1] *A. astaci* infection of PsI genotype reported by Svoboda *et al.* (2014).
[2] Harlıoglu (unpublished).
[3] Kokko et al. (2012) published the qPCR results from sampling year 2011.

with a devastating effect on the crayfisheries and crayfish export (Harlıoglu, 2004; Harlıoglu and Harlıoglu, 2006; Aydın *et al.*, 2012; Kokko *et al.*, 2012). Later, it was discovered, that some of the collapsed narrow-clawed crayfish populations recovered (Harlıoglu, 2008; Güner and Harlıoglu, 2010). However, recent reports indicated, that some populations were chronically infected with *A. astaci* (Kokko *et al.*, 2012; Svoboda *et al.*, 2012, 2014). Furthermore, it has been reported that both freshwater crayfish and freshwater crabs are *A. astaci* carriers in Turkish waters (Svoboda *et al.*, 2014).

Initially, some of the Turkish *A. leptodactylus* populations collapsed and never recovered (*e.g.*, Harlıoglu, 2008), an indication of limited resistance against *A. astaci* infection. On the other hand, some Turkish *A. leptodactylus* populations chronically infected with *A. astaci* are productive, such as those in Lake İznik (Bursa) and Lake Hirfanlı Dam (Kırsehir) (Kokko *et al.*, 2012). The observed latent infections may indicate past and contemporary partial resistance in the host, as has been argued by Unestam (1969) or even virulence evolution of *A. astaci* (Jussila *et al.*, 2015).

The aim of this study was to investigate the distribution of *A. astaci* in wild narrow-clawed crayfish populations in Turkey. We selected narrow-clawed crayfish from eight populations, which were either showing potential gross symptoms of *A. astaci* infection, *i.e.,* melanisation, necrosis or erosion of carapace, or were reported to be *A. astaci* infected (Tab. 1). Crayfish ($n = 35$) were bought from commercial crayfishermen from each location. They were caught during summer 2011 and 2012 from Lake Hirfanlı Dam, Kırsehir (39°11′ N 33°33′ E), Lake Iznik, Bursa (40°43′ N 29°52′ E), Lake Egirdir, Isparta (38°00′ N 30°53′ E), Lake Çıldır, Ardahan (41°03′ N 43°14′ E), Lake Porsuk Dam, Kütahya (39°38′ N 30°11′ E), Lake Sarımsaklı Dam, Kayseri (38°53′ N 35°44′ E), Lake Yenikarpuzlu Dam, Edirne (40°49′ N 26°19′ E) and Lake Keban Dam, Elazıg (38°38′ N 39°28′ E) (Fig. 1). The *A. astaci* prevalence in the first two populations was studied earlier (Kokko *et al.*, 2012; Svoboda *et al.*, 2012), while the last six populations' infection status has not been reported earlier.

Samples for the qPCR analyses of *A. astaci* were taken by cutting a piece of melanised cuticle or, in case of no melanised spots, a uropod from each crayfish. The samples were stored in absolute ethanol (Merck) by the Firat University staff in Turkey. The dissection tools were disinfected after every sampled crayfish tissue. The preserved samples were stored at −21 °C and then shipped by airmail to the University of Eastern Finland, Kuopio campus, for further analyses. In addition, a previous sample set from year 2011 (Kokko *et al.*, 2012) with two locations (Tab. 1) were included into further analyses.

Before the DNA extractions, the tissue samples were rinsed in sterile water to remove the ethanol. DNA extractions were conducted with E.Z.N.A Insect DNA isolation kit (Omega Bio-Tek) following manufacturer's instructions. The quantity and quality of the extracted DNA was measured with a NanoDrop-spectrophotometer (Thermo Fisher Scientific). For *A. astaci* prevalence screening, a quantitative TaqMan® minor groove binder (MGB; Applied Biosystems) real-time PCR assay (qPCR) developed by Vrålstad *et al.* (2009) was adjusted to LightCycler 480 II qPCR machine (Roche) and the sample volume was adjusted to 10 µL similarly as in Kokko *et al.* (2012). TaqMan® Environmental Master Mix (Applied Biosystems) was used for the qPCR reactions (Strand *et al.*, 2011) with 2 µL of 1 × and 10 × diluted DNA. A calibrated standard curve (Vrålstad *et al.*, 2009) was applied to determine the PFU values and agent levels for sampled crayfish tissues. Agent level A0 and A1 (<5 PFU's) indicated negative samples, A2 (5–50 PFU's) very low level infection, A3 (50–1000 PFU's) low level infection, A4 (10^3–10^4 PFU's) moderate infection and agent level A5 (10^5–10^6 PFU's) a high level infection.

Three different PCR amplicons of each sample showing agent level A3 or higher in qPCR were sequenced to further characterize the infections. The chitinase gene was amplified and sequenced for selected samples according to Makkonen *et al.* (2012b) and mitochondrial ribosomal small and large subunits, rnnS and rnnL, according to Makkonen *et al.* (2018). The obtained PCR amplicons were purified with QiaQuick

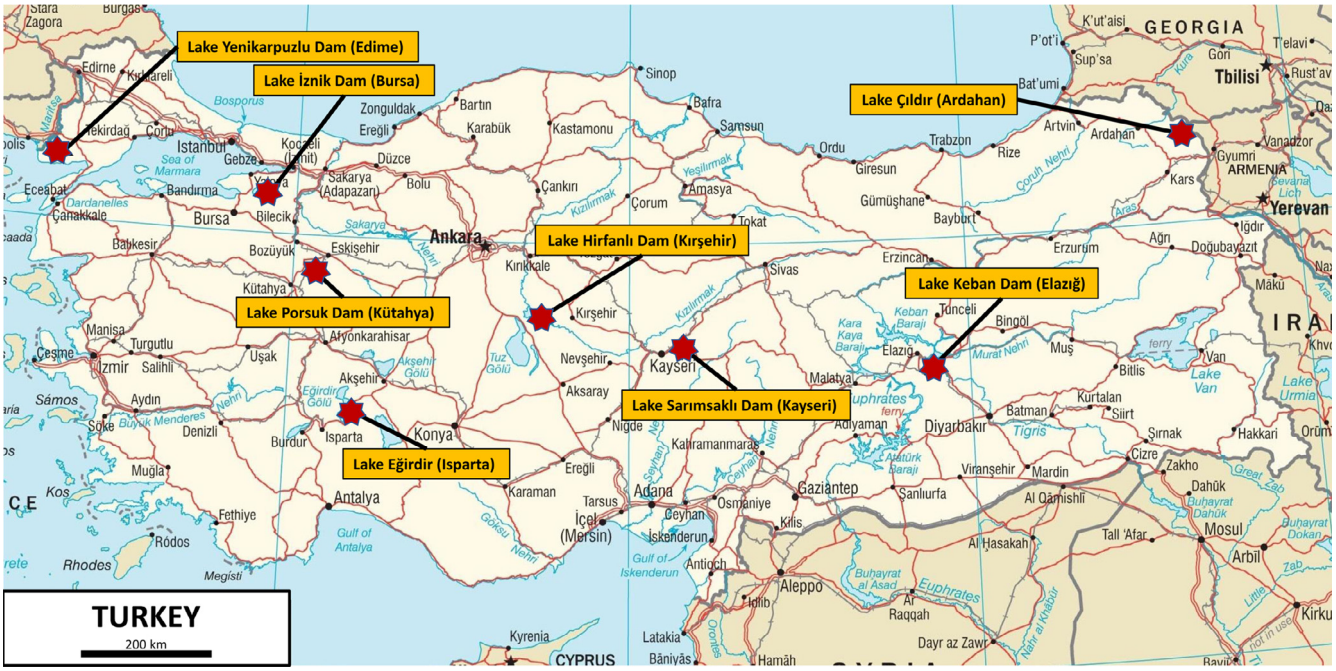

Fig. 1. Locations of the sampled Turkish narrow-clawed crayfish (*Astacus leptodactylus*) populations: Lake Çıldır, Lake Egirdir, Lake Hirfanlı Dam, Lake İznik Dam, Lake Keban Dam, Lake Porsuk Dam, Lake Sarımsaklı Dam and Lake Yenikarpuzlu Dam. Lake Hirfanlı Dam and Lake İznik Dam were analyzed earlier with qPCR by Kokko *et al.* (2012). Red stars indicate the proximate locations of the sampled water bodies.

PCR purification kit (Qiagen) following manufacturer's instructions and sequenced in GATC Biotech (Cologne, Germany). Sequences were submitted to GenBank with access numbers MG596357-MG596378.

Aphanomyces astaci infection was detected in seven of the eight narrow-clawed crayfish populations (Tab. 1), and, in six of those cases, also the haplotype of the disease agent was characterized with at least one sequence. In four cases, *i.e.*, Lake Iznik, Lake Porsuk Dam, Lake Çıldır and Lake Sarımsaklı Dam, haplogroup A (As-genotype) was causing the latent infection. In two cases, *i.e.*, Lake Yenikarpuzlu Dam and Lake Hirfanlı Dam, the infection was caused by the haplogroup B (PsI-genotype) of *A. astaci*. In Lake Hirfanlı Dam case, the haplogroup B infection was detected from both years' 2011 and 2012 samplings, indicating a latent infection caused by the haplogroup B in this site. In addition to *A. astaci*, single rnnS sequence similar to *Saprolegnia ferax* (99.1%) was detected from Lake Porsuk Dam and a sequence similar to *Pythium insidiosum* (96.5%) from Lake Yenikarpuzlu Dam. Furthermore, rnnS amplicon showing 97.4% similarity to *A. astaci* was detected from Lake Egirdir sample that was showing very low agent level (A2) in qPCR. Lake Keban Dam narrow-clawed crayfish population did not show infection gross symptoms, and also the qPCR detected only trace levels of *A. astaci* DNA, *i.e.*, agent level A1 considered as negative (Tab. 1).

The detected infections can mainly be defined as latent *A. astaci* infections (Jussila *et al.*, 2014), as the studied populations have been reported to be productive (Kokko *et al.*, 2012; Svoboda *et al.*, 2012) and show low level of infection gross symptoms (Tab. 1), even though they have variably collapsed since *A. astaci* spread into Anatolian peninsula (Köksal, 1988; Rahe and Soylu, 1989; Timur, 1990). The latent *A. astaci* infections might be caused by *A. astaci* strains of low virulence, while one of the *A. astaci* strains causing latent infection in Slovenian stone crayfish (*Austropotamobius torrentium*) has been shown to be virulent against noble crayfish (Jussila *et al.*, 2017). The haplogroups of the observed *A. astaci* infections were determined according to Makkonen *et al.* (2018) using mitochondrial rnnS ad rnnL sequences. The grouping of *A. astaci* haplogroups is similar to the genotypes (Makkonen *et al.*, 2018), and the detected haplogroup B (genotype B/PsI) strains carried by the signal crayfish (*Pacifastacus leniusculus*) are in most cases considered highly virulent (Aydın *et al.*, 2012, 2014; Makkonen *et al.*, 2012a, 2014) and no latent infections in European crayfish species caused by this haplogroup have been previously observed.

Our study shows a geographically widespread distribution of *A. astaci* among Turkish narrow-clawed crayfish populations. As those populations are still productive and have thus been commercially exploited, it seems that the Turkish narrow-clawed crayfish could have considerably high resistance against both haplogroup A and B *A. astaci* infections, as has already been indicated (Unestam, 1969; Kokko *et al.*, 2012; Svoboda *et al.*, 2012, 2014, 2017), whilst there are also studies reporting significant susceptibility (Schikora, 1906; Alderman *et al.*, 1987). This host-parasite co-evolution and adaptation possibility opens avenues for further studies on the relationship between *A. astaci*, other co-infecting pathogens and its native European crayfish hosts (*e.g.*, Edgerton *et al.*, 2004).

Regardless of the implications of a possibly elevated resistance of the Turkish narrow-clawed crayfish, the conserva-

tion of the native European crayfish requires swift actions against the spreading of different strains of *A. astaci* among native European crayfish populations. On the other hand, our finding together with the recent latent crayfish plague observations from native European crayfish populations (Jussila *et al.*, 2011; Viljamaa-Dirks *et al.*, 2011; Kokko *et al.*, 2012; Svoboda *et al.*, 2012; Kušar *et al.*, 2013; Jussila *et al.*, 2017) might be indicating a brighter future for the European crayfish under the pressures from the crayfish plague disease.

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References

Alderman DJ. 1996. Geographical spread of bacterial and fungal diseases of crustaceans. *Revue Sci Tech OIE* 15: 603–632.

Alderman DJ, Polglase JL, Frayling M. 1987. *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *J Fish Dis* 10: 385–393.

Aydın H, Harlıoglu MM, Deniz T. 2012. Harvest, export and economic status of freshwater crayfish (*Astacus leptodactylus* Esch. 1823) in Turkey. *Afr J Agricult Res* 7: 2463–2468.

Aydın H, Kokko H, Makkonen J, Kortet R, Kukkonen H, Jussila J. 2014. The signal crayfish is vulnerable to both the As and the PsI-isolates of the crayfish plague. *Knowl Manag Aquatic Ecosyst* 413: 10.

Edgerton BF, 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. *Conserv Biol* 18: 1466–1474.

Güner U, Harlıoglu MM. 2010. Status of freshwater crayfish distribution in Thrace region of Turkey. *Rev Fish Sci* 18: 1–6.

Harlıoglu MM. 2004. The present situation of freshwater crayfish, *Astacus leptodactylus* (Eschscholtz, 1823) in Turkey. *Aquaculture* 230: 181–187.

Harlıoglu MM. 2008. The harvest of the freshwater crayfish *Astacus leptodactylus* Eschscholtz in Turkey: harvest history, impact of crayfish plague, and present distribution of harvested populations. *Aquacult Int* 16: 351–360.

Harlıoglu MM, Harlıoglu AG. 2006. Threat of non-native crayfish species introductions into Turkey: global lessons. *Rev Fish Biol Fisher* 16: 171–181.

Jussila J, Makkonen J, Vainikka A, Kortet R, Kokko H. 2011. Latent crayfish plague (*Aphanomyces astaci*) infection in a robust wild noble crayfi sh (*Astacus astacus*) population. *Aquaculture* 321: 17–20.

Jussila J, Makkonen J, Vainikka A, Kortet R, Kokko H. 2014. Crayfish plague dilemma: how to be a courteous killer? *Boreal Environ Res* 19: 235–244.

Jussila J, Vrezec A, Makkonen J, Kortet R, Kokko H. 2015. Invasive crayfish and their invasive diseases in Europe with the focus on the virulence evolution of the crayfish plague. Chapter 8. In: Canning-Clode J, ed. Biological invasions in changing ecosystems. Vectors, ecological impacts, management and predictions. Warsaw/Berlin: De Gruyter Ltd, pp. 183–211.

Jussila J, Vrezec A, Jaklič T, Kukkonen H, Makkonen J, Kokko H. 2017. *Aphanomyces astaci* isolate from latently infected stone crayfish (*Austropotamobius torrentium*) population is virulent. *J Invertebr Pathol* 149: 15–20.

Kokko H, Koistinen L, Harlıoglu MM, Makkonen J, Aydin H, Jussila J. 2012. Recovering Turkish narrow-clawed crayfish (*Astacus leptodactylus*) populations carry *Aphanomyces astaci*. *Knowl Manag Aquat Ecosyst* 404: 12.

Köksal G. 1988. *Astacus leptodactylus* in Europe. In Holdich DM, Lowery RS, eds. Freshwater Crayfish: biology, management and exploitation. London: Croom Helm Ltd, pp. 365–400.

Kušar D, Vrezec A, Ocepek M, Jenčič V. 2013. *Aphanomyces astaci* in wild crayfish populations in Slovenia: first report of persistent infection in a stone crayfish *Austropotamobius torrentium* population. *Dis Aquat Organ* 103: 157–169.

Makkonen J, Jussila J, Kortet R, Vainikka A, Kokko H. 2012a. Differing virulence of *Aphanomyces astaci* isolates and elevated resistance of noble crayfish *Astacus astacus* against crayfish plague. *Dis Aquatic Organ* 102: 129–136.

Makkonen J, Jussila J, Kokko H. 2012b. The diversity of the pathogenic oomycete *(Aphanomyces astaci)* chitinase genes within the genotypes indicate adaptation to its hosts. *Fungal Genet Biol* 49: 635–642.

Makkonen J, Kokko H, Vainikka A, Kortet R, Jussila J. 2014. Dose-dependent mortality of the noble crayfish *(Astacus astacus)* to different strains of the crayfish plague *(Aphanomyces astaci)*. *J Invertebr Pathol* 115: 86–91.

Makkonen J, Jussila J, Panteleit J, Keller NS, Schrimpf A, Theissinger K, Kortet R, Martín-Torrijos L, Sandoval-Sierra JV, Diéguez-Uribeondo J, Kokko H. 2018. MtDNA allows the sensitive detection and haplotyping of the crayfish plague disease agent *Aphanomyces astaci* showing clues about its origin and migration. *Parasitology*, In Press.

Rahe R, Soylu E. 1989. Identification of the pathogenic fungus causing destruction to Turkish crayfish stocks (*Astacus leptodactylus*). *J Invertebr Pathol* 54: 10–15.

Schikora F. 1906 Die Krebspest. *Fisch Ztg* 9: 529–583.

Strand DA, Holst-Jensen A, Viljugrein H, Edvardsen B, Klaveness D, Jussila J, Vrålstad T. 2011. Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Dis Aquat Organ* 95: 9–17.

Svoboda J, Kozubíková E, Kozák P, Kouba A, Bahadir KS, Diler Ö, Diler I, Policar T, Petrusek A. 2012. PCR detection of the crayfish plague pathogen in narrow-clawed crayfish inhabiting Lake Egirdir in Turkey. *Dis Aquat Organ* 98: 255–259.

Svoboda J, Strand DA, Vrålstad T, Grandjean F, Edsman E, Kozák P, Kouba A, Fristad RF, Koca SB, Petrusek A. 2014. The crayfish plague pathogen can infect freshwater-inhabiting crabs. *Freshw Biol* 59: 918–929.

Svoboda J, Mrugała A, Kozubíková-Balcarová E, Petrusek A. 2017. Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *J Fish Dis* 40: 127–140.

Timur G. 1990. Crayfish plague in some lakes of Turkey. *Bull Eur Ass Fish Pathol* 10: 100–103.

Unestam T. 1969. Resistance to the crayfish plague in some American, Japanese and European crayfishes. *Rep Inst Freshw Res Drottningholm* 49: 202–209.

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. *Bull Eur Ass Fish Pathol* 31: 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*. *Vet Mic* 137: 146–155.

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