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Optimisation of diagnosis of the reportable crayfish plague (Aphanomyces astaci) by genotyping

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Key words
Crayfish plague, Aphanomyces astaci, genotyping, eDNA

Aim of the study
The aim of the project was to optimize and complement current diagnostic methods for the reportable crayfish plague in Switzerland by testing and establishing state-of-the art methods for the identification of *Aphanomyces astaci* genotypes. Knowledge about the distribution of *A. astaci* genotypes will help to understand past, present and future spread scenarios of *A. astaci* in Swiss rivers and lakes. The new protocols will also allow to predict outcome scenarios of crayfish plague outbreaks and will enable molecular epidemiology of the disease in Switzerland.

Material and methods
Genotyping: Various commercial kits for DNA extraction were compared to determine the most suitable extraction kit in terms of DNA concentration, fragment length and *A. astaci* DNA detection probability. DNA from all five reference strains (A-E), kindly provided by the WOAH reference laboratory in Finland, was extracted using three different extraction kits. Afterwards, long-read sequencing (PacBio) was performed and analysed by bioinformatics.
To investigate genotype distribution in Switzerland, we performed a prospective and a retrospective study. For the prospective study, 1240 crayfish from 81 populations all over Switzerland - 26 native crayfish populations, 55 invasive crayfish populations - were dissected and various samples were archived at -20°C for further investigation. For the retrospective study, diagnostic cases archived at FIWI between 1958 and 2020 were evaluated, in total 209 submissions of 1502 crayfish (125 submissions of native crayfish, 66 submissions of invasive crayfish and 17 populations of unknown origin). DNA from paraffin blocks of submissions between 1990 and 2020 was extracted and qPCR (Vralstad et al. 2009) was performed for detection of *A. astaci* DNA. In case of positive results, conventional PCR (Oidtmann et al. 2006) and Sanger sequencing were performed. Genotyping according to DiDomenico et al. 2021 was applied to positive samples.
Environmental DNA: To establish an eDNA protocol, two recent outbreaks of crayfish plague were examined, Sissle (Argau) and Waldbiotop Wolfwil (Solothurn). Water samples were taken regularly at the two river sites (daily, weekly, 2-weekly, monthly - depending on location and time after outbreak), at the location of the outbreak, upstream and downstream and in tributaries. 560 water samples were collected. eDNA was extracted and examined by qPCR.

Results and significance
Background: During the course of the study, we found evidence that the internationally accepted and WOAH recommended methods (qPCR according to Vralstad et al. 2009), PCR according to Oidtmann et al. 2006, sequencing) had serious problems with low specificity (qPCR) or low sensitivity (PCR). In the qPCR, false positive results by cross reaction with Aeromonas spp. and other Aphanomyces spp. were recognised. PCR showed low sensitivity, not detecting low agent concentrations, like present in carrier animals. Analyses to identify sequence regions to develop a new sensitive and specific detection method at FIWI are still ongoing. Further re-

search to establish a valid method to reliably detect *A. astaci* in native (sick) and invasive (carrier) crayfish is urgently needed.
Genotyping: Analyses by bioinformatics to identify the best suited extraction method are also still ongoing. However, first results show that even if DNA concentration is low using Insect Kit, fragment length and detection probability is higher.
During the prospective study, *A. astaci* DNA was detected in 4/26 populations of native crayfish and 4/4 populations of invasive crayfish. First results show genotype B involvement in the crayfish plague outbreak in the Sissle (Aargau).
In the retrospective study, qPCR was considered positive in 50 paraffin samples originating from 30 submissions. Using PCR, 11 samples revealed to be positive by PCR and sequencing confirmed presence of *A. astaci* DNA. In 10 cases, the genotype could be identified: Genotype B (3 cases native crayfish, 2 cases invasive crayfish; 1997, 2007, 2017, 2021), Genotype D (1 case native crayfish, 1 invasive crayfish; 1996, 2018) and Genotype E (3 cases native crayfish; 1994, 2011, 2018). One case from 2018 in native crayfish showed a co-infection with two genotypes (D and E). The occurrence of genotypes B, D and E in Switzerland resembles the situation in our neighbouring countries and in Central Europe. Genotype A, which is mostly associated with a chronic course of disease in native crayfish populations, was not found in our archive material. One of the most likely reasons is that only native crayfish showing high mortality were submitted to FIWI for diagnostics.
Environmental DNA: At least 1 filter collected during 31 days in the Waldbiotop Wolfwil was considered positive. The first positive result was found 8 days following detection of crayfish plague outbreak. Positive results were obvious until 374 days post first detection.

Publications, posters and presentations

Simone Roberto Rolando Pisano, Manon Zürcher, Jonas Steiner, James Ord, Pamela Nicholson, Heike Schmidt-Posthaus. Epidemiologie und Ökologie der Krebspest in der Schweiz. 19. Gemeinschaftstagung der deutschsprachigen Sektionen der European Association of Fish Pathologists (EAFP), Hannover, 05.-08.10.2022. Oral presentation

Simone Roberto Rolando Pisano, Nabila El Hassani, Heike Schmidt-Posthaus. Optimierung einer nicht-destruktiven feldbasierten Probenahmemethode zum Nachweis von Aphanomyces astaci. 19. Gemeinschaftstagung der deutschsprachigen Sektionen der European Association of Fish Pathologists (EAFP), Hannover, 05.-08.10.2022. Poster

Simone Roberto Rolando Pisano, Workshop 2023 – improving detection of eDNA from crayfish and crayfish plague - Epidemiology and ecology of crayfish plague and genomics of *Aphanomyces astaci* in Switzerland, Stockholm 23-24.05.2023.

Simone Roberto Rolando Pisano, Heike Schmidt-Posthaus. EURL conference, Copenhagen 30.05-01.06.2023. Oral presentation

Simone Roberto Rolando Pisano, Nabila El Hassani, Heike Schmidt-Posthaus. Optmisation of a nondestructive sampling method for the detection of *Aphanomyces astaci.* The Zoo and Wildlife Health Conference, Valencia, 05.-09.06.2023. Poster

Simone Roberto Rolando Pisano, Manon Zürcher, Jonas Steiner, James Ord, Pamela Nicholson, Heike Schmidt-Posthaus. Epidemiology and ecology of crayfish plague in Switzerland. European Association of Fish Pathologists, Aberdeen, 11.-15.09.2023. Oral presentation

Simone RR Pisano, Jonas Steiner, Elodie Cristina, Zoe Delafortrie, Armin Zenker, Raphael Krieg, Heike Schmidt-Posthaus. 50 years of crayfish plague in Switzerland. Planned publication, Journal of Invertebrate Pathology

Simone RR Pisano, Tatiana Zingre, Torsten Seuberlich, Heike Schmidt-Posthaus. Virus occuring in selected population of a native endangered species, the noble crayfish (*Astacus astacus*) in Switzerland. Planned publication, Journal of Invertebrate Pathology,

Bruno auf der Mauer, Simone RR Pisano, Adrian Gross (2023) Factsheet Neomyceten – Crayfish plague

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