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Prof. RNDr. Bohuslav Gaš
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Referee Report on the PhD thesis of Mgr. Lenka FILIPOVA, titled

Genetic variation in North American crayfish species introduced to Europe and the prevalence of the crayfish plague pathogen in their populations

Dear Prof. Gaš,

You kindly invited me to write a report on the thesis submitted by Mgr. Lenka Filipova, which I am sending you in this letter. Unfortunately, I shall not be able to come to Prague when Lenka will be defending her thesis. I therefore included a few questions that you might find useful at the defense.

My first and overall impression of the submitted PhD thesis has been overwhelming. Lenka addresses one of the big current topics of global conservation biology, the problem of non-native invasive species and their associated pathogens. What is more, it is one of the long-term hot topics in European freshwater conservation, namely the problem of introduced invasive crayfish species and their role as reservoir and vector of *Aphanomyces astaci*, the crayfish plague agent. Her geographic approach is global, instead of the local or regional one usually seen in PhD or master theses. She analyzed samples not only from many European countries, but also studied reference populations from North America in order to understand the nature and number of introductions from a genetic perspective. I was impressed by the sheer number of specimens analyzed by various methods in several independent studies. If I counted correctly, the thesis is based on 683 crayfish individuals examined for mitochondrial DNA variation, 222 individuals studied for allozyme polymorphism, and 993 individuals in which the presence of the crayfish plague pathogen was tested by

quantitative real-time PCR. These are numbers that several times exceed the usually seen sample sizes. Equally impressive is the variety of different methods and approaches. Lenka used several molecular techniques such as classical DNA amplification and sequencing, allozyme electrophoresis, and TaqMan minor groove binder real-time PCR. The analytical work is truly interdisciplinary as it requires knowledge of phylogenetic and phylogeographic methods, DNA-barcoding, classical morphological taxonomy, biogeography and parasitology. I can only admire such a broad methodological knowledge. Not surprisingly, such a huge amount of high-profile research work yielded excellent and very useful results. Thanks to Lenka's effort, for the first time a clear picture of the genetic background for two of the most important non-native invasive crayfish species in Europe (*Pacifastacus leniusculus* and *Orconectes limosus*) is emerging. In addition, a reference framework for taxonomic identification and DNA barcoding of all North American introduced species and major lineages is set, including the less common and less known ones. And finally, a new and most promising method for highly sensitive detection of the crayfish plague agent was devised and tested, providing evidence of its widespread occurrence in Europe and its high, up to 30 per cent prevalence. Equally prolific as Lenka's research was her writing, resulting in no less than seven published or submitted scientific papers, all but one of which with her as the first author.

Formally, of course, this thesis meets all requirements. In fact, it exceeds them by far. Five of the papers are published in peer-reviewed journals indexed by ISI. Three of them are top journal in their subject area, with an IF as high as 3.47. This fact alone leaves little doubt about the high scientific quality of Lenka's work.

It is understandable that with such a broad range of methods used and topics studied, not all questions could be addressed in much detail. Personally I was missing a bit more insight into the demography of the introduced populations, their possible interconnections, recent gene flow, as well as a more explicit treatment of the genetic diversity of introduced vs. source populations. The high number of analyzed individuals would offer a good basis to address this kind of questions. However, I do not see the lack of these points as a weakness of the thesis, rather as an opportunity that still needs to be realized.

Another point I would like to encourage Lenka to work on and think of a bit more is a synthetic and synergistic perspective on her results. As the results are put now, they offer an array of very useful and interesting new discoveries, tools and recommendations. But they mostly stand alone, each part is self-sufficient. Lenka herself writes (Conclusions, 1st paragraph) that "it is necessary to study the whole system, not only the invasive host species but also their parasites, as such host-parasite association may have amplified negative effect on invaded ecosystem". In this context, I would like to ask a few questions that can in part be answered based on the results at hand, and in part as hypotheses that yet remain to be tested.

(1) With so many successful and aggressive invasive species, the native crayfishes of Europe seem to have meager chances of recovery. Is there a single most dangerous NICS, or does the true threat come from the fact that there are several ecologically different NICS?

(2) Is a single successful introduction (like in *Orconectes limosus*) likely to be more disastrous than several successive one from different source populations? What role might genetic diversity of the introduced populations play?

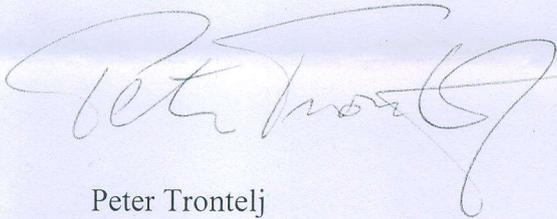
(3) How do new introductions of the same or different NICS affect the already established populations of NICS? Are there any direct or indirect, positive or antagonistic interactions?

(4) If, hypothetically, we would be able to eradicate all North American NICS in Europe, would the crayfish plague pathogen remain?

Conclusive Remarks and Recommendation

The PhD thesis submitted by Mgr. Lenka Filipova is an outstanding scientific achievement. Its results are of high practical importance and a valuable contribution toward conservation of endangered European freshwater biota. This thesis fulfills all the criteria needed to obtain a PhD degree. I highly recommend it for defense and acceptance.

Sincerely,

A handwritten signature in black ink, appearing to read 'Peter Trontelj', written in a cursive style.

Peter Trontelj