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Evaluation of Mgr. Jiří Svoboda's Ph.D. thesis

I am giving my evaluation of Mgr. Jiří Svoboda's Ph.D. thesis based on the invitation by Professor David Storch, chairman of the committee. As an evaluator, I am, to my best knowledge, not incompetent due to the likelihood of bias in this matter. I wish that the following comments would be taken into account, when considering the validity of this work as a Ph.D. thesis.

Assessment of the thesis

The science presented in the thesis is solid and based on four (4) published, one (1) manuscript and two (2) additional scientific papers. Mgr. Jiří Svoboda is the first author in all scientific papers attached to the thesis and has also contributed to additional scientific papers. The author lists of the publications, as such, are convincing and based on international collaboration in four (4) out of five (5) scientific papers attached to the thesis. Due to the pre-review process, the scientific background of the thesis can be considered as well founded. As the scientific papers have already been critically evaluated by several pre-reviewers, I will not focus on the published papers detailed merits or interpretations of individual paper's results, but rather try to evaluate the thesis as the synopsis Mgr. Jiří Svoboda's research.

The publications and appendices attached to the thesis can be assessed according to their originality as follows (numbers refer to those in the list of the publications attached to the thesis):

1. The first paper is a molecular verification of the chronic *Aphanomyces astaci* infection in Turkish narrow clawed crayfish, a study truly needed to boost the understanding of the chronic crayfish plague infections in native European crayfish. The case of the Turkish crayfish in relation to the late crayfish plague infections in that region is a hot topic and should be carefully investigated to understand the adaptation of the *A. astaci* to European conditions. I personally like this paper, since the studies were carried out concurrently with my group, unaware of each others, and resulting in similar findings and conclusions. A true example of ideas brewing among different scientific groups and science well done, too!
2. The issues of other crustaceans than freshwater crayfish or organisms with calcified parts acting as carriers or reservoirs for the *Aphanomyces astaci* have been long discussed. This paper, in addition to having a wonderful background story about developing of the international collaboration, also fills its place nicely in the development of the theories on the *A. astaci* life cycle and sheds light to this relevant issue of alternative hosts for the *A. astaci*.
3. This paper is another example of novel research covering theoretically discussed topics and allowing conclusions of practical scale and relevance. Novel methods based on molecular analyses allow for better understanding of the disease dynamics. One of the key arguments regarding chronic crayfish plague infections in water bodies has here been partially proven wrong and in need for further studies.
4. The topic of the fourth publication was intensively investigated during the period when this study was carried out. The results are again obtained thanks to the improved molecular methods for the detection of the *Aphanomyces astaci* spores. The discovery of one more invasive species acting as a constant source of *A. astaci* spores verifies some of the previous studies and highlights the fact



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that all invasive alien species are posing a permanent threat to the European native crayfish.

5. It has been stated in the past, that *Aphanomyces astaci* cannot survive the digestive track of the warm blooded animals, while not all potential species have been studied. The last one of the attached papers adds to the prevailing knowledge and verifies that two terrestrial predators, otter and mink, are unlikely to pass infective life stages of *A. astaci* through their digestive track. Thus, as predators, they also are unlikely carriers of their prey crayfish disease agents such as *A. astaci*. Quite cunningly, some avenues of the studied animals as carriers are left open, as part of the proper scientific speculation.
6. (Appendix) The survey in Netherlands adds to knowledge on the spread of *Aphanomyces astaci* among wild crayfish stocks in Europe and adds another carrier species to the unfortunately long list of alien carrier crayfish present in Europe, namely *Orconectes cf. virilis*. These findings are part of the baseline studies and reveal unfortunate facts, but have to be done.
7. (Appendix) The crayfish are bound to carry several parasites and pathogens, which do not routinely affect their wellbeing. In addition to having a porous exoskeleton, they also harbor water in their gill chambers, which allows for the transport of micro-organisms. The crayfish are frequently transported for stocking and other purposes and thus act as transport platforms for various micro-organisms. The studies trying to describe their co-travelers are needed as they shed light to distribution of these often ignored micro-organisms accompanying freshwater crayfish.

Specific critical comments

I would like to comment on a few matters been discussed in the synopsis section of the thesis. The matters are raised, because I would like to encourage Mgr. Jiří Svoboda and actually any of my colleagues for intensive and fruitful debate on any matters crayfish. Hopefully as part of the celebrations of this doctoral thesis process. Debates and discussions are bound to create positive competitive situations and improve our work as crayfish researchers. Thus, I would like to see positive collaborative competition among European crayfish research groups instead of competition against each others as there are way too many and too complicated problems to solve, which do not allow wasting of the resources or time.

The exchange of the genetic information, especially in the form of asexual vs. sexual reproduction in *Aphanomyces astaci*, has been long debated and is also discussed in this thesis (pages 8-9). The pathways for evolutionary adaptation of the different *A. astaci* genotypes and isolates, i.e., individual *A. astaci*, allow the speculations and actually encourage these speculations. In his thesis Mgr. Jiří Svoboda concludes that *A. astaci* life cycle does not include sexual processes. Fair enough, but I would still leave the door ajar and I would encourage my colleague to explore alternative pathways, too.

The role of *Aphanomyces astaci* infection in boosting disease resistance of crayfish of North American origin is interesting (page 10). Recently, it has been reported, that signal crayfish seem to be more susceptible to both *A. astaci* infection and multiple infections of *A. astaci* and other parasites under the Nordic Countries' conditions. The outcome of the baseline infection with *A. astaci* in signal crayfish can be seen as severe gross symptoms and increased mortality, which has been reported to cause population collapses both in Sweden and Finland and also increased mortality during laboratory infection trials. There are also reports indicating other diseases accompanying *A. astaci* infection in signal crayfish, included the novel finding of eroded swimmeret disease



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(ESS). Thus, I would rather conclude that *A. astaci* infection is harmful to alien crayfish species under European conditions, at least in case of the signal crayfish.

The list of crayfish indicating their resistance or susceptibility against *A. astaci* infection (Table 1) includes one rather crucial mistake, since a reference by Aydin *et al.* (2014) has been overlooked when stating that signal crayfish are resistant, together with other findings of similar nature. Signal crayfish should be listed as at least resistant-susceptible.

The last sentence of the second to last paragraph on page 15 is hard to understand. Maybe this should be clarified?

The crayfish plague episode dynamics, including killing rate, is depended on ambient temperature and dose, among other matters. In page 16, second paragraph, last sentence, you claim that your colleague did not observe any differences in the progress of the crayfish plague epidemic or subsequent population recovery. Would it be that the differing conditions in wild habitat and crayfish stock might have obscured the possible differences?

The chronic crayfish plague has been long discussed and theories developed. Your speculation on alternative hosts in page 17, first paragraph, is adding to this chain of speculations. The topic, as wide as it is, is definitely worthy of much closer look, not least because the risks of failure in the introductions and re-introductions of native European crayfish have to be diminished. Any further comments on this issue?

The transport of wet or moist matter, especially the transport of fish for stockings, is a potential pathway for pathogen spreading. The fish farming industry has thus a big responsibility here. This has been scarcely discussed, maybe because of the rather big value of stockling production and resulting fish stockings. How would you address this issue of convincing the fish farming industry of the best code of practice regarding safe and pathogen free stocking process?

The issue of crabs being alternative hosts for *A. astaci* opens a new, frightening avenue. Are we facing an even worse future in regard to attempts to limit the spread of *A. astaci* and resulting loss of the native European crayfish?

I especially like the statement that we have to face the fact that alien invasive crayfish have to be stopped in order to be able to discuss possibilities for European native crayfish conservation. One of the hopes here is the implementation of the European Union Regulation 1143/2014 and firm and widespread acts to prevent the trade of live invasive alien crayfish. Is there still hope for the native crayfish and should we have strong stand against further spreading of alien crayfish as it has been argued that the economic value of alien crayfish outcasts their possible damage?

I really liked the list of future perspectives and during the first reading I ticked seven (7) items that would have been in my list, too. And included more with every reading since. Good stuff and research time well spent, as it actually should, resulting in focused and thoroughly considered interpretations of the study outcome and future perspectives.

General questions and specific issues

I would like to hear the candidates, Mgr. Jiří Svoboda's, comments, speculation and also philosophical viewpoints on the following issues

1. The relationship between the North American crayfish species and *Aphanomyces astaci* has been somewhat balanced in their native distribution in North America. This balanced relationship has been reported to have been changed and is currently causing trouble to signal crayfish among its European



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- populations. Do you agree with this statement and if you do, what might have been causing this imbalance? Or do you disagree and why?
2. How would you describe the relevance of your thesis in regard to deepening the knowledge on one of the most devastating aquatic animal diseases, the crayfish plague. Your response should be addressing specifically the possibility of limiting and minimizing the adverse effects of the *Aphanomyces astaci* on the European aquatic ecosystems.
 3. Could you speculate on the future of the native European crayfish, especially bearing in mind the possible future spreading of the *Aphanomyces astaci* and its chronic carriers, the invasive alien crayfish species?
 4. You have mentioned and speculated on the possibility of sexual reproduction in *Aphanomyces astaci*. Do you see any relevance in the attempts to study this issue?
 5. The European Union Regulation 1143/2014 aiming for the eradication of selected invasive alien species (IAS) or minimization of their damage to the biodiversity in Europe is currently debated. The list of the most harmful IAS of EU concern, the 50-list, includes currently signal crayfish, red swamp crayfish, marbled crayfish and spiny-cheek crayfish. How do you see the relevance of this EU Regulation?

My recommendation

I am honored to recommend, based on the merits of his thesis, that Mgr. Jiří Svoboda will be awarded a Ph.D. title.

My best crayfishes

A handwritten signature in black ink, appearing to read 'Japo Jussila'.

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