

PhD thesis review report – Agata Mrugala

Overall assessment

Agata Mrugala has in her PhD dissertation “The crayfish plague pathogen *Aphanomyces astaci* in its introduced ranges: vectors, introduction pathways, genetic variation and host-pathogen interactions” made a substantial and valuable contribution to broaden the knowledge on the *A. astaci* carrier status in populations of non-indigenous American crayfish species not only in Europe, but also in Asia. Furthermore, her work demonstrates that aquarium trade not only is a source to the introduction of alien crustacean species, but also poses an introduction pathway to serious crustacean diseases. Finally, Agata’s work also makes progress towards a better understanding of crustacean immunity in response to *A. astaci* strains of different virulence. Thus, Agata fully prove through her thesis excellent competence for scientific work. The thesis is a collection of published papers supplemented with a very well written general introduction and conclusion. While the requirement for papers in a PhD thesis at the Charles University is set to at least 3 (preferably published) papers, Agata has included 8 peer-reviewed papers that are recently published or in press. In addition, she includes another 3 published papers as appendices. She is the first author of five papers in the thesis, two of which with shared first authorship, and second author on the remaining 3 papers. This is very impressive in such a short period of time. In the papers in the appendices (which has been given less attention in the further assessment) Agata is co-author no. 9 or below. Thus, it is no doubt that Agata’s thesis is well within the requirements in terms of scientific volume. I also regard it as a strength that different journals is used, indicating that Agata most likely has acquired diverse publishing competence though various revision processes. The journals include:

1. *Journal of Invertebrate Pathology* (Impact Factor: 2.198) (Paper 1 & 5 & 7), 2. *Aquatic Conservation: Marine and Freshwater Ecosystems* (Impact Factor: 2.415) (Paper 2), 3. *Journal of Limnology* (Impact Factor: 1.725) (Paper 3), 4. *Biological Invasions* (Impact Factor: 2.855) (Paper 4), 5. *Aquatic Invasions* (Impact Factor: 1.955) (Paper 6), 6. *Journal of Fish Diseases* (Impact Factor: 2.053) (Paper 8), 7. *Conservation Biology* (Impact Factor: 4.267) (Paper in Appendix 1), 8. *BioScience* (Impact Factor: 4.294) (Paper in Appendix 2) and 9. *PLOS Biology* (Impact Factor: 9.343) (Paper in Appendix 3).

Thus all papers in the thesis has already been evaluated by external referees and found worthy of publication. In that respect, the overall scientific merit of this thesis is indeed very good.

The introductory chapter is a very well written overview of the research field and put all presented papers of the thesis into a context and order that is logic and easy to follow. Agata demonstrates accuracy, overview, and ability of critical scientific thinking. At the same time, the chapter tends towards a rather descriptive style with focus on crayfish conservation and management issues. If I miss something from the scientific viewpoint, that is 1) a more critical discussion regarding choice of methods and statistics in the PhD thesis, 2) more ambitious and/or original future perspectives in the research field and 3) overall hypotheses/aims/research questions of the PhD thesis.

The originality of the approaches and results in papers differs from average to very good, as do the experimental design. Some of the papers present new, important but at the same time rather expected results obtained through molecular screening surveys for the presence/absence of *A. astaci* in American crayfish species where *A. astaci* is confirmed (paper 1, 2) or not confirmed (3) in previously not tested American hosts or geographic areas. These studies present no study design as such, but the chosen molecular methods are adequate and carefully carried out. However, a larger sample size per crayfish population would have strengthened these studies and allowed for more powerful statistic tests than conducted here. Paper 4 has a similar but more offensive approach. Here, also a broader range of methods is used; including DNA sequence based phylogenetic identification of ornamental crayfish species. Important and original findings in this paper include that ornamental crayfish species in aquaria trade was found positive with regards to two serious crustacean pathogens; crayfish plague (*A. astaci*) and white spot disease virus (WSSV). Further, ornamental crayfish species identity is sometimes erroneous when sold in pet shops. The papers 5-7

represent experimental work (infection studies), with clear hypotheses and well-planned study design and adequate statistics. In particular paper 5 contributes with original and novel results regarding the immune responses of *Astacus astacus* towards virulent and a-virulent *A. astaci* strains. The paper further establishes new knowledge regarding the virulence of previously not tested strains of *A. astaci* (e.g. genotype E and non-Finnish strains of genotype A). This paper also offers the broadest choices of methods, including cultivation, infection experiments, protein analyses (immune parameters) and molecular analyses, and is indeed a very important and well-performed 1st author contribution in the thesis. The well planned experiment, carefully monitored observations in the mortality experiment and the various analytical results obtained during downstream analyses allows for statistical analyses, which seems carefully performed. Since I miss statistics in the previous papers, it is very good that adequate statistics is in place here. It is not clear to me however, if Agata has actively performed the statistics, or if this is done by the other shared 1st author or some of the other co-authors. Paper 6 and 7 present also infection experiments of Australian crayfish and exotic scrimps, respectively, with clearly expressed hypothesis and study design, and adequate statistics. Paper 8, the review paper, is a nice but not crucial addition to the PhD thesis. For the scientific papers presented in this thesis, the choices of methods are adequate and mastering of methods very good. It has been a team-work, thus Agata's part in mastering of methods is for obvious reasons narrower than the total amount of methods presented. This is satisfactory explained in the author contribution statements, perhaps apart from the statistics. In general, the interpretation of results for all papers is carefully performed and the quality of documentation adequate and precise, although not particularly sophisticated or graphically impressive.

Specific critical comments

It is neither easy nor fair to criticize a thesis that presents 8 (+ 3) papers already evaluated by external and independent referees, and found worthy for publication in international peer-reviewed journals. The comments below are thus not meant as severe deficiencies of the thesis as such, because the thesis is great and substantially more voluminous than can be expected or demanded from a PhD-candidate within a 4 year period. Still, with the large body of presented papers it is of course possible to find aspects for some hindsight criticism or at least constructive feedback.

- There are likely cultural differences between countries with regards to a setup for the introductory chapter of a PhD thesis. With the "Scandinavian" traditions in mind, I miss a more critical discussion on the choice of methods and statistics (or bioinformatics), including experienced pitfalls and/or eventual methodological developments conducted during the study. Further, I miss one or several clearly stated hypotheses and/or main objectives of the PhD work.
- Several of the papers are rather similar screening studies (*A. astaci* presence/absence) with large overlaps in introductions, material and methods, and discussions, and with no statistics apart from very broad confidence intervals for prevalence. They have in common a relatively low sample size per tested population, and lack of statistic tests. It would seem a better priority for a PhD study to focusing more thoroughly on a few selected topics, and giving higher weight on basic scientific methodology and thinking (hypothesis testing, experimental design, sample volume & balance, and statistics) – as done in paper 5 in particular, and also paper 6-7.
- In the screening papers evaluating presence/absence of *A. astaci*, I miss more thorough planning of fieldwork and sample size. With higher sampling effort, perhaps more general questions could have been tested statistically across related papers and given some new perspectives/insights?
- In paper 8, I do not see the rationale in advising to define crayfish to have either high or low susceptibility to *A. astaci* when "moderate" actually seems to be the case both for the A-genotype & European crayfish, and for many *A. astaci* genotypes and some Asian crayfish species (shown in this thesis and in other papers). The "moderate" susceptible maybe the most dangerous with respect to disease transmission and spread. As always in biology, putting "nature" in boxes is rarely very meaningful.

- My last comment is regarding priorities and focus: Even though Agata tie all papers nicely together, I believe the thesis would have been more focused and still excellent without all papers included. The papers I have in mind are 3, 8 and appendices.

General questions to the defendant

I will ask several specific questions to each paper during the defence (not listed here). Here, I list some general questions that could be interesting to discuss with the defendant. Few if any of these questions has a "correct" answer as such, but will hopefully give Agata the opportunity to demonstrate reflective ability, overview and scientific courage, and create a good discussion.

1. You have presented many more papers than needed for a PhD. An alternative would have been to go deeper into 3-4 papers. What would you in case have skipped and what would you prioritize to focus more on/go deeper into?
2. Based on what you know now, and if should start up a new project tomorrow where money is no limiting factor, what would you regard the most scientifically interesting and/or urgent hypothesis to test within this research field, and how would you proceed?
3. You state a hope that your thesis can contribute/prove useful in management efforts towards crayfish plague. What do you regard as the most important result of your thesis in a crayfish (and crayfish disease) management perspective?
4. It seems that you believe it is only a matter of time before all American populations are infected and carriers of *A. astaci*, and that the observed varying prevalence or failure to detect the agent in some populations is a result of introduction history. What other factors could impact? If *A. astaci* was equally "successful" in all water bodies provided access to crayfish, how can you explain very low (+/- 10% prevalence) in some populations?
5. Ten years ago, diagnostics of crayfish plague moved from culture based methods with low success rate to molecular detection methods with high success rate. Still, we are only detecting the problem faster, and we have no functional cure if an outbreak emerges or if we find a positive carrier population. What do you think (qualified guess/speculations) we are doing in 10-50 years from now with regards to crayfish plague diagnostics, surveillance, control, eradication and eventual treatments?
6. We have a good overview of the crayfish and crayfish plague story in Europe during the past 100 years. If you should try to make a qualified guess ("best case" & "worst case" scenario), how will the situation for native and invasive crayfish in Europe look like in 100 years from now, and what have happened with *A. astaci* virulence and European crayfish immunity?
7. In the papers in the appendices, you bring in the term "human values" and discuss whether these should be included or excluded when defining impact of alien species. Do you have examples where "human values" create conflicts and different views regarding management of freshwater crayfish? Would you advise to "remove" human values when defining impact of alien crayfish? How could that in case influence management (including eradication) strategies of "alien" American crayfish and "only a little bit introduced and almost native" crayfish such as noble crayfish in Norway and narrow-clawed crayfish Western Europe?

Statement

The quality of this thesis fulfills indeed the criteria necessary for obtaining PhD degree, and I have no hesitation in recommending this thesis for public defense. I look forward to September 2nd!

Oslo August 24th 2016


Dr. Trude Vrålstad