



Subject: Review of Jiří Svoboda Ph.D. thesis entitled **Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci***

1) Short overall assessment of the thesis

The thesis includes 7 publications (6 published in the peer reviewed journals and 1 prepared for submission). In five of these publications candidate Svoboda is the first author. Thesis also comprises: Abstract with an outline of publications and manuscripts, Introduction to the topic that covers different aspects of crayfish plague research ending with Future perspectives and References used. Besides the thesis, candidate produced a Summary that offers a short overview of the most important information on the studies he conducted: Introduction, Aims, Material and methods, Results and discussion and Conclusions.

After reading all of the thesis parts I can state that this is an original work of a high scientific quality. All of the issues studied are thoroughly thought through, the problems (aims) to be solved are clearly defined, the experimental work is well designed, the applied methods are novel, relevant and well chosen, the results are sound, and their interpretation and the discussion clearly shows that candidate has both excellent knowledge and understanding of the topic which he is studying. Also, Jiří Svoboda's writing style is fluent as he expresses himself with ease, clearly, logically and precisely, and it is easy to follow and understand the texts.

2) Specific critical comments

Since all but one manuscript (Experimental evaluation of the potential for crayfish plague transmission through the digestive system of warm-blooded predators ) have been published in the scientific peer reviewed journals, I will only comment on this one.

The idea of studying the possibility of *A. astaci* transmission through the digestive system of mammals and birds is original and as yet unstudied, this experiment is well designed and the methods well chosen. Although I understand why the authors wanted to include into the manuscript both the results of experiment *in vivo* and *in vitro*, I think that this approach makes



the MS in parts a little bit hard to follow and oversaturated (confusing). The latter specifically refers to the material and methods and results of the part concerning “High-temperature tolerance of *A. astaci* cultures“. Therefore I would suggest perhaps taking into consideration splitting the MS into two MSs, one including experiments with transmission through the digestive system of mammals, and the other dealing with the tolerance of different *A. astaci* strains to high temperatures. This approach would offer space to elaborate on the results in more detail (the differences between the different strains, the temperatures and influences of minor changes in experimental design) and consequently would offer a clearer understanding of this topic to the audience.

Another minor thing that I noticed is that the name of one *A. astaci* strain used in the Material and methods is different from the one mentioned in the Results and Discussion; strain 185 (Material and methods) and strain 187 (Results, Discussion, Table S3).

### 3) General questions to the defendant

Your study, presented as chapter 1, was the one of the first that revealed the presence of the crayfish plague pathogen in the apparently healthy looking population of native European narrow-clawed crayfish. You have stated that these results indicate a long-time co-existence of *Aphanomyces astaci* and native European crayfish species. What did you mean by long time? Could you give some possible scenarios (explanations) on the co-existence of *A. astaci* and native European crayfish? Could you think of the way (an approach) to calculate the time of divergence between different *A. astaci* strains and/or strains and crayfish species?

As you have mentioned, North American crayfish species obviously benefit from being infected with *A. astaci*. Do you expect that more resistant European species or populations that are known to be latently infected also benefit from infection? How would you test it? Would it be possible to stimulate (in advance) native crayfish species innate immune system?

In Croatia, we have discovered a latently infected population of the white-clawed crayfish in an isolated river within the karstic area. Human mediated introduction of the pathogen is



highly unlikely because it is situated in remote, barely reachable and uninhabited region. As this river is situated within the karst, there is a slight possibility that pathogen may have entered the river through the underground waters from the continental part of Croatia, or through infected freshwater shrimps that were flushed through underground waters from continental part. Another possibility is that the pathogen is naturally present within Europe. What do you think about the possibility that some of the *A. astaci* strains may have existed in Europe even before introduction of the North American crayfish?

Taking into consideration your knowledge and experience in dealing with crayfish plague, do you think native European crayfish species have a chance to survive?

### **Recommendation**

To conclude, I unequivocally declare that Jiří Svoboda's PhD thesis entitled Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci* is a valuable scientific contribution to the research of the crayfish plague pathogen, and that **Jiří Svoboda is suitable for award of PhD academic degree.**

Assoc. Prof. Ivana Maguire

In Zagreb, 26<sup>th</sup> of August 2015