

**Review of Dr. Antonín Kouba's habilitation thesis titled
"Marbled crayfish, an emerging invasive species"**

In his habilitation thesis with the title "Marbled crayfish, an emerging invasive species", Dr. Antonín Kouba presents (1) an introduction to the topic, (2) a summary of the six publications that form the core of the thesis (see point 4), (3) future perspectives and (4) six relevant publications of which he is either the first or last author. In the following, I will review the contents of each of these sections and will conclude with a summarizing evaluation of the thesis as well as specific comments and questions.

In the **Introduction** section, Dr. Kouba gives an excellent overview of the topic. He starts with the general issue of the freshwater biodiversity crisis and how it is related to biological invasions which are indeed one of the major global biodiversity threats. The section continues with the more specific threats posed by invasive crayfish and the crayfish plague, a disease caused by the pathogen *Aphanomyces astaci* which is carried and translocated by invasive North American crayfish. These are largely resistant against the disease and typically show no symptoms when being infected, whereas other crayfish, including those native in Europe, are frequently killed by the disease. The Introduction continues with an overview of 'old' non-native crayfish species in Europe, i.e. those introduced a longer time ago. A minor remark I'd like to make is that it would have been better to explicitly define what 'old' means here already in this part. This is clarified in the next part of the Introduction though, so it is really only a minor point. This next part focuses on 'new' non-native crayfish species which were introduced since 1980. One example is the marbled crayfish (*Procambarus virginalis*), the focal species of this thesis. It is a unique crayfish species, as it is the only one that reproduces parthenogenetically and also does not have a native population: it first appeared in the German pet trade in the 1990s and is a descendant of the North American slough crayfish (*Procambarus fallax*). The biology and ecology of the marbled crayfish is very well explained in the Introduction's next and final part. The Introduction is very well written throughout. It also includes numerous relevant references, indicating Dr. Kouba's thorough understanding and expertise in the topic.

A **summary of the six publications** forming the core of the thesis is provided in the following section. These publications focus on the marbled crayfish. They are very well summarized by Dr. Kouba.

Future perspectives on the topic are presented in the next section. This section is fine as well, but could have been a bit more detailed from my perspective.

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The **first** of the six **publications** has in addition to Dr. Kouba as first author two co-authors. This paper appeared in 2014 and has already been frequently cited (>230 citations in Google Scholar). It is indeed a paper that we also often refer to in our own working group, as it is an important and excellent contribution with a highly relevant overview of the spatial distribution of all (native and non-native) crayfish species in Europe, including of course the marbled crayfish.

The **second publication** of the thesis appeared 2017 in the well-known journal *Biological Invasions*. Dr. Kouba is this paper's last author. As outlined above, the pet trade played an important role for the emergence of the marbled crayfish as an invader, and this is also true for other non-native crayfish, particular those introduced since 1980 (i.e. the 'new' non-native crayfish species, see above). The second study of this thesis takes a close look at the role of the pet trade for crayfish invasions with a focus on the Caspian Sea and adjacent water bodies. This area has previously been largely overlooked, thus the study is a very welcome and solid contribution towards closing this knowledge gap.

Dr. Kouba is also the last author of the **third publication** which was led by Boris Lipták, a former PhD candidate under Dr. Kouba's supervision. This publication appeared in 2016 in the well respected *Journal of Limnology*. It provides important evidence of new occurrences and thus spatial spread of the marbled crayfish in Slovakia. Captured crayfish were also investigated for potential crayfish plague infection. This is another solid publication of the habilitation thesis.

The **fourth publication** appeared 2015 in the open-access journal *Scientific Reports*. Its first author is Lukáš Veselý, another former PhD candidate supervised by Dr. Kouba who is the last author of this very good paper which experimentally addresses the question under which winter conditions warm-water non-native crayfish species can survive in temperate Europe. This issue is of high practical relevance due to the European invasion of a number of warm-water crayfish species, e.g. the marbled crayfish.

The **fifth publication** also appeared in the journal *Scientific Reports*. It was published in 2016, with Dr. Kouba being the first author, and focuses on the ability of three native and five non-native crayfish species, one of them the marbled crayfish, to survive severe drought conditions and constructing vertical burrows. This experimental study thus nicely complements the above study number IV which focused on another type of adverse abiotic conditions (low winter temperatures). It is also of high practical relevance due to an expected increase in drought frequency in many European countries as a result of climate change.

Finally, the **sixth publication** was published in 2019 in the prestigious journal *Freshwater Biology*. Its first author is Wei Guo, a PhD candidate currently supervised by Dr. Kouba who himself is last author. As study number V, this experimental study looks at the effects of drought on crayfish, however in this case not on survival but on reproduction. The focal species is the marbled crayfish. Using a clever experimental setup with artificial burrows the study shows that marbled crayfish are able to go through several stages necessary for reproduction (egg incubation and early post-embryonic development) in the absence of free standing water (high air humidity is sufficient). Such an ability is

not known for native European crayfish species, suggesting another key advantage of marbled crayfish and possibly other invasive burrowing species like the red swamp crayfish (*Procambarus clarkii*, see study V) over native European crayfish.

Summarizing evaluation: For the reasons outlined above, I consider this to be a very good habilitation thesis with a focus on one of the most unique and interesting freshwater invaders, the marbled crayfish. Dr. Kouba has presented an array of important publications which have strongly contributed towards a better understanding of non-native crayfish species in Europe, particularly of the marbled crayfish, and are of high practical relevance. The six studies included in this thesis are of very high scientific quality, and I **strongly recommend to accept this habilitation thesis**.

Specific questions and comments:

- The marbled crayfish is still spreading in Europe. Do you think it will become as widespread in Europe as some other invasive crayfish, e.g. spiny-cheek crayfish (*Faxonius limosus*)?
- Which potential management actions do you consider to be most relevant and promising for the marbled crayfish?
- Is there a chance that native European crayfish species will at some point become immune against the crayfish plague, given that some individuals already can be chronically infected without lethal effect (as mentioned in the Introduction of the thesis)?
- With respect to study I of this thesis: Which crayfish species that is either not yet present in Europe or only in very low numbers do you consider to be particularly relevant as a potential future invader of European inland waters?
- In study IV of this thesis, the effects of low winter temperatures on the survival of crayfish species were experimentally investigated. Survival in the field could be affected by the burrowing ability of crayfish, which as shown in study V of this thesis, differs strongly among crayfish species. To which degree, do you think, would the results of an experiment differ from those of study IV if such differences in burrowing ability are considered in the experimental design?
- In the section “Future perspectives”, it says that “Assessment of trophic roles of co-occurring NICS is another important direction of future research” (p. 17). Could you please outline this a bit further?

With best wishes,

Prof. Dr. Jonathan Jeschke