

To  
Prof. RNDR. Jaroslav Vrba, CSc.  
Chairman of the Habilitation Committee  
Katedra biologie ekosystému PrF JU,  
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Innsbruck, 17. September, 2021

**Ref: Evaluation Report for the Habilitation Thesis of Ing. Martin Bláha, PhD**

Dear Prof. Vrba! Dear Members of the Habilitation Committee!

In response of your kind request from June 21, 2021, I have prepared this written review for the habilitation thesis submitted by Ing. Martin Bláha, Ph.D., entitled "The diversity of crayfish and major threats they face" for his habilitation in the field of Hydrobiology. Herein, I am providing my opinion on his habilitation thesis, which I was doing with pleasure.

***Formal presentation, contents, strengths/flaws of the submitted habilitation thesis***

The habilitation thesis is organized in several chapters, followed by an inclusion of 15 appendices (publications). In the "Introduction" a general review, background information and state-of-the-art knowledge for the herein presented scientific contributions, as well as the recent and actual status of native and introduced and/or invasive crayfish species around the world is presented. The following chapters, Martin Bláha reviewed in detail the "Phylogenetic relationships and diversity of freshwater crayfish" (Chapter I) and the "Diversity of crayfish under threat" (Chapter II). In these two chapters he gives a general summary of the existing knowledge in the field but also elaborates these facts along his own contributions (publications). This is followed by "Conclusions and future perspectives", "Acknowledgements", and a comprehensive list of the scientific publications ("References") relevant for his field. The 15 (!) publications are attached.

The scientific review is comprehensive, well presented and provides very accurate and important information on the existing knowledge about the topic. Martin Bláha provides a comprehensive picture of his broad scientific and methodological knowledge, brings this – as proven by the national and international scientific cooperations in the individual contributions as well as the world-wide considerations – to a global perspective. His habilitation thesis is also very strong, because it combines traditional and state-of-the-art methodologies with innovative approaches. In this respect, his studies on crayfish can serve as a keystone study in modern ecological theory and application. His and his colleagues' work contributes to several important themes, like taxonomy, phylogenetic relationships, genetic diversity, scientific description of species new to science, conservation issues, threats and invasion biology.

There are no flaws.

### ***General evaluation of the scientific publications***

The habilitation thesis includes 15 publications, which are in most cases co-authored by well-known scientists in the field. Martin Bláha is the first author on four articles. All articles demonstrate the importance of national and international cooperation when covering a wide spectrum of methods, scientific knowledge and expertise.

All contributions are published in peer-reviewed scientific journals, well-known and with high impact in the field. These publications span a wide picture from European crayfish biodiversity, phylogenetic relationships, genetic methodologies, conservation issues and the ever existing threats to native freshwater crayfish. Here the articles on invasion history and potentials with the potential spread of crayfish plague will be highly recognized in the field.

### ***Originality, significance of the results for the development of science***

All the presented results are highly significant. Many details were not known, and the herein presented methodology and approaches for the understanding of phylogenetic characteristics, the combination of morphological characteristics with molecular methods, the application of these techniques in remote and less known regions are of great importance for science. His collaborative research has yielded much valuable information, and it seems, that the number of unanswered questions increases with each investigation. This is stimulating further research, and Martin Bláha is already full of ideas, provided by his explanation of future research topics and collaborations.

### ***Signs of plagiarism***

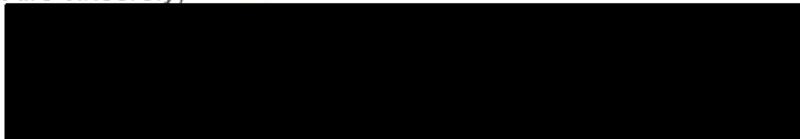
I am sure that the Habilitation Committee will test the thesis for plagiarism. My personal evaluation proofed all the presented material, methodologies and results being original.

### ***Conclusion and overall evaluation***

In general, a broad coverage of the relevant literature and existing knowledge is provided, but also in the individual publishes studies. All the specific chapters are without exception a comprehensive compilation of important results, moreover proof to cover traditional in combination with innovative methodologies. The results are on the one hand important for crustacean science but on the other hand will be very useful for management advice and implementations. Moreover, this presented work is a great piece of modern science.

Based on the herein described qualities, the habilitation thesis itself, but also the performance of the candidate at several scientific conferences and events, where I met him as a highly interacting and critical scientist, satisfy significantly the criteria for evaluating Martin Bláha's habilitation thesis as an "EXCELLENT" contribution to science!

Yours sincerely,

A large black rectangular redaction box covering the signature of the reviewer.

Prof. Mag. Dr. Leopold Füreder

**Reviewing of the Habilitation Thesis**

Submitted by **Ing. Martin Blaha, PhD**

Entitled

**The Diversity of crayfish and major threats they face**

For habilitation in the field of Hydrobiology

**Martin Blaha**

**University of South Bohemia in České Budějovice:**

**České Budějovice, CZ**

academic researcher (Faculty of fisheries and Protection of Waters)

<https://orcid.org/0000-0003-3597-6604> (54 papers referenced)

Researcher ID: G-5015-2015 (<http://www.researcherid.com/rid/G-5015-2015>)

Scopus Author ID: 26422755400

Martin Blaha is an academic researcher working on freshwater species. The number of freshwater species studied is substantial and concerned fishes and crustaceans (copepoda, shrimps and numerous crayfish species). Works are performed both in the field and in the laboratory. Topics concerned mainly diversity of crayfish species, protected environment, native and invasive species, pet trade. Outside the Czech Republic, collaborations were undertaken in Eastern countries and worldwide with a focus on southern hemisphere.

With 48 papers listed in the manuscript he has reported significant IF up to 5.589. He signed 10 papers as first author and 12 as second author. He was involved in three books (in Czech with two as first author). He was the last author concerning 4 papers but he did not specified if it was as a supervisor. Comparing to the number of papers written, a few oral communications were made (only 3 and two as a speaker).

For the Habilitation Thesis, Martin Blaha selected 15 papers (2 with IF under 1, 9 with IF more than 1 and 4 with an IF of very good level i.e more than 2.5, the highest being 3.13), in order to offer a coherent and easy to read manuscript concerning only crayfish.

The introduction is well conducted leading to the fact that freshwater biodiversity is extremely threatened, and probably more so than in other systems. This appears to be largely a result of the high degree of connectivity within freshwater systems, such that threats like

pollution and invasive alien species can and do spread more rapidly and easily than in terrestrial ecosystems. It could be useful to precise that any freshwater species remain 'Data Deficient' in IUCN terminology, in particular due to lack of taxonomic expertise and of information on species distributions. Results from the recent 2010 update to the IUCN Red List of Threatened Species (Richman et al., 2015), indicated for the first time by the worldwide crayfish researchers involved (see the complete reference listing these authors) that crayfish are one of the most threatened freshwater taxa yet to be assessed.

Overall Martin Blaha gives new insights about phylogenetic relationships and genetic diversity of European crayfish species particularly about Eastern European species up to date poorly investigated. Consequently it was a novelty to collaborate with colleagues from Eastern Europe particularly from Ukraine, Georgia, Hungary. Another panel of active collaborations was also initiated in New Guinea in order to identify the species collected with a phylogenetic investigation, a preliminary study absolutely necessary for protecting native species and identifying non native species. It was possible to describe for the first time the diversity of New Guinean crayfish.

If we consider more precisely the content of the manuscript, Martin Blaha demonstrated that, owing to the molecular tools -but without neglecting morphological characters- the state of knowledge of the phyleogeography is in constant progress. Despite the large number of publications available, it was possible to describe a new family (Cambaroididae) in Northern Hemisphere. It remains to state more accurately their historical biogeography with both molecular tools and fossil records. It will be interesting to know if Martin already has contacts with museums etc.....Collaborations with Eastern Europe allowed to study more precisely *Astacus pachypus* and *Astacus leptodactylus*, both species being related evolutionary lineages. The collaboration with Georgia gave innovative results concerning *Astacus colchicus*. The whole results effectively allowed to give new insights about European crayfish diversity and open the field of additional investigations.

I now propose to comment on certain points of the manuscript which were of particular interest to me:

The last part of the chapter 1 about northern hemisphere concerned management of native crayfish populations and it would be nice to highlight this important piece of work with a title to show that it has addressed the problem of conservation of populations of *Astacus astacus*. Particularly it is not common to have managed a pilot project during 10 years. In this part it would be essential to specify that the first concept of ark site was devoted to Peay

(2009): in Britain, in addition to designated 'No-Go' areas, smaller secure locations or 'Ark sites' are being developed into which threatened populations of Indigenous Crayfish Species are being moved. For example an Ark site for the white-clawed crayfish is an isolated, self-contained site with running water, still water, or both, which can support a healthy, self-sustaining population with little need for ongoing management. Suitable sanctuary sites are best looked for in isolated pristine headwaters, remote from human activities. The current UK focus on Ark Sites - new, isolated sites, such as abandoned quarries, where populations can be established in relative safety from the widespread signal crayfish - is now backed by a stage-by-stage methodology for ark site selection (Buglife, 2010).

The chapter 2 concerned Southern hemisphere crayfish and the impact of pet trade of these crayfish in Europe. Owing to the molecular tools two new species of the genus *Cherax* were discovered in pet trades: *Cherax gherardii* (leading to a great debate and showing how it is a hard task to distinguish species with morphological characters) and *Cherax subterigneus*. Once the panel of species found in pet trade was identified, the second step is to identify if released in the field in Europe. Five *Cherax* species were recorded in Hungary. Investigations in the field in New Guinea allowed to describe another species *Cherax acherontis*, the first cave-dwelling crayfish in Southern Hemisphere. It would be interesting to report in the manuscript that the invasive crayfish *Procambarus clarkii* was found for the first time in caves of Europe, as in Portugal and Italy. The presence of *P. clarkii* in caves is noteworthy, representing a new threat for the groundwater ecosystems due to the possible negative impacts on the native communities (Mazza et al. 2014).

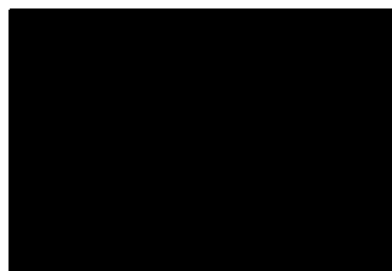
Concerning the crayfish plague - a pathogen considered as one of the 100 world's worst invasive alien species- it was very important to undertake its detection in *P. clarkii* from South-East-Asia. Its first detection is a high potential risk to native crayfish as we found in South America (in Brazil: see Peiró et al. 2016). Concerning the fact that other decapods could harbour and transmit the crayfish plague, recent papers could be cited about crabs (Svoboda et al., 2014 who found a native semiterrestrial species *Potamon potamios* in Turkey, and an invasive catadromous *E. sinensis* in Sweden; Shrimpf et al., 2014) and ornamental crayfish investigated by Keller et al., 2014 and Panteleit et al., 2017. Considering the problem of crayfish plague dissemination by non indigenous crayfish, the last part of the manuscript considers the situation in Europe. The most striking example is Ireland as one of the few locations in Europe where 'old' non-indigenous North American crayfish species have not been introduced, and considered as a refuge for the endangered white-clawed crayfish

*Austropotamobius pallipes* (Lereboullet, 1858). The parthenogenetic crayfish species Marmorcrebs, *Procambarus fallax* f. *virginialis* (Hagen, 1870), is sold in the pet trade in Ireland within the recorded range of *A. pallipes*. Marmorcrebs risk being introduced into Irish waters, where they could threaten *A. pallipes* populations, particularly as a vector for crayfish plague (Faulkes, 2015, 2017). In Czech Republic, Martin Blaha published that this parthenogenetic species was effectively present as well as in Slovakia and this is really a great threat. The new arrival of species such as *Cambarellus patzcuarensis* in Hungary is an alarming event because the species can be established because of no European regulation.

### **Conclusion**

Genovesi (2007) has pointed out that the main obstacles to fight against non indigenous crayfish are a lack of transboundary cooperation, limited ability to detect species early enough, ineffectual or delayed responses to the early stages of invasions, limited tools for eradicating or controlling invasive species in freshwater and inconsistency of legal provisions and the difficulty of trade regulation. With Martin Blaha, taking the opportunity of the advent of powerful molecular tools, we have the pleasure to see that all these mentioned obstacles are largely bypassed and that the manuscript reveals how his results are of first importance for managing native crayfish and fighting against non native crayfish with the control of pet trade, a hard task.

Of course results and particularly discoveries of new species open a panel of perspectives that Martin Blaha perfectly describes at the end of the habilitation thesis and it is without hesitation that I consider him worthy of being empowered for habilitation in the field of Hydrobiology.



**Catherine Souty-Grosset**

## Annex : references cited in the report

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## Referee's Report

### Habilitation thesis: The diversity of crayfish and major threats they face

Author: **Ing. Martin Bláha, Ph.D.** (Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice)

#### General comments

The submitted habilitation thesis is composed of 15 scientific papers and Introduction in which the applicant summarizes the results in the field of crayfish phylogeny, distribution, species diagnostics and identification, as well as the problem of non-indigenous and invasive species and the threat of ornamental crayfish trade on native species as well as local aquaculture. The focus of the thesis is impressive and highly variable covering the situation not only in the Czech Republic but also in other European countries like Ukraine, Hungary, Georgia, much of the work has been done in crayfish hotspot, in Indonesia (esp. PNG). The Introduction is nicely written and serves as an excellent proof that the applicant is well familiar with all the topics presented in the thesis as well as that he is both an enthusiastic scientist in the above mentioned fields of crayfish biology and also conservationist. The later role, however, looks little bit funny (cf. Supplement 10, e.g. the potential threat of *P. clarkii* which may pose to Indonesian freshwater diversity and appeal to local authorities what should they do) in case of a scientist from the country of widespread, abundant and even heavily stocked common carp (*Cyprinus carpio*), superabundant stone moroko (*Pseudorasbora prava*), spiny cheek crayfish (*Faxonius/Orconectes limosus*), Chinese pond mussel (*Sinanodonta woodiana*) or e.g. coypu/nutria (*Myocastor coypu*), one of the most invasive species on the planet Earth (water habitat examples only).

The applicant contributed to the problem of phylogenetic relationship among Northern Hemisphere freshwater crayfish (Astacoidea – Astacidae, Cambaridae, Cambaroididae) as well as to the phylogenetic position of thick-clawed crayfish (*Astacus pachypus*) and endemic *Astacus colchicus*. He also solved the problem of successfully translocated populations of native noble crayfish (*Astacus astacus*) which are, however, limited by low population size and high degree of inbreeding in the source populations. The applicant co-discovered three new Southern Hemisphere crayfish species – blue-legged crayfish (*Cherax/Astaconephrops gherardii*), *Cherax subterigneus* (*Ch. snowden* by Lukhaup et al. – the story of the first description of this species is amusing and sad simultaneously) and Yumugima crayfish (*Cherax acherontis*), the first cave crayfish species of the Southern Hemisphere. A number of contributions described the problem of ornamental crayfish trade and a problem of aquaculture as a potential threat for local/native crayfish fauna (non-indigenous crayfish species, frequently vectors of dangerous crayfish plague pathogen *Aphanomyces astaci*). As demonstrated, this problem is of high importance both in

Indonesia, the hotspot of crayfish diversity, ornamental trade and aquaculture, and also in e.g. Central Europe (CR, SK, H). First findings of parthenogenetically reproducing marbled crayfish (*P. f. virginialis*) in Bílina and Prague reveal the authentic danger of ornamental trade and hobbyist illegal activities even for the Czech Republic.

**The present habilitation thesis is well composed and extremely inspiring.** The only problem is the real contribution of the applicant, Martin Bláha, being a first (and to my feeling crucial) author of four out of 15 papers included. In one case, the applicant is the last, i.e. senior author. In the rest of selected papers (10), the applicant is a member of the research team with no other significant role (e.g. as a corresponding author, or having equal role in manuscript composition and writing as the first author). Moreover, the role of individual authors is not specified in any of selected papers and any attempt like this has been done by the applicant himself, e.g. in the Introduction part of the thesis (which would be nice and really helpful for correct evaluation). I understand that the applicant was responsible mainly for genetic/molecular part in all relevant papers and was definitely involved during the demanding and adventurous fieldwork. For example, the fact that six papers from this habilitation thesis were written by Jiří Patoka, who is all the time also the corresponding author, looks (at least) peculiar.

#### Special comments and questions

(Introduction, Figure 1). Why is the distribution of freshwater crayfish on the Earth so patchy with the group totally missing in continental Africa, most of Asia and South America? Even a very patchy distribution of the family of Parastacidae being present regionally on four continents is interesting and makes little sense (a historical result of Pangea?).

(Supplement 9) What is the reason for a number of cave crayfish species in the Northern Hemisphere and just one in the Southern Hemisphere? Just a substantial difference in a research effort?

(Supplement 11) What are the proofs that populations of *Cherax quadricarinatus* west of the Wallace line are out of their native range? I would expect that this is simply a function of low scientific effort in this region in the past (e.g. south-east Borneo).

(Supplement 12) So, what to stock to garden ponds when i) native species are mostly protected and ii) non-native species are dangerous to local biota? (a case of crayfish, amphibians, etc.)

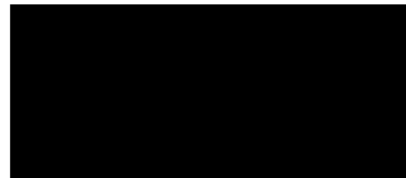
(Supplement 13, 14) I missed a suggestion (better solution) how to eradicate non-indigenous, invasive and dangerous, parthenogenetically reproducing marbled crayfish (*P. f. virginialis*) from affected, non-native localities.

(Supplement 13, 14) Any suggestions why newly established populations of marbled crayfish (*P. f. virginalis*) in both Czech Republic and Slovak section of Danube are free of crayfish plague pathogen *Aphanomyces astaci*?

(Supplement 14) What is the real threat of marbled crayfish (*P. f. virginalis*) for the Slovak section of Danube in the situation of presence of spiny cheek crayfish (*Faxonius/Orconectes limosus*) infected with the crayfish plague pathogen *Aphanomyces astaci*?

#### Final comment and recommendation

The applicant, Martin Bláha, is a well recognizable researcher in the field of hydrobiology (48 IF papers on WoS, 10 IF papers as a first author incl. Science of the Total Environment, H-index 14, over 440 citations excluding self-citations). He is an enthusiastic scientist and lecturer. From this reason but also the reason mentioned above, I recommend the submitted habilitation thesis as a base for the **associate professor** (doc.) degree with caution.



doc.RNDr. Martin Čech, Ph.D.

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Institute of Hydrobiology

&

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In Prague, 19 August 2021