



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Jan Kohout, Dipl.-Ing.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Petr Rab, DSc.
Title of PhD thesis: Population genetic structure of brown trout as groundwork for efficient management of fisheries in central European salmonid waters	
REVIEWER:	
Surname: Sušnik Bajec	Institution: University of Ljubljana Dept Anim Sci – Biotech faculty Domzale 1230 Ljubljana Slovenia
Name: Simona	E-mail: Simona.Susnik@bf.uni-lj.si
Titles: Assoc. Prof. Dr., Ph.D.	
Please describe your professional relationship to the PhD student: No professional relationship	Please describe your field of expertise: Population genetics, phylogeography, conservation genetics, genomics

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

PhD thesis of Jan Kohout (candidate) represents the first in-depth study of genetic structure of brown trout in central European and eastern Balkan region. In this context it could be considered as a very important contribution to knowledge about brown trout diversity in these regions and brown trout evolutionary history, especially in the framework of its conservation. Besides basic research the thesis also included one case where the candidate showed the usefulness of genetic analyses in applied projects, i.e. efficient management of fisheries.

The results and conclusions of the PhD thesis are on the level comparable to the current state of the art in the field of brown trout population genetics/phylogenetics/conservation genetics.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

PhD thesis is structured into five Chapters, including General introduction with literature review, three scientific papers and General discussion. General introduction is well structured covering all the issues important for the thesis and also reviewing the most important and relevant literature from these field of research. Three scientific papers included in the thesis well addressed the initial aims of the thesis. In General discussion candidate covered all his results in a comprehensive way and compared them to already known facts from the literature. This enabled him to sufficiently address all the aims of the thesis.

In order to answer his main aims, candidate used three different types of genetic markers that are used worldwide in similar studies: maternally inherited mtDNA, highly variable microsatellite loci and a nuclear coding locus. He applied different statistical approaches to analyze the results. All methods are contemporary and applied in numerous population genetic, phylogenetic and conservation genetic studied. Types of genetic markers and statistical methods used are adequately selected.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

PhD thesis of Jan Kohout deals with characterization of brown trout population structure and the influence of stocking in the Danube river basin. PhD thesis is composed of General introduction, three scientific papers, two of them already accepted/published in peer-reviewed journals, General discussion and Summary.

The aims of PhD thesis were.

- 1) To reveal the genetic structure of brown trout populations in the Czech Republic and Slovakia based on mitochondrial and nuclear DNA markers.
- 2) To compare the genetic diversity of Central European populations and populations from eastern Balkans, where stocking activities have probably been limited.
- 3) To answer the phylogeographic questions regarding the colonization of the Danube River basin.
- 4) To outline management strategies for brown trout in the Czech Republic on the basis of results of the genetic analyses.

As written bellow all the aims were sufficiently answered by the studies performed.

(1) To elucidate the impact of stocking on central European populations of brown trout and to outline further management strategies, PhD candidate analyzed using mitochondrial (control region) and nuclear DNA (microsatellites, *LDH-C1**) markers of 25 wild populations and five hatchery stocks from Czech Republic and Slovakia were. Candidate concluded that stocking



practices have caused massive hybridization between the Atlantic and Danube brown trout populations in the middle Danube basin and have led to a loss of among-population genetic variability in Slovakia and Moravia. However, the populations from the Elbe River basin seem to be much less affected by stocking compared to the Danube River basin populations.

(2) **To compare the genetic diversity of Central European populations and populations from eastern Balkans**, PhD candidate analyzed samples from the lower parts of the Danube River basin using the same mitochondrial and microsatellite markers. The samples from Aegean Sea basin were included in order to reveal genetic variability of eastern Balkan populations and estimate the impact of stocking in this area. Candidate pointed out to very low level of introgression from Atlantic and other non-indigenous trout in the eastern Balkan populations. Furthermore, he concluded that the genetic differentiation among the populations in this area is substantially higher compared to the central European populations and that these populations are much less affected by human-mediated transfers and stocking compared to most European brown trout populations.

(3) **To answer the phylogeographic questions regarding the colonization of the Danube River basin**. Based on his results the candidate supposed a late or post-Pleistocene penetration of the Atlantic lineage trout to the Danube River basin. Furthermore, his results indicate that the divergences among haplotypes within the Black Sea basin are even higher than the divergences among the three basins. The haplotypes from the upper and middle Danube River basin and the Caspian and Aral Sea basins created statistically well supported cluster. Assuming the results of demographic analyses provided by Bernatchez (2001) and new results, candidate proposed a scenario of the Pleistocene expansion of this subclade across the Danube River basin, Caspian Sea basin and Aral Sea basin. The expansion was not statistically supported for the whole sample set including also the newly found haplotypes from the lower Danube and non-Danube Black Sea basin locations. This result may indicate long-term isolation of populations of brown trout in the southern part of the Black Sea basin and supports the findings of sympatric, reproductively isolated groups of populations.

To my opinion PhD thesis is well presented, the scientific papers well addressed and answers initial aims of the thesis. I have one minor remark for the Chapter 3: it would be interesting to see phylogenetic relationship among mtDNA haplotypes on tree, especially position of two DA cluster. The only chapter that to my opinion is not fully prepared and therefore contains several weaknesses is Chapter 4. Here are some remarks that might improve the paper (that I guess is not submitted to scientific journal yet):

- How did the candidate select the number of clusters? With K or ΔK ?
- The candidate states: „Bayesian analysis in STRUCTURE did not reveal any population structuring using only the samples from headwaters of the Otava River ($K=1$).“ but on the other hand, these populations showed to be highly divergent based on significant F_{st} values? I would suppose that also structuring between these populations would be revealed.
- Were the samples from Borova Lada included in Structure analyses?
- There was a typo mistake in sentence: „The cluster **ONE** may represent native trout of the Otava headwaters, whereas the cluster two may reflect gene flow from other parts of the



Elbe River basin due to migration and/or stocking.”

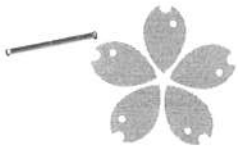
- Please, give significance values in Table 2. Pairwise *FST* values for the brown trout populations from the Elbe River basin.
- To my opinion GeneClass analyses and phylogenetic tree are not necessary for this data, as the candidate has already mentioned in discussion. So, I am wondering why you used this programs for this data set? I think it would be more appropriate to expand the Structure analyses and results.

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

.....31.5.2013, Domžale, Slovenia
Date and place

.....Simona Sušnik Bajec.....
Name and signature



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Jan Kohout, Ing.	First name(s), surname, titles of supervisor: prof. Ing. Petr Ráb, DrSc.
Title of PhD thesis: Population genetic structure of brown trout as groundwork for efficient management of fisheries in central European salmonid waters	
REVIEWER:	
Surname: Zima	Institution: ÚBO AV ČR
Name: Jan	
Titles: prof. RNDr., DrSc.	E-mail: jzima@brno.cas.cz
Please describe your professional relationship to the PhD student:	Please describe your field of expertise:

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

See attached general comments.

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See attached general comments.



OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

A review of the doctoral thesis by

Kohout J., 2013: Population genetic structure of brown trout as groundwork for efficient management of fisheries in central European salmonid waters.

Thesis submitted at the Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice.

The thesis is structured into five sections starting with introductory chapter and proposed aims of the study, followed by three scientific papers, and concluded in general discussion, summary and author's curriculum. Altogether the thesis covers 75 pages.

The introductory section reviews the problematic of diversity and taxonomy of the brown trout, approaches to stocking and farm-rearing of the fish, and their effects on genetic variation. This overview shows clearly that the topic of the thesis is topical, and the aims are proposed clearly. The results of such a research design are potentially valuable and promise to be implied in various practical issues of fisheries.

The first paper was published in Fisheries Management and Ecology in 2012. This study revealed that the genetic structure of brown trout is strongly affected by stocking in Central Europe and a loss of variation between populations is demonstrated. It is remarkable that this effect is apparently more distinct in the Danube basin than in the Elbe basin.

The second paper has been accepted for publication in Biológia and the study reports genetic diversity and phylogenetic origin of brown trout in eastern Balkans. The results are employed in discussion of colonization of the Danube basin by the species and relationships of the Danubian populations with those occurring in other major river systems.

The last paper is an unpublished manuscript reporting genetic data of brown trout in headwaters of the Otava River. The origin of brown trout populations in this river system is examined using a set of microsatellites and the impact of migration barriers and geographic distance is evaluated in respect of the relationships of wild and hatchery populations.

All the papers included are multi-authored but the contribution of the Candidate is apparent from the fact the he is the first author in all the studies.

The moderate incidence of misprints and grammatical errors can be found in the text, and I feel that a thorough revision of English style would improve the level of the thesis. It is rather surprising that,



except of the enclosed manuscripts, the text does not include almost any figures or tables which could be useful for readers' understanding. General conclusion comparing the proposed aims and the achieved results would be useful.

General discussion is rather confusing in some parts and it is often difficult to derive clear and unequivocal conclusions. Some of the findings merit more imaginative discussion. Considerations based on single loci (LDH-C1*) or single haplotypes (A3) may be misleading or results in oversimplification with respect of the complicated history of domestication, transfers and introductions. Different time scales are discussed in considerations of the historical evolutionary changes (e.g. postglacial expansion in the Danube basin vs. assumed interconnection of the Black, Caspian, and Aral Seas 270-290 thousand years BP). What is the evidence for the species distinction between *Salmo trutta* and *S. labrax*? Has the reproductive isolation between these presumptive species been really proved? Extensive intermixing between the Atlantic and Black Sea trouts in the upper Danube river basin (p. 61, l. 5-6) does not seem to support this assumption.

In the view of the proposed aims of this theses I suppose that the first two dealing with the genetic structure of brown trout in Central Europe) were achieved satisfactorily. The third goal (phylogeography in relation to colonization of the Danube basin) was elaborated but clear conclusions have not been apparently achieved. The last goal seems a little bit questionable. In fact, I miss in the thesis, particularly in general discussion, an outline of management strategies for brown trout in the Czech Republic proposed on the basis of genetic research. I understood from the discussion that there is no reliable evidence about the origin of fish kept in the Czech hatcheries. It is obvious that strict regulation of the management of hatcheries, transfers and introductions is urgent and necessary (if it is not too late for such measures). In my view it is a pity that the author has not made more efforts to imply the data achieved in such practical questions.

In spite of some critical comments, I am convinced that the submitted PhD thesis constitutes an original contribution to learning and gives evidence of systematic study and of ability to relate the results of such study to the general body of knowledge in the subject. The practical work is commendably thorough and appropriate; the analysis seems intelligent and sound.

I consider the work to be appropriate for the award of a PhD degree, both in terms of its scientific quality and in the breadth of the investigations carried out. Therefore, I can recommend the thesis for successful defence.



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a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

FINAL RECOMMENDATION

- PhD Thesis can be recommended for defence
- PhD Thesis can be recommended with reservations for defence
- PhD Thesis can not be recommended for defence

Brno, June 15, 2013

.....

Date and place

prof. RNDr. Jan Zima, DrSc.

.....

Name and signature