



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Tomáš Tichopád, Mgr.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: The effect of polyploidization and hybridization on reproductive physiology in fish	

REVIEWER:

Surname: Mikulíček	Institution: Univerzita Komenského v Bratislavě, Slovensko
Name: Peter	
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Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: phylogeography, population genetics, asexual vertebrates, hybridization, hybrid zones

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):

Tomáš Tichopád's dissertation deals with issues of hybridization, polyploidization, gametogenesis and reproductive physiology of model fish groups. The relationship between hybridization, polyploidization and different modes of asexuality in vertebrates has been known since the discovery of the first asexually reproducing vertebrate, the fish *Poecilia formosa*, in the 1930s. Since then, approximately one hundred species (or forms) of fish, amphibians, and squamates have been described that reproduce by parthenogenesis or similar modes such as gynogenesis and hybridogenesis. With the exception of one species, they are of hybrid origin, show aberrant modes of gametogenesis and increased ploidy levels. Asexual vertebrates began to be studied more significantly in the second half of the last century, while the studies at that time were mainly based on the use of classical methods of allozyme electrophoresis and karyology. In the 1980s, the first studies based on molecular markers (e.g., mtDNA) were published, which are now being extended by genomic approaches and advanced molecular-cytogenetic analyses. I appreciate very much that Tomáš Tichopád's dissertation is based on advanced and new techniques, the application of which makes it possible to give answers to classical (e.g., the course of clonal gametogenesis) and new scientific questions (e.g., gene expression in asexual forms). An unexplored area of asexuality



research that has only recently begun to receive attention is epigenetics and development. The first paper included in the dissertation (published in *Biology of Reproduction*) is unique in this regard because it asks how somatic tissues near the developing germ cells influence clonal gametogenesis. We know from developmental biology that in early ontogeny the development of a group of cells is influenced by other tissues (often genealogically unrelated). The question of whether clonal gametogenesis is affected by similar developmental processes is thus original and of great importance not only for asexual forms. The third manuscript included in the dissertation confirms apomictic egg formation in gynogenetic *Poecilia formosa* and compares gene expression between this form and its sexual parental species *Poecilia mexicana*. The authors analyze specific meiosis genes that are differentially expressed between the two forms and hypothesize which genes might be key in the evolution of ameiosis. I appreciate that the authors also discuss how allelic bias in expression and allele specific expression may relate to the Bateson-Dobzhansky-Muller (BDM) model of hybrid incompatibility. Thus, the hypotheses presented in this manuscript could explain the more general topics of genome evolution and speciation. From the methodological point of view, originality, scientific importance, and research perspectives, I consider the dissertation to be excellent.

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):

The dissertation consists of an Introduction, which on about 15 pages introduces the reader to the issue of asexual reproduction of vertebrates, the link between hybridization, asexuality and polyploidy and the genetic consequences of these processes, including the impact on gene expression. The Introduction is relatively brief, but given that additional information is provided in the introduction of individual papers, I consider it sufficient. The Introduction is followed by three already published works and one manuscript, in two of which Tomáš Tichopád is the first author. The publications are relatively heterogeneous, but they are united by the central topic of hybridization and polyploidization. The final chapter contains a general discussion and summary. However, the general discussion represents only a brief version of the discussions in individual studies and, in my opinion, is not that important.

As a whole, the dissertation seems compact and consistent, the chapters follow each other logically. A minor critical comment can be directed towards the absence of a section focused on the use of polyploid forms in aquaculture, since the 5th chapter of the dissertation is devoted to this issue.

The objectives of the dissertation are clearly formulated and have been met. The results were published in high quality impact journals.



OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

In general, I consider the dissertation of Tomáš Tichopád to be of high quality and based on publications in high-impact journals. One manuscript that has not yet been published is carefully prepared and I believe it will be submitted soon. The dissertation brings original results, is consistent and logically ordered. It fulfills the criteria set for PhD thesis and can be recommended for the defense.

Since Tomáš Tichopád's dissertation mainly consists of published papers that have passed the regular review process, I have a few comments only on the general introduction and two questions to discussion.

1. In the General Introduction, including Figures 1 and 2, the author summarizes hypotheses on the origin of auto- and allopolyploid forms. It would be useful to give some examples from species living in nature.
2. Parthenogenetic lizards formerly assigned to the genera *Lacerta* and *Cnemidophorus* are now assigned to the genera *Darevskia* and *Aspidoscelis* (page 10).
3. The author gives examples of asexual animals with parthenogenetic, gynogenetic, androgenetic and hybridogenetic reproduction on page 10. The list is not exhaustive, and the author could have given other well-documented examples (e.g., *Phoxinus*, *Menidia*, *Squalius*, *Ambystoma*). However, I assume that the purpose of the general introduction was not to make a comprehensive list of asexual animals.
4. Fish of the genus *Poeciliopsis* (page 10) can reproduce by both gynogenesis and hybridogenesis. The first method of reproduction is known for triploids, the second for diploid hybrids.
5. Males of some asexual forms (e.g., *Corbiculla*, *Pelophylax*) can produce diploid sperm. In the paper of Tichopád et al. (2020), published in *Aquaculture*, the authors found higher sperm velocity and motility in hybrids between common carp and gibel carp, which can be attributed to the presence of diploid sperm. Can the production of diploid sperm be advantageous for asexual forms? For example, might the higher speed and motility of diploid sperm be advantageous to hybridogenetic males in egg fertilization compared to males producing haploid and potentially less fast and motile sperm?
6. In a study by Dedukh et al. the authors found differences in the expression of meiotic genes between sexual *P. mexicana* and asexual *P. formosa* and identified specific genes whose downregulation is associated with the ameiotic course of gametogenesis. In this context, a hypothetical question arises whether we would be able to detect the downregulation of the same genes in other gynogenetic forms of fish and amphibians.



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FINAL RECOMMENDATION

- ☒ PhD thesis can be recommended for defence
- ☐ PhD thesis can be recommended for defence with reservations
- ☐ PhD thesis cannot be recommended for defence

July 07, 2022, Bratislava

doc. Mgr. Peter Mikulíček, PhD.