

## Opponent Review of Doctoral Thesis

Thesis author: Anatólie Marta, M.Sc.

Title: Triggers of asexual reproduction: on the crosslink between hybridization, asexuality, polyploidy, and speciation on the example of Cobitidae loaches

Supervisor: Mgr. Karel Janko, Ph.D.

This doctoral thesis addresses the ways of reproduction in *Cobitis* fish species and their hybrids to learn how the hybrids avoid sterility using asexual reproduction. The main task of the author were cytogenetic analyses of gametogenesis, particularly of meiotic prophase I.

A. Marta has published on the subject of his doctoral studies at least four articles (one as the first author) in renowned, peer-reviewed journals, thereby fulfilling the requirements of the study program to submit doctoral work.

First, the author took part in cytogenetic and flow cytometry experiments (Dedukh et al., 2020) to uncover meiotic processes occurring in fertile and sterile *Cobitis* fish hybrids of both sexes. The publication revealed that while hybrid males were sterile due to failed meiotic synapsis, endoreplication in oogonia rescued female hybrid fertility but not gene flow between species, as it lead to asexual reproduction.

Second, A. Marta participated in developing centromeric satellite markers discerning *Cobitis* species and hybrids (Marta et al., 2020), an important prerequisite of speciation studies in *Cobitis*.

Third, these cytogenetic markers were used by the author to dissect the relationship between endoreplication and fertility of clonal *Cobitis* hybrids (Dedukh et al., 2021).

Fourth, the author analyzed the cytogenetic markers in the gonads of fish with implanted manipulated and control stem cells to aid in demonstration of the importance of sex and somatic gonadal cells for the fertility and endoreplication of *Cobitis* hybrids (Tichopad et al., 2022).

Moreover, the author included a manuscript (Marta, under preparation), dealing with the analysis of clonality and sterility in Cobitidae hybrids with a wide range of chromosomal and nucleotide divergence. Hybrid female fertility due to clonal reproduction via endoreplication has been detected in most of the crosses, which is unexpected given the limited occurrence of clonal reproduction in natural species.

In addition, A. Marta participated in a study characterizing the gametogenesis of Amazon molly (*Poecilia formosa*), which uses asexual reproduction but is of different order than the *Cobitis* fish (Dedukh et al., 2022).

I found an acceptable number of minor errors (misspelled headline REFEREnCES, missing spaces etc.) in this work, suggesting that its proofreading was not neglected. However, word order and word selection could be improved, which would lead to higher clarity, e.g., “While in China diploids and tetraploids reproduce sexually...” should read “While diploids and tetraploids from China reproduce sexually...”. Moreover, many formulations are not clear, because they do not reveal, to what to compare the given facts - e.g., “Spined loaches are distinguishable by the elongated body shape and movable suborbital spine.”: distinguishable from what? I found one unexplained abbreviations (“BDM” on p.130) and some expressions, which do not sound right: “gametogenetic abortion” (p.131), “incompatibilities between

autosomal and cyto-nuclear incompatibilities" (p.137). The ending sentence of Discussion of Chapter 6 ("should be based targeted experimental crosses.", p.139) is missing something.

The author and/or supervisor should explain why the *Poecilia* fish article is included in thesis, although it deals with fish from another order than Cobitidae.

My evaluation conclusion is that this work presents sound results and can be accepted as a doctoral thesis. In addition, I suggest - after a successful thesis defense - to confer the degree of Ph.D. on Anatólie Marta, M.Sc..

#### Questions for discussion:

1. The author discusses on page 138 Chapter 6 result, which suggests that hybrid spermatocytes displayed decreased numbers of synapsed homologs compared to hybrid oocytes. This result is attributed to "pairing mechanisms and affinity among orthologous chromosomes fundamentally differ between males and females, as previously reported in the zebrafish model (77)." Could a sex-specific repair of meiotic breaks, meiotic synapsis, or meiotic checkpoint lead to similar result? Could this be related to heterogametic sex?
2. Have you detected any intraspecific polymorphism regarding hybrid sterility in Cobitidae (some individuals from the same species producing fertile hybrids and some sterile hybrids)?
3. Does asexual reproduction already have some practical application - in fish or in another animal species?
4. What was the first vertebrate species shown to reproduce asexually? When? First animal species?
5. Who and when first described a vertebrate species using endoreplication to avoid hybrid sterility? First animal species?



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