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Review of the master thesis:

## ***Daphnia* hybridization in canyon-shaped reservoirs**

**by Štěpánka Ruthová**

The MSc thesis consists of two manuscripts, which are attempted to be later submitted to the peer-reviewed scientific journals. The main focus of this thesis is a molecular description of the hybridizing *Daphnia longispina* complex.

The first chapter consist of a detailed genetic study of the *Daphnia* hybridizing complex, in three reservoirs in Czech Republic. Using 12 microsatellite loci, the species composition was studied along the horizontal gradient of each canyon-shaped reservoir. It was concluded, that the population structure is caused by local adaptation and clonal selection facilitated by presence of ecological gradients.

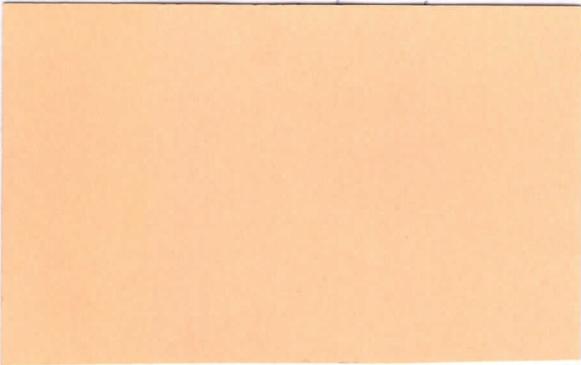
The second chapter focuses on the taxa identification issues. In order to conclude, which identification method is reliable in this *Daphnia* species complex, the 'species-specificity' was compared across a wide range of molecular markers. The results obtained by the allozymes and microsatellite markers correspond well with each other. There are, however, consistent discrepancies between the ITS-RFLP and other markers. Finally, the morphological identification seems to be the least reliable method, as an extreme phenotypic similarity was observed across individuals belonging to different taxa.

In summary, this is one of the best theses I ever read. The performed analyses were not trivial, as various computation software were used. Also, I was very impressed with the writing style. In both chapters, an introduction was extensive and clear, and the review of literature was on a

very high level. About 50 articles, I could see it from the text, were truly read and comprehended.

The work presented in this thesis has clear international significance and I regard it as fully meeting any requirements for the MSc degree. In my opinion, the two of the manuscript have a big chance at getting published in the high impact factor journals (I have few minor comments and suggested changes, which I present below).

Dr. Justyna Wolinska



Some comments, questions and suggested changes:

Page 14: “All samples used for the purpose of this study were sampled”, better “were collected”.

Page 14: “(...) by two allozyme markers” - which ones? Provide details.

Page 14: change “Zooplankton was colleted” to “zooplankton was colleCted” (it was misspelled).

Page 16: “and homogenized”, sounds like they pooled all together, clarify.

Page 17: “As it has been shown that genotypic richness of natural *Daphnia* assemblages is high (Thielsch et al., in review), we disregarded for the purpose of this study factors which may occasionally slightly overestimate the real clonal diversity, such PCR artefacts, somatic mutations and scoring errors.” – can one correct for these artifacts otherwise? How?

Page 17, while explaining the term “genotypic richness” write what is the minimum, maximum possible value of R.

Page 17: “In order to estimate differences in genotypic richness on the horizontal gradient of the Stanovice Reservoir, we computed both characteristic also for pooled dataset including all three sampling sites of the dam region”, so how was it done for two other reservoirs? The data set were not pooled?

Page 19: Change “at two localities, the Stanovice Reservoir (...), and the Vír Reservoir (...)” to “in both the Stanovice Reservoir (...), and the Vir Reservoir”, in order to avoid the confusion caused by usage of the word “localities” (which otherwise refers to the sampling stations within the same reservoir).

Page 19: “In general, nutrient concentrations in studied reservoirs decreased in the downstream direction from the inflow region towards the dam (Figure 2a, 2b; Table 2), which is in concordance with general characteristics of canyon-shaped reservoirs” – I do not really see that the N concentration “decreased in the downstream direction” (Fig. 2b).

Page 19: “the changes of phosphorus concentration (Figure 2c)”, should be Figure 2d, I think.

Page 20, Figure 2: “Increasing concentration of the total nitrogen (b) from upstream to downstream in the Vranov Reservoir suggests that nitrogen is not limiting element” – shouldn't it go to the Methods rather?

Page 19: “651 individuals (...) were screened using 12 microsatellite markers”. Do you mean here “successfully screened”? You mentioned in the M&M section that there were more animals initially screened.

Page 22, fix some inconsistencies on a Figure 4 legend. For example, what kind of hybrid does “D.gal x cuc” refer to: F1 or F2? (compare with the “F1 D. gal. x long.” provided).

Page 24: “>97% if all hybrid” – “if” should be replaced with “of”

Page 24: “>97% if all hybrid classes were pooled together as ‘hybrids’ ” – which hybrid classes are meant in the first part of this sentence? Does it refer to hybrid class defined by allozymes?

Page 25, first sentence: what does a “G” refer to?

Page 29: change “reported by Schwenk” to “performed by Schwenk”.

Chapter 2, general: why all the analyses were performed on 10, and not 11 lakes? In the two previous publications (Seda et al. 2007, Petrussek et al. 2008), 11 lakes were screened. Which lake (and why) was excluded from your study?

Page 39: there is an inconsistency between the two sentences. It is written that samples were first used “(...) for DNA based identification”. In the following up sentence, it is written that “additional samples were used for DNA methods”.

Page 40: what does the ‘s’ refer to in ‘sAAT’ allozyme locus? Shouldn’t it be just ‘AAT’?

Page 40: “Each individual was determined as one of the parental species (*D. galeata*, *D. longispina*, *D. cucullata*) or as a hybrid genotype” – it is not clear here, based on which marker set.

Page 41, Table 1: the polish lake is “Mikolajskie” not “Mikolajkie” ☺

Page 44: Something wrong in the first sentence: how many animals were identified with the microsatellites and ITS-RFLP markers? I guess the “444” refers to “all three markers” and not to “microsatellites and ITS-RFLP”, or?

Page 46, Table 2: I do not think you need to provide two sets of information of this table, i.e. the percentage and total numbers. It is a bit confusing. I would keep the “%” only. Also in Tables 3, 4 and 5, consider providing the “%” only. Another option would be “xx%, n”, where “n” is a total number of animals screened.

Page 47, Figure 3: I would test, for which lakes the two sets of classifications are statistically different. For example, with the Fisher Exact test.

Page 48: delete “and no other taxa were found“. This information is redundant with a previous sentence.

Page 51, Figure 5: this figure shows that different species form one cluster in the PCA analyses, based on the morphological measurements. I wonder if the pattern would not become clearer, if such an analysis was done for daphnids from a single population only. Have you tested that?

Page 56: “We expected that for pure species and their hybrids” – shouldn’t that be “We expected that, but only for hybrids”?

Chapter 1 and 2, general: Why do you think there are no *Daphnia longispina* x *cucullata* hybrids present in the Czech reservoirs?