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Review of MSc thesis by Zuzana Faktorova: "Historical changes in species composition and interspecific hybridization of the *Daphnia longispina* species complex (Crustacea: Cladocera) in Lago Maggiore"

The Master thesis of Ms. Zuzana Faktorova focuses on a characterization of changes in the *Daphnia* species composition in Lago Maggiore, over a period of 60 years. Specifically, 2150 *Daphnia* individuals were picked from a long-term collection of preserved zooplankton samples and body shape and body size of these specimens were recorded. Body shape was used to classify these specimens to different parental species and hybrids from the *Daphnia longispina* complex. Although Lago Maggiore is considered a model lake, there was no study thus far which would systematically look at a variation in relative abundances of *Daphnia* taxa. The analysed samples were mostly preserved in formaldehyde, so it was not possible to use classical genetic methods for *Daphnia* species assignment. Morphological assignment was thus the only applicable solution.

The goal of this thesis was to evaluate changes in *Daphnia* community composition of Lago Maggiore, and to relate these changes to environmental challenges that this lake has experienced. Ms. Faktorova reports the prevailing presence of *D. longispina* in the 1940s, a dominance of *D. galeata* after 1980s, only occasional presence of *D. cucullata* in 1986 and 1992, as well as common intermediate phenotypes that likely represent interspecific hybrids since the 1940s. This species succession was likely driven by a combination of different environmental factors.

I find this project very interesting and innovative (it is hard to believe, that nobody has explored these valuable historical samples yet!). Applied methods were well chosen. Ms. Faktorova processed an impressive number of samples which all needed to be photographed. She then conducted morphometric analyses and applied scores of these analyses to multivariate statistics. Ms. Faktorova proved a good knowledge of literature; she was able to present her expectations and results in a general context. After an extensive
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review of the existing studies, she finished Introduction part with a presentation of clear research hypotheses. In the Discussion, Ms. Faktorova provided a careful interpretation of the results. Importantly, she avoided any over-statements. In some places, however, the Discussion part was rather long and repetitive.

The results of this project were nicely illustrated, in a form of numerous figures and photographs. Figure 9, for example, nicely summarizes important results; I especially appreciate seeing there the proxies of different shapes of *Daphnia*. Figure 10 wisely visualizes rather complex results. On Fig. 16, I find marking of significant events on the time axis a very good idea.

Unfortunately, figure legends and table captions are not always fully complete. For example: Figure 8 - there is no information why no month is provided for 2012. Figure 12 – what do error bars and "su." vs "sp." stand for? Figure 14 – what does darker or lighter blue colour in the boxes mean? Table 1 – what do "s" and "q" stand for? Additionally, some parts of the thesis seem to be written in a rush, leading to repetitions, spelling and grammar errors. Some sentences appear awkward and incomplete. On the other hand, however, it is highly appreciated that Ms. Faktorova decided to write her thesis in English, which is a very challenging task!

I have three sets of questions to Ms. Faktorova:

Question set no. 1) One ANOVA test was conducted. Were assumptions for a parametric test met? Please list these assumptions and describe how you tested them.

Question set no. 2) The ANOVA test is described in a following way: "The differences in PC1 scores between taxon distribution and between years were tested by analysis of variance (ANOVA)" and then: "PC1 score values are significantly different between the taxon distribution and even between years". It is unclear to me, what kinds of treatments were included in this analysis. As the Results sentence is written, I would assume "taxon" and "year"? However, there is only a single p-value provided in the Results. Please explain how exactly this ANOVA test was conducted, listing the dependent and independent variables. How many degrees of freedom does this test have?

Question set no 3) Could different fixation methods contributed to the obtained results (i.e. older samples were preserved in formaldehyde whereas newer samples were preserved in ethanol)? Maybe *Daphnia* size and/or shape are differently affected by these two fixatives?

Overall, I recommend that this thesis should be accepted by the examination board. I grade this thesis as good (2.0).

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