

## ***Interactive comment on “Impact of climate and hydrochemistry on shape variation – a case study on Neotropical cytheroidean Ostracoda” by Claudia Wrozyzna et al.***

**Claudia Wrozyzna et al.**

claudia.wrozyzna@uni-graz.at

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We thank the referee for his/her constructive comments on the open discussion paper. Although he/she thinks it is an interesting study, criticism is formulated on the methodology of the statistical analyses and the discussion of the relationship between climate and shape changes.

In particular, reviewer's major concern is that our analyses focused on PLS analyses and not (as usual) MANOVA/MANCOVA or Procrustes ANOVA.

- The suggested types of analyses (MANOVA/MANCOVA as well as the inclusion of

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phylogenetical data) are used to discriminate groups since they maximize the variance between groups. We know already from previous studies (which are cited in the manuscript) that the groups are different. The present study aims to investigate potential relationships between environmental factors and phenotypes on a regional scale. This requires different kind of analyses. Maybe this was overlooked by the referee. Nonetheless, we will carefully check our introduction in order to emphasize this important difference to conventional studies on morphological variability.

Referee 1 also criticizes that we performed regression analyses on each relative warp.

- This approach enables direct evaluation which environmental factors are associated with which kind of shape variation. We will add this information into the manuscript in order to highlight the strength of this approach.

“In addition, further inspection on the result of PLS analysis is recommended. The authors emphasize the similarity between Florida and Brazil based on the plot of RW1 vs RW2, but Fig. 5 indicated the similarity between Florida and Mexico. Graphical presentation of shape deformation indicated by PLS singular axis of shape variables should be added for further discussion. Loadings of environmental variables in PLS singular axis will be helpful for understanding the effects of climatic and hydrochemical factors. The authors reported first PLS singular axes, but second PLS singular axes might also indicate relationship between shape and environmental factors.”

- We will include a sentence about the results of the second PLS axis in the manuscript. However, we suggest providing the detailed results and graphics in the supplements because of the minor relationship between morphology and environment. - We will add the loading values into the supplementary material. We concede that the selection criteria for the warps were not clearly formulated in the manuscript. Therefore, we decided to consider relative warps with PLS loading values higher than the mean loading value (based on absolute values). Accordingly, we added the regression analysis for RW3 of females LV. The values will be changed in the table. These changes do not

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affect the interpretation in any way.

“Discussion in the paper descriptively indicates that there are morphological differences that possibly relates to environmental variables, but there are little discussions on why shape of ostracod valves differ depending on environments (e.g. are there any functional meanings? or merely due to physicochemical consequences?) and how ostracods respond to environmental changes. It is impossible to achieve definitive conclusions in the paper, but at least proposing some hypotheses is required.”

- Showing that the shape of ostracod valves can vary with respect to differences in environmental conditions is a key finding to understand how these organisms react to external influences. However, the knowledge about physiological processes, including molting and calcification of ostracod valves in relation to environmental parameters, is quite scarce. A further limitation that hinders a straightforward interpretation is the inconsistent results of different approaches of mesocosm experiments. Nonetheless, we will extend our discussion according to the referee's comments, including the consideration if the shape changes might be the result of functional advantages or if they are the result of physiological processes. Since we took our samples from many different habitats, a functional adaptation of carapace shape changes would cause a pattern of similar habitats associated with similar morphotypes independently of the region. Yet, each region is characterized by its own morphotype. This discrepancy rather contradicts the idea of functional morphology. It is therefore more likely that the observed shape changes are caused by physiological processes. However, we would like to avoid discussing hypotheses that cannot be tested in the frame of this paper and prefer a rather tentative way of discussion.

Specific comments Page 3 Line 1: The authors focus on “the causes for the regional differences”, so how the factors cause the morphological differences should be more discussed in Discussion. - We carefully revise our discussion and, as mentioned above, try to deepen discussion how the factors cause morphological differences.

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Line 8: For identifying “the morphological characteristics and environmental variables that contribute most to the relationship”, my recommendation is to inspect further the result of PLS analysis and to apply MANOVA/MANCOVA or Procrustes ANOVA, rather than regression analyses conducted in the study.

- As we discussed already above, MANOVA/MANCOVA or Procrustes ANOVA would be suitable analyses if we were interested in group differences. However, the present study focuses on individual relationships between morphological and environmental parameters, and regression analysis are a convenient tool to investigate those.

Line 20: Add explanation more about “water sampling”.

- We will add more detailed information.

Page 5 Lines 9–10: Even if selecting variables that contain high loading values in the PLS analysis, separately applying regression analyses might diffuse the environmental effects on shape.

- The PLS analysis is an overall indication that there is a relationship but the regression analyses allow a more detailed perspective. High PLS loading values indicate a strong relationship between shape and environment. Therefore, we consider it indeed necessary to go into more detail about the relationships with specific warps (and thus morphological traits).

Lines 11–12: The authors concern multicollinearity. One idea is to conduct principle component analysis of environmental variables, and use PC scores as explanatory variables.

- Multicollinearity is a natural phenomenon in datasets with many variables, particularly environmental parameters use to be correlated to some extent. As stated clearly in the respective paragraph, we apply stepwise selection of variables based on the variance inflation factor. This procedure prevents misinterpretation from correlated variables.

Page 6 Line 18: Show the result of statistical testing of the “clear relationships” in the

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PLS analyses. Statistical testing is possible by using resampling technique, such as permutation test.

- We will apply permutation tests on the PLS results.

Lines 27–28: Table 1 does not show that higher warps (the authors mean RW4, RW5, etc.?) have minor influence on shape variation. In this paragraph, do the authors show the results of relative warp analysis?

- This paragraph is about the PLS analysis. We will reformulate it to avoid further confusion.

Page 8 Lines 5 & 23: The authors only noted that the reasons why shape differences relate to environmental factors are not or poorly understood. However, at least proposing some hypotheses is required.

- See above.

Page 9 Lines 22–29: Incorporating phylogenetic information in the analysis may be helpful.

- This is an interesting point but this was not the intention of the present study.

Page 10 Lines 6 & 19: The authors emphasize the similarity between Florida and Brazil, but the results of PLS analyses seem to indicate the similarity between Florida and Mexico.

- This contradiction is caused by mixing two different aspects. The emphasis of the similarity between Florida and Brazil refers to the morphologies (given in the Relative Warps Analyses), both regions provide rather shortened carapace outlines compared to Mexican carapaces which are strikingly different. The PLS indeed reflects the relationship between environmental gradients and shape changes. The higher 'similarity' of Florida and Mexico in the PLS is caused by similar environmental factors. For instance, Florida and Mexico cover wider conductivity ranges (205 to 2360  $\mu\text{S}/\text{cm}$ )

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whereas Brazil is represented by low conductivities ( $\leq 279 \mu\text{S}/\text{cm}$ ). We will rephrase this to avoid misinterpretation.

Technical corrections will be done according to the referee's comments.

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