MS #2016:01:8642:0:1:REVIEW

Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed in the coastal subtropical waters around Kuchierabu-jima Island, southern Japan

PeerJ

Dear Academic Editor

Dr. James Reimer

We received the comments on our revised manuscript on May the 16th, with the major revisions as kindly concerned by both referee #1 and yours, as the academic editor in charge of our work.

Please, find below our replies to each comment, in *italic*. Also, other important concerns were addressed in the revised version of the manuscript, highlighted in red, especially regarding the material and methods and the discussion sections.

Also, we had incorporated other minor issues, not addressed before, as we realized these only after resubmission.

We hope that the present version fits all requirements for publishing with PeerJ.

On behalf of the authors,

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Editor Decision

I have received comments back from one reviewer who had reviewed the previous version of this manuscript. The reviewer has noted you have made much progress addressing the concern on the number of specimens, but there are still some outstanding issues remaining.

In particular, the reviewer makes an excellent comment on the preservation issues surrounding the use of specimens from different collection events, and possibly under different protocols or fixatives. At a bare minimum, you must address how such issues could influence your results, and temper your discussion accordingly. I realize you are in somewhat of a 'no-win' situation, as the sample numbers from the first submission resulted in you adding these new specimens from other collections. Still, as reviewer 1 has correctly pointed out, such issues could directly and strongly influence your results, and they must be addressed.

*A. Thank you very much for your time and efforts on evaluating our manuscript. Indeed, no comment on the status of preservation of samples was addressed in the former version of the manuscript. Most of the specimens pictures were provided by the Kagoshima University Museum (KAUM), and were taken while samples were still fresh, before fixation. Please, refer to such statement in lines 137-145 in the restructured Material and Methods section, with a new literature cited referring to such issue (lines 448-450).*

*Indeed, we are facing now a ‘no-win’ situation, as further efforts beside the collaboration of the KAUM are impossible. However, the academic editor himself suggested increasing of N size with museums and collections samples, and we see no further move. Actually, acquisition of such material was already a hard task, as (1) these fishes, while juveniles, are cryptic, thus their observation/sampling is very limited; (2) in order to keep our data to a single population, we did not use any sample from other areas beyond the surroundings of Kuchierabu Jima Island.*

*We honestly hope by addressing all the concerns raised, our MS is eligible for publication within PeerJ.*

Reviewer #1 (Anonymous)

**Basic reporting**

The authors have done a strong effort to fix all issues raised during the reviewing process, however some points remain unclear (see below).

**Experimental design**

The authors have done a strong effort to fix all issues raised during the reviewing process, however some points remain unclear (see below).

**Validity of the findings**

I am still concern about the sample size and sampling procedures. There are a lot of papers showing that animal fixation protocols can modify and alter morphological structures (e.g. ethanol, formaldehyde etc…). Authors explain that some of the samples are coming from museums or other samplings, but nothing its said about animal preservation. Were all the samples preserved following the same protocol? If not, how different protocols can affect results?

*A. Thank you for your concern. Indeed, such issue was not properly addressed in our previous version of the manuscript. Most of the specimens pictures were provided by the Kagoshima University Museum (KAUM), and were taken while samples were still fresh, before fixation. However, a few specimens were photographed after fixation in formaldehyde and preservation under ethanol 70%. These were taken in the Laboratory of Biology of Aquatic Resources, Hiroshima University, after requesting the respective samples from KAUM. Please, refer to such statement in lines 137-145 in the restructured Material and Methods section, and again discussed in lines 231-234, with a new literature cited referring to such issue (lines 448-450).*

After checking Figure 2 (ANCOVA), there is something missed. Red and both green groups don’t follow lines plotted, and have a greater slope.

*A. The hyperbolic expression “highly significant interdependency” in line 207 was replaced for “significant interdependency”, same line. Also, we have added a brief comment on the round leaf models deviation (lines 211-212).
Both trend lines were generated by the statistical software employed.*

I am still concerned about the proportion between landmarks and sample size. Sixteen landmarks and 20 *Lobotes surinamensis*, 18 *Platax orbicularis*, 12 *Canthidermis maculata*.

*A. Please, notice that the juveniles of the species approached are cryptic, due to their mimetic interaction with plant parts. Thus, increasing the N size may be only possible under (1) loan from museums or collections; or (2) enhancing sampling efforts in the study area. As (2) is not an option (the skilled observer responsible for sampling is out of Japan since 2014, and even if he was still there, the occurrence of such individuals is seasonal, often occasional. Back to 2011, we had this exceptional occurrence of this huge aggregation of specimens at once, never observed before. Obviously, we can still reduce the number of landmarks for our analyses. However, as addressed in the manuscript (please, refer to lines 166-172, “…The morphometric comparisons among the fish and models were not intended to analyse homologous patterns, as we were interested in shape similarities randomly shared among the mimetic fish and their respective models distributed in the same environment, from a geometric morphometrics perspective. Therefore, the necessity of marking peripheral anatomic structures in the mimetic fish, instead of fins insertions only, in order to check for general appearance of mimetic fish with the plant models.”), the main purpose of this study is not to check for homologies, nor amongst fish species, neither amongst then with their respective associated plant parts (which would be rather impossible), but only to test for shape similarities among the groups (mimetic fishes + plant models). Despite the heterogeneity observed within and amongst groups, the actual number landmark is necessary, required to reach for all peripheral structures involved in the similarities amongst fish and plants.*

I a followed the procedures you get 15 sampled individuals, 24 from Kagoshima University Museum and 11 from previous surveys. The total sampled fish is 50 not 51 as present in the paper. According to additional data there are 12 additional individuals for *Platax orbicularis*, not 11 as indicated in Methods section.

*A. Regarding the N of* P. orbicularis*, we have mistyped this information as 11 instead of 12. Please, accept our apologies, and find the corrected N in the present version of our manuscript (line 146). Thus, the current N of fish specimens used for this work was 52, and not 51, as addressed in the previous version (see line 145* *and also S2 dataset).*