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

Morphometric comparisons of plant-mimetic juvenile fish associated with plant debris observed on Kuchierabu-jima Island, southern Japan

PeerJ

Dear Academic Editor

Dr. James Reimer

We received the referees comments and suggestions to our manuscript on March the 19st, and here we present our revised version. We have considered all suggestions, comments and criticism made by the three referees and by the academic editor, and tried to incorporate the most of them, in order to improve our manuscript, making it suitable for publication in PeerJ.

We have proposed a new, slightly changed title, as we had to enhance N size with other samples from the surrounding areas. In the tracked version, all revisions following the suggestions from referee #1 were highlighted in green, in yellow for referee #2, and in red for referee #3. Whenever it was the case, comment boxes accompany each change, in order to better clarify some of the referees concerns and suggestions. A clear version with all changes is also available.

Below, you can also find the referees comments and questions regarding each item, followed by our answers (*A. italics*) for each one of them. Also, as we have increased sample N with specimens from museums and collections, we have provided a new dataset as supplementary material (Dataset\_S2), this time in format \*.xlsx, in order to facilitate replication. We have reduced the number of Figures to only three, as we have noticed a wrong application of Linear Discriminant Analysis (LDA) after enhancing the sample size. We have also provided a new supporting material with a video file (Video\_S3.wmv), with a number of examples of plant-mimetic interactions by fish observed in the study area, replacing the previous statement “data not shown”, line 229.

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

On behalf of the authors,

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Reviewer 1

**Basic reporting**

References: The paper is too long and there are too much references that difficult the reading of the manuscript.

The discussion is too long and repetitive in some points. I think that it can be shortened about a 25 % without losing information.

Discussion is too ambitious, more if you are taking into account that only 15 specimens from 3 species were sampled, one of which with only two individuals.

*A. Thank you very much for your criticism and comments on our MS. We were pleased in trying to incorporate your concerns as much as we could, and we wish this revised version of the manuscript fulfills your expectations as an acceptable contribution to PeerJ.*

*Considering the MS length only, as another referee (#2) considered it as satisfactory, we tried to shorten a little in every section, in order to attend to both referees arguments.*

**Experimental design**

My major concern refers to the small sample size, only 15 individuals belonging to 3 fish species were captured in one week field sampling. One of the species is only represented by two individuals. At this point is difficult to justify the paper and the conclusions assumed.

How is the natural shape variability affecting results? It is not possible to measure natural variability.

Due to the small sample size but the large number of landmarks the results are completely overestimated.

Additionally, no information about the sampling is provided, are for instance fish and mimetic plants captured in the same samples?

Statistical methods are not the most appropriate for reduced sample sizes.

*A. We have asked more samples from the Kagoshima University Museum, which has kindly provided all necessary specimens for adequate sample size and statistical methods. Also, we have added twelve more* Platax orbicularis *specimens, sampled in previous periods (year 2006), from the same study area (Kuchierabu jima Island). Please, refer to the revised version of the MS, lines 124-133. Specific details on these new samples are also available within the supporting information* S2 Dataset.csv *(sampling location and date).  
Despite our observations concerning the present study are due to July 2011, we have been dealing with that locality for the last few decades (yet focusing only one species,* P. orbicularis*, since 2004*)*, where such fish-plant associations have been observed in several occasions. In the year 2011, it was probably the first time we did observe so many specimens, of different taxa, at the same time. All additional samples regarding* C. maculata *and* L. surinamensis *to the present dataset were collected in or nearby Japan, yet not from our study area (Kuchierabu-Jima Island). All fish were sampled along with their accompanying plant models (please refer to lines 111-112 in the revised version of the manuscript).*

**Validity of the findings**

My major concern refers to the small sample size, only 15 individuals belonging to 3 fish species were captured in one week field sampling. One of the species is only represented by two individuals. At this point is difficult to justify the paper and the conclusions assumed.

How is the natural shape variability affecting results? Can you measure variability with only 2 fish species?

Due to the small sample size but the large number of landmarks the results are completely overestimated.

Additionally, no information about the sampling is provided, are for instance fish and mimetic plants captured in the same samples?

Statistical methods are not the most appropriate for reduced sample sizes.

*A. Please refer to our previous answer.*

**Comments for the author**

Interesting MS dealing with fish morphometrics. However, some majors concerns arisen after my reading of the manuscript:

- Methods and Data analysis. My major concern refers to the small sample size, only 15 individuals belonging to 3 fish species were captured in one week field sampling. One of the species is only represented by two individuals. At this point is difficult to justify the paper and the conclusions assumed.

How is the natural shape variability affecting results? Can you measure variability with only 2 fish species?

Due to the small sample size but the large number of landmarks the results are completely overestimated.

Additionally, no information about the sampling is provided, are for instance fish and mimetic plants captured in the same samples?

Statistical methods are not the most appropriate for reduced sample sizes.

*A. Please refer to our previous answer.*

- References: The paper is too long and there are too much references that difficult the reading of the manuscript.

*A. Some repetitive references were cut from the revised version. However, a new one concerning the importance of Kuroshio current* (Kimura et al. 1998) *was added, as requested by the referee #3.*

Specific Comments:

Abstract

Lines 24-25: Change “*Lobotes surinamensis* (Lobotidae), *Platax orbicularis* (Ephippidae) and *Canthidermis maculata* (Balistidae), three plant-mimetic fish species, were compared” to “Three plant-mimetic fish species, Lobotes surinamensis (Lobotidae), Platax orbicularis (Ephippidae) and Canthidermis maculata (Balistidae) , were compared”.

*A. Proceeded as suggested by the reviewer.*

Line 27: Change “plant parts” to “plant debris”.

*A. Proceeded as suggested by the reviewer.*

Line 31: by a linear model?

*A. “Linear model” was rephrased by “analysis of covariance”, as concerned by the reviewer.*

Keywords

Avoid using the same words as in title, thus remove Morphometrics.

*A. Keyword “morphometrics” removed, as suggested by the reviewer.*

Introduction

Here and trough the manuscript. Remove some references, for instance in lines 47-48, 5 references are provided, and in lines 54-55, nine references are provided. The amount of references is unnecessary, difficulting the reading.

Introduction needs to be reworded and maybe shortened, for instance lines 48-50 (“Therefore, many…”) can be deleted without losing information.

Lines 68-104: Can be shortened and summarized (up to ca. 33 %) without losing information.

*A. We have proceeded as recommended by the reviewer, trying to shorten the whole introduction section as much as we could. Also, repetitive references were removed from the revised version. However, we did not change much of the contents from lines 68-104, as we judge addressing such background on the knowledge of mimetic interactions among each species may be necessary for a number of potential readers. We tried to compact the information provided though.*

Methods

Lines 113 and through the text: add space between number and %.

*A. Proceeded as recommended by the reviewer*

Lines 124-126: Only 15 fish individuals were captured during one week sampling. This is a major issue (see general comments).

*A. We have increased the N with samples from museums and collections. Please, refer to Material and Methods section, lines 124-133, containing all information regarding these newly added samples.*

Lines 124-125: What is the number between brackets? SD or SE? Please include.

*A. These are AVE ± SDEV values. This information is now provided in the revised version (Material and Methods, Ln 121).*

Line 127 and through the text: Change “n” to “n” in cursive.

*A. Proceeded*

Lne 133: Why left and not right when photos were taken?

*A. This is a general protocol for photographing fish samples, excluding some taxa which shape does not allow taking pictures from the left lateral view. Also, standardizing the same lateral view would reduce possible biases in geometric morphometric.*

Line 141: Sixteen landmarks but only 6, 7 or 2 specimens… the ration is completely overestimated.

*A. We have increased the N with samples from museums and collections. Please, refer to Material and Methods section, lines 124-133, containing all information regarding these newly added samples.*

Line 158: Data analyses methods are not the most appropriate to your small sample size

*A. We have increased the N with samples from museums and collections. Please, refer to Material and Methods section, lines 124-133, containing all information regarding these newly added samples. Also, detailed information regarding sampling area and collection reference number of each added new specimen can be found at the* Dataset\_S2.csv *file. We hope by increasing the N with such samples would allow us to keep with the chosen statistical methods for establishing comparisons among shapes of different fish taxa and plant debris.*

Line 164: Why are you using TL in statistical analyses, but relative body are is standardized by SL?

*A. When calculating relative body area, we have mistyped TL for SL (Ln. 161). This information was already revised in this new version, and we do apologize for that.*

Results

See previous comments.

Additionally I recommend joining results to discussions in a results and discussion section.

*A. According to the submission guidelines, PeerJ does not allow to join Results and Discussion in a single section. However, we have tried to restructur the Discussion section, under the light of the new results (after the museum reference samples were added).*

Discussion

The discussion is too long and repetitive in some points. I think that it can be shortened about a 25 % without losing information.

Discussion is to ambitious, more if you are taking into account that only 15 specimens from 3 species were sampled, one of which with only two individuals.

*A. We have tried to adapt the discussion under the light of the new results, after having enhanced N size. We hope this revised version fulfill your expectations in order to acceptance and further publishing by PeerJ.*

Reviewer: Even Moland

**Basic reporting**

First, I would like to congratulate the authors on an extremely well prepared manuscript.

The abstract is well written and provides a good window to the paper. I note that the authors use plant-mimetic, mimicry, mimesis and mimetic fish for cases that some purists would sort among protective resemblance given a strict definition of fish mimicry as only occurring when a fish evolves to resemble another. However, I agree with the way the authors have dealt with this issue and for the sake of readability I think the terms chosen are useful.

The introduction is well written and gives sufficient background for the study. The references chosen are appropriate and different views are well represented.

Results: Figures are relevant and the quality is high. I would have liked to see the nice study site figure provided in the supplements included in the main paper!

Line 170: repeat what the LM abbreviation means to keep the reader on track.

*A. Thank you very much for your compliments and suggestions! We were deeply pleased, and honoured, to have caused you such an impression! We have slightly modified some parts of the manuscript, as kindly observed by the other two reviewers. Might this improved, revised version may still cause the same impression, hopefully fulfilling and requisites for publishing in PeerJ.*

Raw data is supplied.

**Experimental design**

The hypotheses are well defined and within reach in terms of what the study can deliver.

The research is original and useful as an example of taking what subjectively meets the eye into a quantitative framework and thus seek to increase the objective validity of a science deemed largely anecdotal by some.

Materials and methods are described with sufficient detail and information. Analyses seem appropriate and are clearly and credibly presented.

*A. After increasing our sample N, we have kept the same analytic approach.*

**Validity of the findings**

The discussion is interesting and well written.

Line 194-198: This sentence is useful and true but could be rewritten to state simply that these are cases where the plant-mimetic species outgrow their plant debris model and thus the mimesis ceases. There are plenty of examples where fish are only mimics during vulnerable life stages, and reference to juvenile fish mimicry could fit in here.

Line 202: Roberston, a typo.

*A. Corrected*

Line 204-205: personal observation of behaviour, is there video material available? A link to a video clip made available online would have been nice if it exists, to support the case for “mimetic behaviour” (line 207). Cases where mimetic fish modify their behaviour in order to increase the resemblance to a model are well known and could be cited to lend further support.

*A. We do have several footage hours for* P. orbicularis*, a few minutes for* L. surinamensis*, and unfortunately no video record for* C. maculata*. Of course we can make such material available as supplementary material, if the absence of video data for this particular species is accepted. As the three species have been reported as mimetic before, I don't see any problem in not uploading video examples, yet by doing so means not an issue at al.*

Data is robust, statistically sound and controlled.

**Comments for the author**

My suggestions above are for consideration only and whether they are used in a minor revision or not should by no means preclude acceptance of the paper which in my opinion may be published forthwith.

*A. Thank you so much. Your comments and criticisms were much appreciated, and we are quite sure they are a valuable contribution to this revised version of the MS.*

Reviewer 3

**Basic reporting**

This manuscript focused the relationships between plant-mimetic fish species and plant parts, and was interesting philosophy for the fish morphological work on the bases of statistic methods.

However, some of the parts on the manuscript are difficult to understand, and need more careful explanations, especially in the results and discussion.

*A. Thank you very much for your concerns and advices. We hope this revised version, after increasing sample N and improving statistical methodologies, this may fulfill all requirements for publication in PeerJ.*

**Experimental design**

The selection of the species is bit difficult, the number of C. maculata are fewer than other two species, and may making problems on the statistic methods.

However, I can understand to collect a number of this species are so difficult, so is nonsense to order to add more data for this species. Nonetheless, this manuscript seem diverting to touch this species especially in results. The authors should carefully explain "what can read from figure" in results, and express their opinion of why the results differed (or not differed) in discussion.

*A. Thank you very much for your concerns. Indeed, observing and sampling such events in nature require both observation skills and luck, and we were fortunate to register so many events in a single week of observation. However, as pointed by both anonymous reviewers #1 and #3, such a low sample N may incur to statistical biases, compromising the analysis interpretation. Thus, we have added data of specimens from the Kagoshima University Museum, in order to increase data quality and statistical power, allowing us to keep with the same statistical approach. Please, refer to lines 124-133, in the Material and Methods section, and also to the new dataset file* S2\_dataset.csv*, containing specific details on all samples (collection reference number, location and sampling dates).*

**Validity of the findings**

Of cause, the facts these three fish species are mimicking plant parts, are widely known in many publications. However, the interest of the manuscript is to show the fact in science.

*A. Thank you very much. We were pleased with your comments, and we hope this revised version of the manuscript is enough for acceptance and further publication with PeerJ.*

**Comments for the author**

The maps and photos (Fig. 1) needs to modify. This figure may good for power point presentation, but no good for manuscript.

*A. This figure is provided as supplement material (Answering to your query from the annotated draft, line 111 -- The reason why we address to the figure as "S1 Fig.") . We have tried to fix it in this revised version, in order to clarify all missing information from the map.*

Other comments are written in the manuscript.

*A. Thank you. We have tried to incorporate all of then, as presented below:*

Abstract, line 28 - Please explain the abstract of Kuchierabu-jima Island and Kuroshio Current in introduction. The latter written in many fish publications from Ryukyu Archipelago.

*A. Please, refer to the revised version: Introduction, lines 70-72; and again in the Discussion section, lines 264-267. We also have included a new citation: Kimura et al. (1998); lines 374-376.*

Material and Methods, line 111 – “…. (S1 Fig.)” – What is this meaning?

*A. S1 Fig refers to a Figure file which is a supporting or supplementary information, as explained above. This figure is not part of the main text of the manuscript, and serves only the purpose of addressing the basic geography of Kuchierabu-Jima Island.*

Material and Methods, line 134 – “…. digital pictures of left lateral view…”– What is this meaning?

*A. Fish pictures are standardly shown from the left view of the body. As the other two referees did not have any comment on this matter, we have decided to leave it the way it is described. We hope only the present comment addressed to your concern is enough to clarify any doubts you may have.*

Material and Methods, line 148 – “We established equidistant 16 semilandmarks…” - Why the positions of numbers not corresponded with LM? No. 10 is caudal tip of fish, but no. 9 is caudal tip of reef.

*A. Indeed, it was a mistake when numbering and marking the figure. Please, refer to the revised Figure 1 in the present version of the MS, with all LM and SLM equally distributed in both mimetic fish and plant models.*

Results, line 175 – The authors should carefully explain "what can read from figure" in results, and express their opinion of why the results differed (or not differed) in discussion.

*A. We have tried to improve our Results and Discussions based on the new analyses after increasing sample size, as suggested by both referee #1 and #3, and also the academic editor.*

Results, line 197 – Nothing written about C. maculata. When they have no significant difference, you should show in thje resultd, and mention why they don’t have difference (caused by number of specimens or some other reason). If not, you can’t include this species in this manuscript.

*A. As the N of C. maculata was very low, we thought we should better refrain from any detailed statement on that species.* *We have tried to improve our Results and Discussions based on the new analyses after sample size was enhanced, as suggested by both referee #1 and #3, and also the academic editor.*

Discussion, Line 201 - The authors should carefully explain "what can read from figure" in results, and express their opinion of why the results differed (or not differed) in discussion.

*A. We have tried to improve our Results and Discussions based on the new analyses after increasing sample size, as suggested by both referee #1 and #3, and also the academic editor.*