PONE-D-20-30389  
Species identification based on a semi-diagnostic marker: evaluation of a simple conchological test for distinguishing blue mussels Mytilus edulis L. and M. trossulus Gould  
PLOS ONE  
  
Dear Dr. Khaitov,  
  
Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we feel that it has merit but does not fully meet PLOS ONE’s publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process.  
  
While both reviewer are excited about the general idea behind this study, both are concerned about methodological approaches (allozyme markers that are not fully diagnostic) and lack of discussion of the hybrid status of many mussel populations / species concept in mytilid mussels. I would suggest to follow the reviewers' advice to incorporate a hybrid category in your analysis and, potentially, to re-analyze parts of the data using diagnostic DNA markers, if possible. All underlying sequence data should be deposited in publicly available databases.  
  
Please submit your revised manuscript by Jan 24 2021 11:59PM. If you will need more time than this to complete your revisions, please reply to this message or contact the journal office at plosone@plos.org. When you're ready to submit your revision, log on to https://www.editorialmanager.com/pone/ and select the 'Submissions Needing Revision' folder to locate your manuscript file.  
  
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[Note: HTML markup is below. Please do not edit.]  
  
Reviewers' comments:  
  
Reviewer's Responses to Questions

**Comments to the Author**  
  
1. Is the manuscript technically sound, and do the data support the conclusions?  
  
The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: Partly

Reviewer #2: Partly

2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: Yes

Reviewer #2: Yes

3. Have the authors made all data underlying the findings in their manuscript fully available?  
  
The [PLOS Data policy](http://www.plosone.org/static/policies.action" \l "sharing" \t "https://mail.rambler.ru/folder/INBOX/INBOX_74164/_blank) requires authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception (please refer to the Data Availability Statement in the manuscript PDF file). The data should be provided as part of the manuscript or its supporting information, or deposited to a public repository. For example, in addition to summary statistics, the data points behind means, medians and variance measures should be available. If there are restrictions on publicly sharing data—e.g. participant privacy or use of data from a third party—those must be specified.

Reviewer #1: No

Reviewer #2: Yes

4. Is the manuscript presented in an intelligible fashion and written in standard English?  
  
PLOS ONE does not copyedit accepted manuscripts, so the language in submitted articles must be clear, correct, and unambiguous. Any typographical or grammatical errors should be corrected at revision, so please note any specific errors here.

Reviewer #1: Yes

Reviewer #2: Yes

5. Review Comments to the Author  
  
Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: This is an interesting manuscript describing a new semi-diagnostic character that could be used to roughly distinguish between the two mussel species Mytilus edulis and Mytilus trossulus in the field without the need for genotyping. I want to commend the authors for collecting this broad sample set. The analyses are in my opinion well done, although I had a few questions and concerns, especially regarding the use of allozyme markers and the lack of available raw data, that should be addressed.  
  
- The manuscript is a bit long. Sometimes it is hard to follow what was done.  
- L94: unambiguous  
- L191: Mussels in the Baltic Sea represent a hybrid swarm and are not pure M. trossulus. This is explained later in the discussion, but I think this should be recognized already here.  
- L198: I did not find a table with the allozyme and SNP genotypes and also no accession numbers for Illumina sequence data for samples that were new to this study. Could you please provide and deposit the raw data for full reproducibility? I think the input STRUCTURE files should be provided as well.  
- L203: Why were the allozyme markers used? These markers are also only partially diagnostic and provide less resolution than multilocus DNA markers. It does not make sense to me to try to confirm a semi-diagnostic characteristic with methods that are not fully diagnostic themselves. Then another part of the dataset is genotyped with a genome-wide SNP panel. I think the authors need to give a better explanation / justification of their analyses and a reason why they did not choose a consistent method across samples.  
- L269: What does a few genotyped samples mean? Please specify the sample size for this analysis.  
- Would it not be interesting to see if a general model with pools of all samples is possible, using salinity and location as variables? I don’t completely understand why this was not tested. I would think that such an analysis would be useful for researchers that work on regions not included in this study.  
- L309-323: This seems redundant. Can you merge this section with the one on the Kola Peninsula?  
- L601-603: I don’t think this statement is justified. Mytilus species have emerged as a non-model system for hybrid zone research, so for many scientists knowing the exact genotype composition is very important. I would soften this statement.  
- L672: links  
- L728: But probably there are some published data on salinity for those locations, even though it was not measured in this study?

Reviewer #2: Species identification based on a semi-diagnostic marker: evaluation of a simple conchological test for distinguishing blue mussels Mytilus edulis L. and M. trossulus Gould  
  
PONE-D-20-30389  
  
Khaitov et al.  
  
The manuscript is concerned with the diagnosis of two species (Mytilus edulis, M. trossulus) that are difficult to distinguish based on morphological characters for several reason. For instance, there is a general lack of phenotypic characters in these closely related bivalves, there is large phenotypic plasticity and there is extensive interspecific gene flow. The current taxonomic status of both species is derived from multi-trait investigations of mussel shells and genetic analyses (basically allozyme investigations). Additional support comes from the fact that both species are show ecological differentiation. Despite of ecological and phenotypic differentiation, they are considered part of the Mytilus edulis species complex together with M. galloprovincialis. Given the great importance of mytilid mussels for marine science (they represent up to 90% of the benthic biomass and hence shape marine ecosystems), an easy-to-apply diagnostic marker would be more than desirable for research. This is the justification of the study presented by Khaitov et al.! The authors follow previous investigations in the White Sea and test the “dark stripe” as a semi-diagnostic shell morphological character. There aim is to investigate, whether this trait is diagnostic in all marine environments. The authors apply a mathematical approach that is common on medicine where semi-diagnostic characters are commonly used for diagnosis. Both, the scientific question and the innovative approach makes the study interesting for (marine) taxonomists and ecologists and will finally guaranty the publication. However, I have serious concerns that the manuscript in its current form is already publishable! I have various questions and recommendations to the authors that need to be addressed before I can recommend the publication of this manuscript.  
  
Species concept  
My first question concerns the species concept behind the study. The authors use either allozymes or DNA markers for a priori species identification. To my understanding, this is used to calculate the Ptros-parameter. Although many of the populations are situated in hybrid zones, the individuals are either classified as M. edulis or as M. trossulus. For instance, STRUCTURE-q values (which represent hybrid indices) are used for a priori species diagnosis and the threshold is 0.5 (lines 214 to 215). This means, the authors classify F1 hybrid like individuals and early-backcross-generations as one or the other species. Furthermore, species diagnostic allozymes may mask the extend of genetic admixture at other genomic loci, e.g. in the Baltic. Consequently, classification into two categories that incorporate ANY hybrid status seems not justified. I had expected classification base on other admixture thresholds into at least three categories (including at least one hybrid category) and with reference to a valid species concept (e.g., referring to genetic characters). To avoid any misunderstanding: I agree that small proportions of introgression do justify allocation to one or the other species; I disagree that F1 like genotypes (including backcross genotypes) are not treated as hybrids but as one or the other species. In this context, what is said the paragraph about the Baltic and Norway (lines 669-687) could be discussed in another light if the hybrid category is considered. This paragraph already suggests that the pronounced hybrid character of these populations make the approach used in this manuscript doubtful.  
  
The “dark stripe character” in obvious hybrid individuals  
[I am aware that “dark stripe” does not fully describe the complexity of the prismatic layer closed to the ligament but I would like to use the term for simplicity.] Including a hybrid category would allow testing how the dark-stripe-character is developed in genetic hybrids! I am not sure whether the length of the “stripe” gives an information?  
  
Morphological characters  
Just a question: Is the presence/absence of the “dark stripe” the basis for PT (T-morphotype)  
  
Taxonomic structure of populations  
I like the idea that the taxonomic structure of a population might be more important for some scientific questions than the genetic affinity of a given mussel (lines 601-603). However, I do not see the reason why mussels in a mixed population should be identified as either M. trossulus or M. edulis (lines 612-615). Again, why is a status of being a hybrid not considered? Hybrids may have properties that allow surviving in extreme marine environments and Hybrids are the rule (not the exception) in mytilid mussel contact zones.  
  
Length of the discussion  
The discussion takes 12.5 pages and is overly long to my opinion. I feel that it need to be shortened substantially. This particularly refers to the part from line 747 to 876 (= 6 pages). I recommend summarizing the applications and the limitations of the morphotype test in a concise way.  
  
Terminology  
The parameters Ptros, PT, P(E/edu), P(T/tros), P(tros/T), P(edu/E) are parts of the calculations and the abbreviations are justified and logic. However, it is hard to keep the overview about their meaning. I ask the authors to give the short explanations (as in lines 229-236) as often as possible to help the readers.

6. PLOS authors have the option to publish the peer review history of their article ([what does this mean?](https://journals.plos.org/plosone/s/editorial-and-peer-review-process" \l "loc-peer-review-history" \t "https://mail.rambler.ru/folder/INBOX/INBOX_74164/_blank)). If published, this will include your full peer review and any attached files.  
  
  
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Reviewer #1: No

Reviewer #2: No

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