

Dear Editor,

We are very thankful for the opportunity offered to resubmit to *PeerJ* our manuscript entitled "**Asymmetrical dispersal and putative isolation-by-distance of an intertidal blenniid across the Atlantic-Mediterranean divide**" by Castilho et al. that we have now uploaded a revised version to comply with the minor revisions decision.

All suggested changes were taken into consideration, and to the best of our ability either incorporated in the manuscript or justified their non-inclusion. Additionally, we have amended the abstract for better reading. Please find below interspersed with the reviewer's comments our detailed reply, which we have highlighted using a blue font color. We would like to thank all referees, but especially referee #1, for his thorough and relevant contribution.

We hope to have answered all raised issues to your satisfaction. We look forward to hearing from you.

Yours sincerely,  
Rita Castilho

Editor's Comments  
MAJOR REVISIONS

As you can see, two of the original reviewers have re-reviewed your submission. Although the comments of Reviewer 1 are mostly 'editorial', Reviewer 2 still has a significant concern which (as they indicate) could be addressed via some additional analyses.

Please address all of the review comments in your revision.

Reviewer 1 (Anonymous)

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**Basic reporting**

The revised version of the manuscript "Isolation-by-distance and asymmetrical dispersal of an intertidal blenniid across the Atlantic-Mediterranean divide" is much improved, with better background and context provided, and a more balanced discussion.

**Reply:** We thank the referee for his positive comment.

I would suggest changing the title to reflect this version, though.

**Reply:** The title was change to incorporate the equivocal evidence from IBD.

**Experimental design**

Experimental design is adequate and the new analyses done provide further support to the findings.

**Reply:** We thank the referee for this positive comment.

**Validity of the findings**

The new analyses done and the changes introduced in the manuscript make it more robust.

**Reply:** We thank the referee for this positive comment.

**Comments for the Author**

The authors of "Isolation-by-distance and asymmetrical dispersal of an intertidal blenniid across the Atlantic-Mediterranean divide" are to be congratulated for their effort in answering all my comments and addressing my concerns. The new version of the manuscript is much improved and provides a more balanced interpretation of the results. I am particularly pleased with the thorough reply considering the IMA-2 and Migrate-N discussion, and I have no further comments on that part. Therefore, I recommend the manuscript to be accepted pending minor (mainly editorial) revisions.

Minor changes:

Title - the new version of the manuscript acknowledged the difficulty in attributing the observed phylogeographic pattern in *Salaria pavo* to a specific model. Therefore, I would recommend for the title to be changed to accommodate the new version.

**Reply:** We thank the referee for the suggestion and we introduced a nuance in the present title: "Asymmetrical dispersal and putative isolation-by-distance of an intertidal blenniid across the Atlantic-Mediterranean divide"

- ✓ Line 67: add a - between sargus and Bargelloni
- ✓ Line 89: Spawn (and not breed)
- ✓ Line 121: were observed - remove be
- ✓ Line 122: returned to the same pool
- ✓ Line 127: remove ( from 1998

- ✓ Line 132: not necessary to show the primer sequences if they are described elsewhere
  - ✓ Line 168: and phiST
  - ✓ Line 168: remove and, make it two sentences
  - ✓ Line 172: add a ,
  - ✓ Line 198: missing a ) at the end
  - ✓ Line 232: add a .
  - ✓ Line 226: it seems that BSP analysis was only conducted for the Atlantic samples, please specify this in the methods. Otherwise, the authors should have estimated BSP for both populations  
**Reply:** We have made that explicit in the Material and Methods section.
  - ✓ Line 243: replace characters by bp
  - ✓ Line 250: replace eleven by 11
  - ✓ Line 253: a nucleotide diversity of 0.036 is not low. Please change accordingly
  - ✓ Line 257: remove )
  - ✓ Line 277 (and 364): Isolation-by-distance
  - ✓ Line 286: remove the extra sentence saying AMOVA results
  - ✓ Line 312: a nucleotide diversity of 0.18 is not low. Was this in %?  
**Reply:** It is percentage, the sign % was added.
  - ✓ Line 381: add (LGM) after last glaciation maximum
- ✓ Figure 5: Not sure why it says GST, when it should be FST and phiST. Also, what are the circles in the figure? I'm assuming they mean non-significant, but that does not show in the legend, which mentions asterisks.  
**Reply:** Gst was changed to Fst, and asterisks were change by circles in legend.

Reviewer 2 (Anonymous)

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### Basic reporting

The revised manuscript is well written and well structured, and figures/tables are clear and appropriate.

**Reply:** We thank the referee for this positive comment.

### Experimental design

I appreciate the significant time and effort the authors put into revising the manuscript, including undertaking additional analyses of population structure and a more detailed assessment of the S7 data. That said, I do not see any indication of results for a pairwise analysis of population structure nor the related IBD analysis for the S7 data.

**Reply:** Results were included in file "Salaria\_Appendix S1.xlsx", in "Pairwise values" sheet. A sentence was now also added to the results section. "Also pairwise comparisons between locations, which returns three significant values, always involving Ria Formosa location (Ria Formosa – Cabo de Gata; Ria Formosa – Cadiz; Ria Formosa - Sado) (Appendix S1) and non-significant values for the isolation-by-distance Mantel test (Appendix S1)."

Likewise, AMOVA was not conducted using Dest for either loci.

I feel this is a non-trivial point as the argument for IBD is based on a significant signal in the D-loop Dest data, yet IBD was not tested using the S7 data and AMOVA/SAMOVA were not run using Dest.

I recommend the authors look into Genodive (Meirmans and Van Tienderen 2004) which

offers an ability to test for hierarchical structure (AMOVA) using several standardized measures of differentiation (including Dest).

Meirmans, P.G., and P.H. Van Tienderen: (2004), GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms, *Molecular Ecology Notes* 4 p.792-794.

**Reply:** We appreciate this suggestion and we would like to implement it; however, we have contacted the author, Patrick Meirmans, and he confirmed that Genodive does not have that capability. (see email below).

From: **Patrick Meirmans** p.g.meirmans@uva.nl  
Subject: Re: AMOVA with D  
Date: 28 February, 2017 at 13:38  
To: Rita Castilho rcastil@ualg.pt



Dear Rita,

You have to disappoint your reviewer, because it is not possible at all to calculate  $D_{jost}$  using an AMOVA. I have been thinking of ways to do this, and actually could not come up with a solution, so I think that it may not be possible at all.

What you can do is to use GenoDive to calculate  $D$  based on heterozygosities. However, that only works for a single population level, not when the populations themselves are clustered into groups (as one often does in an AMOVA).

Cheers, Patrick

On 28 Feb 2017, at 08:58, Rita Castilho <rcastil@ualg.pt> wrote:

Dear Patrick

A reviewer insists I do an AMOVA with  $D_{jost}$ , and suggested your software. However, I do not find a straightforward way of performing this analysis, can you please give me some directions?

Thanks,  
Rita

### Validity of the findings

While I agree with many of the authors' conclusions, my concern is that without additional analyses I feel that there is only marginal support for the IBD model.

**Reply:** We agree with the referee, and the text was modified in the previous submission to tone down the IBD claim. Also, the new title, as suggested by referee 1 also reflects a cautionary approach to the IBD claim.

### Comments for the Author

While I think the manuscript will be well received once published, I feel there is still some ambiguity regarding the importance of IBD versus regional population structure. As I've indicated above, I feel the manuscript would benefit from a few additional analyses. Since the conclusion of IBD was based in part on a significant IBD pattern in the d-loop Dest data set, I think it would be appropriate to test this same data set for hierarchical population structure (AMOVA) using Genodive. I as well think it would be informative to test pairwise population structure and IBD (mantel test) using the S7 dataset, both of which can be run in Arlequin.

**Reply:** We have done an exhaustive search for alternative software that would implement the approach suggested by the referees, but we could not find any such software. As suggested, we have performed the pairwise population structure and IBD test (as referred above).