Genetic structure in the endosymbiont *Breviolum ‘muscatinei’* is correlated with geographical location, environment and host species

Brendan H. Cornwell and Luis Hernández

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Original submission: 19 November 2020
Revised submission: 9 February 2021
Final acceptance: 10 February 2021

**Note:** Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

**Review History**

RSPB-2020-2896.R0 (Original submission)

Review form: Reviewer 1

**Recommendation**
Accept with minor revision (please list in comments)

**Scientific importance: Is the manuscript an original and important contribution to its field?**
Excellent

**General interest: Is the paper of sufficient general interest?**
Good

**Quality of the paper: Is the overall quality of the paper suitable?**
Excellent

**Is the length of the paper justified?**
Yes

**Should the paper be seen by a specialist statistical reviewer?**
No
Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible? 
No

Is it clear? 
N/A

Is it adequate? 
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Summary

Cornwell & Hernandez analyzed the population genetics of congeneric sea anemones and their shared endosymbiont along the west coast of North America using a RADseq approach. They find little population structure in the hosts, instead identifying an isolation-by-distance pattern. But the symbionts exhibit more structure, with at least seven populations across the biogeographic range. Genetic structure in the symbiont is influenced by macroscale (geography), mesoscale (intertidal zonation), and microscale (host specificity) processes. Given the symbiont’s shorter generation time and greater structure (due to low gene flow and/or strong selection), the authors conclude that the symbionts are better positioned to adapt to climate change, which may influence the adaptive capacity of the holobiont.

This is an excellent manuscript: thoughtful, easy to read, and interesting. The methods are sound and the conclusions follow from the data. I wouldn’t say the results are particularly surprising (many of the patterns have been identified previously, albeit among symbiont species, not within species), but there are few studies in this intra-species space and even fewer that take advantage of high-throughput approaches to look at population structure. This focus on within-species variation does provide novel insight into the relative importance of coevolution among partners. I was particularly impressed with the analyses of correlations between genetic and environmental variation in the symbiont, as well as covariance between host and symbiont loci. I have not seen such efforts in the Symbiodiniaceae literature before; I hope this manuscript will popularize them. The Anthopleura-Breviolum association is a fantastic system; it’s fairly unique in that it allows for consideration of macro/meso/micro-scale processes simultaneously. I really don’t have much to critique here—just a few suggestions for minor improvements. Great job.

Minor Comments

You should probably include the Symbiodiniaceae revision in the introduction, as it provides context for the within/between genus discussion. LaJeunesse et al. 2018 Current Biology (“Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts”).

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(because you are talking about variation within B. muscatinei; because the statement holds for intra-specific variation; and because ITS2 is a species-level marker, though it also gives you the genus at the same time).

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When discussing the BBA A. elegantissima symbiont population whose reads did not align with the B. minutum genome, it is worth mentioning that this likely represents a unique species, rather than just a distinct population of B. muscatinei.

For the paragraph on specific environments favoring particular symbiont strains (L413-423), consider incorporating Grupstra et al. 2017 Coral Reefs (“Evidence for coral range expansion accompanied by reduced diversity of Symbiodinium genotypes”), as it’s relevant.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Is it clear?
Yes
Is it adequate?
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Do you have any ethical concerns with this paper?
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Comments to the Author
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The Sanders and Palumbi 2011 paper provided a nice study showing many of the genetic differences in the Breviolum symbiont found in Anthopleura along the coastline from Washington to California. I would like to see more effort to reconcile the present work with this paper. From the preliminary genetic work from this earlier paper, it appears that the northern population in Washington and Oregon may be a different species than that found southern California (and possibly a third). I think that the present work would have benefited from providing at least one phylogenetic marker (LSU?) to help provide a phylogenetic framework for better perspective of their findings. This would also reveal the identity of the symbiont found in the most southern anemones. Clearly that symbiont is of another genus.

There are a few papers that also show how the genetics of the host does not match with that of the genetic structure of the symbiont, please read and include this in your citations:

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Breviolum muscatinei is presently not a valid species as there is no formal characterization. So it should be written in this paper as: Breviolum ‘muscatinei’

A thorough systematic treatment is needed for this dinoflagellate to determine if it indeed constitutes one species over the entire geographic range of the U.S. coastline or comprises two or more.

Please delete the Stat et al. 2008 paper in PNAS, cited at the end of the discussion. This is a misleading work and erroneous at all levels from the experimental design, data collection, to interpretation of results. It is a poster child of how to do bad science and get it published in PNAS if you know a member who is willing to see it though without much review (that practice has since been discontinued for reasons such as this paper). Sorry to be so blunt.
Decision letter (RSPB-2020-2896.R0)

19-Jan-2021

Dear Dr Cornwell

I am pleased to inform you that your Review manuscript RSPB-2020-2896 entitled "Genetic structure in the endosymbiont Breviolum muscatinei is correlated with geographic location, environment, and host species" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

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2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author’s name and journal name, e.g authorname_procb_ESM_figures.pdf

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

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It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.
If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2020-2896 which will take you to your unique entry in the Dryad repository.

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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Daniel Costa
mailto:proceedingsb@royalsociety.org

Associate Editor Board Member: 1
Comments to Author:
Dear Brendan and Luis

Your manuscript has been reviewed by two experts in the field. They both like your manuscript but highlight a number of points that should be addressed before the manuscript is ultimately published. I hope you will find their reviews useful in the final edit of this ms.

Warm Regards

Line

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

Summary

Cornwell & Hernandez analyzed the population genetics of congeneric sea anemones and their shared endosymbiont along the west coast of North America using a RADseq approach. They find little population structure in the hosts, instead identifying an isolation-by-distance pattern. But the symbionts exhibit more structure, with at least seven populations across the biogeographic range. Genetic structure in the symbiont is influence by macroscale (geography), mesoscale (intertidal zonation), and microscale (host specificity) processes. Given the symbiont’s shorter generation time and greater structure (due to low gene flow and/or strong selection), the authors conclude that the symbionts are better positioned to adapt to climate change, which may influence the adaptive capacity of the holobiont.

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Regards
Todd C. LaJeunesse

Author's Response to Decision Letter for (RSPB-2020-2896.R0)
See Appendix A.

Decision letter (RSPB-2020-2896.R1)
10-Feb-2021
Dear Dr Cornwell
I am pleased to inform you that your manuscript entitled "Genetic structure in the endosymbiont Breviolum 'muscatinei' is correlated with geographic location, environment, and host species" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.
If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org
**Appendix A**

Associate Editor Board Member: 1
Comments to Author:
Dear Brendan and Luis

Your manuscript has been reviewed by two experts in the field. They both like your manuscript but highlight a number of points that should be addressed before the manuscript is ultimately published. I hope you will find their reviews useful in the final edit of this ms.

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We thank the reviewer for these suggestions and have consolidated the figures (1 and 2 are now a single figure, as are 4 and 5). The additional materials and methods, as written, are ~1400 additional words. After reducing the discussion, it is ~2600 words. Based on the guidance from PRSB on predicting page counts and article length, we anticipate that this article will fall just under the maximum page limit for PRSB. However, if we are incorrect in that estimation, we would be happy to incorporate more of the SI materials and methods, but have refrained from doing so due to our uncertainty about whether or not they will fit.

When discussing the BBA A. elegantissima symbiont population whose reads did not align with the B. minutum genome, it is worth mentioning that this likely represents a unique species, rather than just a distinct population of B. muscatinei.

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We have added additional consideration of the results of Sanders and Palumbi in the ‘Macroscale’ portion of the Discussion (l.300-304). Sanders and Palumbi found evidence for genetic subdivision of the symbionts roughly on the scale of 100’s of km, similar to the spatial scales over which we found similar levels of differentiation. Sanders and Palumbi 2011 only consider populations from California (Pelican Point to San Diego). We have very small sample sizes from Oregon, and while they do form a cluster in the ADMIXTURE analysis, they do not form their own subpopulation, and form a group with a central CA population, which is also consistent with the PCA. Given our small sample size from Oregon, we are refraining from making assertions about whether those individuals belong to a separate species. Unfortunately we do not have LSU data for these individuals, and were unable to generate additional data to incorporate into this study between submissions to the journal.

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This remained in the citation list after a previous round of revisions where it was no longer cited in the text. The citation has been removed from the references and we thank the reviewer for bringing our attention to problems with the study.

Regards

Todd C. LaJeunesse