

## To see or not to see: molecular evolution of the rhodopsin visual pigment in neotropical electric fishes

Alexander Van Nynatten, Francesco H. Janzen, Kristen Brochu, Javier A. Maldonado-Ocampo, William G. R. Crampton, Belinda S. W. Chang and Nathan R. Lovejoy

### Article citation details

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<http://dx.doi.org/10.1098/rspb.2019.1182>

### Review timeline

Original submission: 26 February 2019  
1st revised submission: 21 May 2019  
2nd revised submission: 10 June 2019  
Final acceptance: 11 June 2019

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

## Review History

### RSPB-2019-0478.R0 (Original submission)

Review form: Reviewer 1

#### Recommendation

Major revision is needed (please make suggestions 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.**

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?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

#### **Comments to the Author**

"To see or not to see: molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" by Van Nynatten et al. presents an interesting finding about the lack of a sensory trade-off between selection to maintain the molecular systems associated with vision and selection to maintain molecular systems associated with electrosensory capabilities. The importance of this study reflects the nature of the maintenance of selective constraint across the genome.

Therefore, my first comment is that an extended discussion of load would benefit the paper. How many molecular systems can be maintained in a genome and how does that impact and how is it impacted by the organismal effective population size? How much deleterious mutation can be maintained in these systems. A discussion of this relevant literature to put the study in context would be helpful.

My second comment concerns the amino acid substitutions that were observed, including the suggested case of epistasis. Differentiating true positive selection that is enabled by epistasis and simple compensatory compensation that may look like positive selection is difficult. Can a formal characterization of the pseudoenergies associated with different combinations of substitutions be used to differentiate between these alternatives?

Overall, this is a strong paper, but I think these suggestions can improve the presented work.

## **Review form: Reviewer 2**

#### **Recommendation**

Major revision is needed (please make suggestions 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?**

Excellent

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

Acceptable

**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?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

### **Comments to the Author**

This paper studies possible trade-off between sensory modalities and I think is of major interest for the scientific community. The gymnotiforms are therefore interesting since developed electrolocation. I found the subject and the question absolutely fascinating.

However, I found the authors only focusing on the Rh1 gene decreased the interest in the paper and the results are not particularly exciting. Rh1 is under strong purifying selection even in lineages in dim-light environments and I think the LWS would provide a more interesting story. So I think the authors should provide this side of the story as well or a good explanation of why only focused on the Rh1 (it was not possible to PCR the LWS gene?). The compensation of a retinis pigmentosa mutation was a very interesting addition to the paper.

The analysis is robust and the dN/dS analysis is the best way to understand the evolution of functional genes. However I would like to see the modalities of electrolocation to be studied as well. From what I know, there are several types of organ discharge and jamming avoidance responses in African mormyrid and South American gymnotid fishes (Alves-Gomes et al., 1995). So It would be good if this has an impact in the evolution of Rh1 (and LWS if this information is provided).

Another correction needed is related the referencing itself. The authors are focusing citations in

papers of lab even when the paper is not adequate. A example is the paper citing Gutierrez et al, 2016 (reference 1) as a review (I'm not sure even if is peer-reviewed since its not in a journal) between line 71-74 instead of the papers that found pseudogenizations in each of the lineages mentioned. Each statement should be properly supported by the \*original\* research and if several papers found the same pattern \*all\* should be cited.

## Decision letter (RSPB-2019-0478.R0)

27-Mar-2019

Dear Dr Chang:

I am writing to inform you that your manuscript RSPB-2019-0478 entitled "To see or not to see: molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. As you will see, there is a consensus that aspects of your manuscript are of high quality and of interest. Nevertheless, there are issues relating to key elements of analysis and interpretation, as well as some additional emphasis on novelty, based on the use of the single gene, that requires your careful consideration. Please note, as indicated below, that our invitation to resubmit, does in no way guarantee the eventual outcome. We therefore will look carefully at the nature and rationale of your response, and look forward to reading the response letter, alongside changes to the manuscript. If you do choose to resubmit your manuscript, please upload the following:

- 1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
- 2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
- 3) Line numbers in your main document.

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Sincerely,  
Proceedings B  
mailto: [proceedingsb@royalsociety.org](mailto:proceedingsb@royalsociety.org)

Associate Editor  
Board Member: 1  
Comments to Author:  
Dear Prof Chang:

I found the manuscript very interesting, and I agree with the reviewers that the trade-off between sensory modalities is a fascinating topic. However, the reviewers have identified some points that need to be clarified (e.g. the discussion of the broader significance of the results and why the focusing on Rh1) before recommending the manuscript for publication.

Best wishes,  
Roberto Feuda

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

"To see or not to see: molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" by Van Nynatten et al. presents an interesting finding about the lack of a sensory trade-off between selection to maintain the molecular systems associated with vision and selection to maintain molecular systems associated with electrosensory capabilities. The importance of this study reflects the nature of the maintenance of selective constraint across the genome.

Therefore, my first comment is that an extended discussion of load would benefit the paper. How many molecular systems can be maintained in a genome and how does that impact and how is it impacted by the organismal effective population size? How much deleterious mutation can be maintained in these systems. A discussion of this relevant literature to put the study in context would be helpful.

My second comment concerns the amino acid substitutions that were observed, including the suggested case of epistasis. Differentiating true positive selection that is enabled by epistasis and simple compensatory compensation that may look like positive selection is difficult. Can a formal characterization of the pseudoenergies associated with different combinations of substitutions be used to differentiate between these alternatives?

Overall, this is a strong paper, but I think these suggestions can improve the presented work.

Referee: 2

Comments to the Author(s)

This paper studies possible trade-off between sensory modalities and I think is of major interest for the scientific community. The gymnotiforms are therefore interesting since developed electrolocation. I found the subject and the question absolutely fascinating.

However, I found the authors only focusing on the Rh1 gene decreased the interest in the paper and the results are not particularly exciting. Rh1 is under strong purifying selection even in lineages in dim-light environments and I think the LWS would provide a more interesting story. So I think the authors should provide this side of the story as well or a good explanation of why

only focused on the Rh1 (it was not possible to PCR the LWS gene?). The compensation of a retinis pigmentosa mutation was a very interesting addition to the paper.

The analysis is robust and the dN/dS analysis is the best way to understand the evolution of functional genes. However I would like to see the modalities of electrolocation to be studied as well. From what I know, there are several types of organ discharge and jamming avoidance responses in African mormyrid and South American gymnotid fishes (Alves-Gomes et al., 1995). So It would be good if this has an impact in the evolution of Rh1 (and LWS if this information is provided).

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## Author's Response to Decision Letter for (RSPB-2019-0478.R0)

See Appendix A.

## RSPB-2019-1182.R0

### Review form: Reviewer 1

#### **Recommendation**

Accept as is

#### **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.**

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?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

**Comments to the Author**

While I would have liked to have seen a more extended discussion of load and sensory trade-offs in selection, I am content to see the manuscript published as is.

## 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?**

Excellent

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

Excellent

**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?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

**Comments to the Author**

After reviewing the paper I found the many changes done by the authors improved significantly the paper. I think this paper will be interesting for the readers of Proc Royal Soc B and I fully support its publication.

I just have a final comment that should be seen as a very small minor correction. I think that sensory trade-offs are quite complex and many times don't appear to be universal or straight-forward. It has been suggested trade-offs are present in bats, the naked mole-rat, the star-nosed mole, and the blind Mexican cave fish but also in primates (Gilad et al., 2004). However the loss of the SWS1 gene driven by HDC echolocation is not as straight-forward as suggested by Zhao et al., 2009 and recent papers (Gutierrez et al., 2018; Simoes et al., 2019) and mostly Sadier et al., 2019 show the loss of the SWS1 in bats is very complex. Although a sensory trade-off between vision and thermal-sensory may be observed in vampire bats (Kries et al., 2018) it doesn't appear to be the case in vipers (Gower et al., 2019). I think paragraph between 59 and 67 could be extended to show the complexity of sensory trade-offs and show an alternative hypothesis (when sensory trade-off are not present). This may help the paper since for example Gower et al., 2019 suggested that there is no sensory trade-offs in vipers because there is an integration of the thermal-sensing with vision in vipers something similar to what is suggested by the authors (line 205-207).

Gilad et al. Loss of Olfactory Receptor Genes Coincides with the Acquisition of Full Trichromatic Vision in Primates. *PLoS Biol* 2, 0120–0125 (2004).

Sadier et al. Multifactorial processes underlie parallel opsin loss in neotropical bats. *eLife* 7:e37412 (2018).

Simões, B. F. et al. As Blind as a Bat? Opsin Phylogenetics Illuminates the Evolution of Color Vision in Bats. *Mol Biol Evol* 36, 54–68 (2019).

Kries, K. et al. Colour vision variation in leaf-nosed bats (Phyllostomidae): Links to cave roosting and dietary specialization. *Mol. Ecol.* 27, 3627–3640 (2018).

Gower, D. J. et al. Evolution of the eyes of vipers with and without infrared-sensing pit organs. *Biol J Linn Soc* 496, 311 (2019).



## Decision letter (RSPB-2019-1182.R0)

03-Jun-2019

Dear Dr Chang

I am pleased to inform you that your manuscript RSPB-2019-1182 entitled "To see or not to see: Molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. 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 us know.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Before uploading your revised files please make sure that you have:

- 1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".
- 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. PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. 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.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation

It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:

- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

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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=\(Document not available\)](http://datadryad.org/submit?journalID=RSPB&manu=(Document not available)) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit <https://royalsociety.org/journals/authors/author-guidelines/>.

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

Sincerely,

Professor Gary Carvalho  
mailto: [proceedingsb@royalsociety.org](mailto:proceedingsb@royalsociety.org)

Associate Editor  
Board Member  
Comments to Author:  
Dear Prof Chang:

Thank you for submitting your manuscript to Proceeding of the Royal Society B. I found this work very interesting, and I would be happy to recommend it for publication after an expansion of the discussion on the sensory trade-offs (please see reviewers' comments for details).

Best wishes,  
Roberto

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

While I would have liked to have seen a more extended discussion of load and sensory trade-offs in selection, I am content to see the manuscript published as is.

Referee: 2

Comments to the Author(s).

After reviewing the paper I found the many changes done by the authors improved significantly the paper. I think this paper will be interesting for the readers of Proc Royal Soc B and I fully support its publication.

I just have a final comment that should be seen as a very small minor correction. I think that sensory trade-offs are quite complex and many times don't appear to be universal or straight-forward. It has been suggested trade-offs are present in bats, the naked mole-rat, the star-nosed mole, and the blind Mexican cave fish but also in primates (Gilad et al., 2004). However the loss of the SWS1 gene driven by HDC echolocation is not as straight-forward as suggested by Zhao et al., 2009 and recent papers (Gutierrez et al., 2018; Simoes et al., 2019) and mostly Sadier et al., 2019 show the loss of the SWS1 in bats is very complex. Although a sensory trade-off between vision and thermal-sensory may be observed in vampire bats (Kries et al., 2018) it doesn't appear to be the case in vipers (Gower et al., 2019). I think paragraph between 59 and 67 could be extended to show the complexity of sensory trade-offs and show an alternative hypothesis (when sensory trade-off are not present). This may help the paper since for example Gower et al., 2019 suggested that there is no sensory trade-offs in vipers because there is an integration of the thermal-sensing with vision in vipers something similar to what is suggested by the authors (line 205-207).

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Simões, B. F. et al. As Blind as a Bat? Opsin Phylogenetics Illuminates the Evolution of Color Vision in Bats. *Mol Biol Evol* 36, 54–68 (2019).

Kries, K. et al. Colour vision variation in leaf-nosed bats (Phyllostomidae): Links to cave roosting and dietary specialization. *Mol. Ecol.* 27, 3627–3640 (2018).

Gower, D. J. et al. Evolution of the eyes of vipers with and without infrared-sensing pit organs. *Biol J Linn Soc* 496, 311 (2019).

## Author's Response to Decision Letter for (RSPB-2019-1182.R0)

See Appendix B.

## Decision letter (RSPB-2019-1182.R1)

11-Jun-2019

Dear Dr Chang

I am pleased to inform you that your manuscript entitled "To see or not to see: Molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" 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](mailto:procb_proofs@royalsociety.org)

Your article has been estimated as being 8 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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### Electronic supplementary material:

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.

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

Board of Editors  
Proceedings of the Royal Society B

May 17, 2019

To the Editors,

We appreciate your consideration of our resubmitted manuscript, and include here a revised version that incorporates the suggestions of the editor and two reviewers. As suggested, we have elaborated the rationale for our focus on rhodopsin, which was of particular interest to us after we found that our gymnotiform dataset included a mutation associated with disease in humans. We now explain in the manuscript that the broad importance of rhodopsin derives from its wide use as a model system for studies of molecular function, the ecology and evolution of vision, and disease biology. We have revised our abstract, introduction and discussion to make this case. We have also revised sections of our discussion to better explain the broader significance of our results, and expanded our references to further address aspects of molecular evolution as suggested by the reviewers. We also include below a point-by-point outline of our revisions, which we have made in response to specific comments from the reviewers. We feel that these revisions have significantly improved our manuscript and strengthened our conclusions.

We look forward to hearing from you.

Sincerely,

Nathan Lovejoy, Professor  
Department of Biology

Belinda Chang, Professor  
Department of Ecology & Evolutionary Biology

## Editor: Roberto Feuda

*I found the manuscript very interesting, and I agree with the reviewers that the trade-off between sensory modalities is a fascinating topic. However, the reviewers have identified some points that need to be clarified (e.g. **the discussion of the broader significance of the results and why the focusing on Rh1**) before recommending the manuscript for publication.*

**Response:** We thank the editor for sharing our interest in the subject of the manuscript, and for the invitation to resubmit. As described below, we have made revisions throughout the manuscript to better explain our focus on the evolution of rhodopsin, as well as its broader significance and relevance to other evolutionary studies.

## Reviewer 1

*"To see or not to see: molecular evolution of the rhodopsin visual pigment in neotropical electric fishes" by Van Nynatten et al. presents an interesting finding about the lack of a sensory trade-off between selection to maintain the molecular systems associated with vision and selection to*

*maintain molecular systems associated with electrosensory capabilities. The importance of this study reflects the nature of the maintenance of selective constraint across the genome.*

**Response:** We thank the reviewer for this positive response and interest in the broader importance of this study.

*1. Therefore, my first comment is that an extended discussion of load would benefit the paper. How many molecular systems can be maintained in a genome and how does that impact and how is it impacted by the organismal effective population size? How much deleterious mutation can be maintained in these systems. A discussion of this relevant literature to put the study in context would be helpful.*

**Response:** We agree with the reviewer and we now mention the importance of mutational load and effective population size for understanding gene complement underlying sensory systems (253-256).

*2. My second comment concerns the amino acid substitutions that were observed, including the suggested case of epistasis. Differentiating true positive selection that is enabled by epistasis and simple compensatory compensation that may look like positive selection is difficult. Can a formal characterization of the pseudoenergies associated with different combinations of substitutions be used to differentiate between these alternatives?*

**Response:** We agree with the reviewer that our interpretation of the putative epistatic effects of these substitutions would benefit from future *in silico* investigations, especially considering their location in a highly dynamic and important domain in rhodopsin structure and function. We have expanded our discussion of these critical aspects of rhodopsin's structure and function in lines 211-219. However, we believe that because these substitutions are likely to alter a dynamic process, investigation of the effects of the substitutions should be accompanied by experimental characterization in order to better understand the mechanistic basis underlying functional shifts. This is a clear future objective that we intend to pursue, but we believe it is beyond the scope of the current study.

## **Reviewer 2**

*This paper studies possible trade-off between sensory modalities and I think is of major interest for the scientific community. The gymnotiforms are therefore interesting since developed electrolocation. I found the subject and the question absolutely fascinating.*

**Response:** We thank the reviewer for these encouraging remarks!

*1. However, I found the authors only focusing on the Rh1 gene decreased the interest in the paper and the results are not particularly exciting. Rh1 is under strong purifying selection even in lineages in dim-light environments and I think the LWS would provide a more interesting story. So I think the authors should provide this side of the story as well or a good explanation of why only focused on the Rh1 (it was not possible to PCR the LWS gene?).*

**Response:** We have revised our manuscript to better explain our focus on rhodopsin in this study system, and to emphasize the broad significance of studies of rhodopsin molecular evolution and our study in particular. We have added text (particularly in the introduction, lines 78-91) to point out: (1) rhodopsin is the key visual pigment for vision in dim-light, and therefore is a likely candidate to exhibit a sensory trade-off with the electrosensory system, (2) rhodopsin is an important model system for studying the evolution of molecular function (and the evolution of GPCRs in general), (3) rhodopsin functional variation in relation to site-specific mutations is better characterized than any other visual pigment, enhancing our ability to make conclusions about the functional effects of observed sequence variation, (4) evidence of a human disease-causing mutation in gymnotiform rhodopsin is an intriguing result that highlights the broad relevance of our study of rhodopsin evolution in this system.

We note that although rhodopsin often evolves under purifying selection in dim-light (as pointed out by the reviewer), some gymnotiform species live in environments that are almost devoid of light (the bottoms of extremely turbid rivers), and relaxed rhodopsin evolution has been observed in similar circumstances. Also, previous studies have shown positive selection in rhodopsin in dim-light occurring taxa (as we did for two branches on the gymnotiform tree). This range of possibilities, in conjunction with the possible interaction of habitat and sensory biology in gymnotiforms, underlie the value of studying rhodopsin evolution in this particular study system.

The reviewer suggests that LWS may be an interesting addition to this story. We agree, and we will pursue investigations of other visual pigment genes in the future (lines 251,252, 261-263). However, as described above, we consider our investigation of rhodopsin to be well-suited to the questions we investigated, and to have yielded findings that will be of significant interest for the readers of Proceedings B.

*2. The compensation of a retinitis pigmentosa mutation was a very interesting addition to the paper.*

**Response:** We appreciate this feedback. This is another reason why we chose to focus on rhodopsin, as emphasized in our abstract and introduction (lines 34, 86, 87).

*3. The analysis is robust and the dN/dS analysis is the best way to understand the evolution of functional genes. However I would like to see the modalities of electrolocation to be studied as well. From what I know, there are several types of organ discharge and jamming avoidance responses in African mormyrid and South American gymnotid fishes (Alves-Gomes et al., 1995). So It would be good if this has an impact in the evolution of Rh1 (and LWS if this information is provided).*

**Response:** We reanalyzed our data to see if modality of electrolocation (pulse type electric organ discharge versus wave-type electric organ discharge) or electric discharge voltage (high versus low) affected patterns of molecular evolution. We found no evidence that variation in selection pressures is associated with these parameters. We have included these additional analyses in our revised manuscript (lines 146-148, supplementary methods, table S1).

4. Another correction needed is related the referencing itself. The authors are focusing citations in papers of lab even when the paper is not adequate. A example is the paper citing Gutierrez et al, 2016 (reference 1) as a review (I'm not sure even if is peer-reviewed since its not in a journal) between line 71-74 instead of the papers that found pseudogenizations in each of the lineages mentioned. Each statement should be properly supported by the \*original\* research and if several papers found the same pattern \*all\* should be cited.

**Response:** We have expanded the references in the introduction and discussion to include additional primary research articles, including the citations listed below. We removed the Gutierrez et al. 2016, reference.

### Eye loss, Sensory Trade-offs, and Electroreception

1. Niven, J. E. & Laughlin, S. B. 2008 Energy limitation as a selective pressure on the evolution of sensory systems. *J. Exp. Biol.* **211**, 1792–1804. (doi:10.1242/jeb.017574)
2. Fernholm, B. & Holmberg, K. 1975 The eyes in three genera of hagfish (*Eptatretus*, *paramyxine* and *Myxine*) – A case of degenerative evolution. *Vision Res.* **15**, 253–IN4.
3. Keeseey, I. W. et al. 2019 Inverse resource allocation between vision and olfaction across the genus *Drosophila*. *Nat. Commun.* **10**, 698. (doi:10.1038/s41467-019-09087-z)
4. Lavoué, S., Miya, M., Arnegard, M. E., Sullivan, J. P., Hopkins, C. D. & Nishida, M. 2012 Comparable Ages for the Independent Origins of Electrogenesis in African and South American Weakly Electric Fishes. *PLoS One* **7**, e36287. (doi:10.1371/journal.pone.0036287)

### Molecular Evolution of rhodopsin in fishes

1. Stieb, S. M., Cortesi, F., Sueess, L., Carleton, K. L., Salzburger, W. & Marshall, N. J. 2017 Why UV vision and red vision are important for damselfish (*Pomacentridae*): structural and expression variation in opsin genes. *Mol. Ecol.* **26**, 1323–1342. (doi:10.1111/mec.13968)
2. Rolland, J., Silvestro, D., Litsios, G., Faye, L. & Salamin, N. 2018 Clownfishes evolution below and above the species level. *Proc. R. Soc. B* **285**, 20171796. (doi:10.1098/rspb.2017.1796)

### Rhodopsin structure and function

1. Goncalves, J. A., South, K., Ahuja, S., Zaitseva, E., Opefi, C. A., Eilers, M., Vogel, R., Reeves, P. J. & Smith, S. O. 2010 Highly conserved tyrosine stabilizes the active state of rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19861–19866. (doi:10.1073/pnas.1009405107)



2. Piechnick, R., Ritter, E., Hildebrand, P. W., Ernst, O. P., Scheerer, P., Hofmann, K. P. & Heck, M. 2012 Effect of channel mutations on the uptake and release of the retinal ligand in opsin. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 5247–5252. (doi:10.1073/pnas.1117268109)

## Appendix B

Board of Editors  
Proceedings of the Royal Society B

May 17, 2019

To the Editors,

We appreciate your consideration of our manuscript and have enclosed a revised version addressing the suggestions made by two anonymous reviewers. We have broadened our introduction to clarify that sensory trade-offs are not always observed in species with alternative sensory modalities and included a reference suggested by Reviewer 2 that highlights an example where this is the case. We feel that these revisions and the suggestions made by the reviewers in our previous iteration of this manuscript have improved the scope and significance of our study.

We look forward to hearing from you.

Sincerely,

Nathan Lovejoy, Professor  
Department of Biology

Belinda Chang, Professor  
Department of Ecology & Evolutionary Biology

Thank you for submitting your manuscript to Proceeding of the Royal Society B. I found this work very interesting, and I would be happy to recommend it for publication after an expansion of the discussion on the sensory trade-offs (please see reviewers' comments for details).

We thank the editor for their enthusiasm, and have included additional text highlighting the complexity of sensory tradeoffs (lines 67-69).

### Referee: 1

Comments to the Author(s).

While I would have liked to have seen a more extended discussion of load and sensory trade-offs in selection, I am content to see the manuscript published as is.

We appreciate this feedback.

### Referee: 2

Comments to the Author(s).

After reviewing the paper I found the many changes done by the authors improved significantly the paper. I think this paper will be interesting for the readers of Proc Royal Soc B and I fully support its publication.

We thank the reviewer for this positive response and their constructive suggestions.

I just have a final comment that should be seen as a very small minor correction. I think that sensory trade-offs are quite complex and many times don't appear to be universal or straight-forward. It has been suggested trade-offs are present in bats, the naked mole-rat, the star-nosed mole, and the blind Mexican cave fish but also in primates (Gilad et al., 2004). However the loss of the SWS1 gene driven by HDC echolocation is not as straight-forward as suggested by Zhao et al., 2009 and recent papers (Gutierrez et al., 2018; Simoes et al., 2019) and mostly Sadier et al., 2019 show the loss of the SWS1 in bats is very complex. Although a sensory trade-off between vision and thermal-sensory may be observed in vampire bats (Kries et al., 2018) it doesn't appear to be the case in vipers (Gower et al., 2019). I think paragraph between 59 and 67 could be extended to show the complexity of sensory trade-offs and show an alternative hypothesis (when sensory trade-off are not present). This may help the paper since for example Gower et al., 2019 suggested that there is no sensory trade-offs in vipers because there is an integration of the thermal-sensing with vision in vipers something similar to what is suggested by the authors (line 205-207).

We appreciate this suggestion, and have added a sentence to the introduction addressing the fact that sensory trade-offs are not always observed in species with alternative sensory modalities, such as the pit vipers (lines 67-69).