

## **Novel vascular plexus in the head of a sea snake (Elapidae, Hydrophiinae) revealed by high-resolution computed tomography and histology**

Alessandro Palci, Roger S. Seymour, Cao Van Nguyen, Mark N. Hutchinson, Michael S. Y. Lee and Kate L. Sanders

### **Article citation details**

*R. Soc. open sci.* **6**: 191099.

<http://dx.doi.org/10.1098/rsos.191099>

### **Review timeline**

Original submission: 21 June 2019

Revised submission: 30 July 2019

Final acceptance: 1 August 2019

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

Note: This manuscript was transferred from another Royal Society journal without peer review.

## Review History

### RSOS-191099.R0 (Original submission)

#### Review form: Reviewer 1

**Is the manuscript scientifically sound in its present form?**

Yes

**Are the interpretations and conclusions justified by the results?**

Yes

**Is the language acceptable?**

Yes

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

No

**Have you any concerns about statistical analyses in this paper?**

No

**Recommendation?**

Accept with minor revision (please list in comments)

**Comments to the Author(s)**

This is an exciting paper as it describes a novel, and entirely unexpected adaptation in sea snakes. The paper is well written, the methods valid, the illustrations expertly prepared and presented, and valid conclusions reached from the data. I recommend publication.

However, in the abstract, where the authors presumably are trying to minimise the number of words to fit within the prescribed limit, there are several constructions that should be revised.

They are:

Line 38 is teleological: I suggest changing "arterial blood to facilitate" to "arterial blood that facilitates"

Line 39: "oxygen" in a noun, not an adjective. I suggest changing "limiting oxygen availability to the brain" to "limiting availability of oxygen to the brain"

Line 41: "sea snake" is a noun, not an adjective. The possessive should be used. I suggest changing "sea snake cutaneous" to "sea snakes' cutaneous"

The body of the paper is relatively free of such constructions.

**Review form: Reviewer 2 (Harvey B. Lillywhite)****Is the manuscript scientifically sound in its present form?**

Yes

**Are the interpretations and conclusions justified by the results?**

Yes

**Is the language acceptable?**

Yes

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

No

**Have you any concerns about statistical analyses in this paper?**

No

**Recommendation?**

Accept with minor revision (please list in comments)

**Comments to the Author(s)**

This is an interesting and very well-written manuscript that reports a novel feature of vasculature in a sea snake. The approach of this investigation is conceptually sound, and the conclusions of the authors are well supported by the findings of the study. In my opinion, this manuscript represents an important, meritorious paper, and the authors have done a very nice job of reporting their findings. I have no suggestions for major changes, but I do have a number of queries and one editorial emendation that I feel might improve an already excellent paper. (Revisions related to the items below might not be feasible and are optional.)

1. Inasmuch as the vascular structure and its proposed function are not yet demonstrated to be an “adaptation for underwater respiration,” I think the title would be more informative if the words “novel adaptation for underwater respiration in” are changed to something like “novel vascular plexus in the head of”.
2. It is not clear from the descriptions and figures how far below the skin are the blood vessels of focus. Evidently these are deep in the dermis. Presumably there is a capillary network close to the epidermis, but the evidence for this is not discussed, nor are the “smaller branches” mentioned in the caption to Fig. 1 distinguishable to my eye (at least, near the epidermis). If the vascular network of larger vessels (MCVN) is indeed respiratory in function, one would expect connecting capillaries that are very close to the epidermis. I suggest the authors might address this issue.
3. Is there any further information concerning the ecology or behavior of this species that might be relevant to the discussion? This species is euryhaline, and there might be features of habitat where this snake occurs or sojourns that possibly relate to environmental oxygen levels, or reclusive behaviors of snakes that might expose them to variable oxygen levels. If there is any information about this, it would be significant to mention.
4. Is there external indication of the location of the foramen that is visible on the scales of the top of the head? I believe this might be so based on photos I have seen, but I am not sure.
5. Is there indication of anything special about the permeability barrier (mesos layer of epidermis; or arrangement of lipids) in the cephalic scales of this snake? This might be expected if the vascular system is specialized for gaseous exchange or uptake. Otherwise I would not expect the thickened scales of the head to be especially permeable.
6. Both items # 2 and 5 above are relevant to the use of Fick’s diffusion equation mentioned on page 8 of the manuscript (both diffusion distance and coefficient).

I do not wish to remain anonymous. – Harvey B. Lillywhite

## Decision letter (RSOS-191099.R0)

22-Jul-2019

Dear Dr Palci

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-191099 entitled "Novel adaptation for underwater respiration in a sea snake (Elapidae, Hydrophiinae) revealed by high-resolution computed tomography and histology." has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

- Ethics statement

If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- Data accessibility

It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (<http://datadryad.org/>), or modify your current submission to dryad, please use the following link:

<http://datadryad.org/submit?journalID=RSOS&manu=RSOS-191099>

- Competing interests

Please declare any financial or non-financial competing interests, or state that you have no competing interests.

- Authors' contributions

All submissions, other than those with a single author, must include an Authors' Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:

AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

- Acknowledgements

Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

- Funding statement

Please list the source of funding for each author.

Please ensure you have prepared your revision in accordance with the guidance at <https://royalsociety.org/journals/authors/author-guidelines/> -- please note that we cannot publish your manuscript without the end statements. We have included a screenshot example of

the end statements for reference. If you feel that a given heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 31-Jul-2019. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/rsos> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create 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 referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees. We strongly recommend uploading two versions of your revised manuscript:

- 1) Identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
- 2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.

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

- 1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document";
- 2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format);
- 3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account;
- 4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript. Make sure it is clear in your data accessibility statement how the data can be accessed;
- 5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (<https://rs.figshare.com/>). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. 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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submitted as part of our collaboration with the Royal Society of Chemistry (<http://rsos.royalsocietypublishing.org/chemistry>).

If your manuscript is newly submitted and subsequently accepted for publication, you will be asked to pay the article processing charge, unless you request a waiver and this is approved by Royal Society Publishing. You can find out more about the charges at <http://rsos.royalsocietypublishing.org/page/charges>. Should you have any queries, please contact [openscience@royalsociety.org](mailto:openscience@royalsociety.org).

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

Kind regards,  
Andrew Dunn  
Royal Society Open Science Editorial Office  
Royal Society Open Science  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

on behalf of Dr Jake Socha (Associate Editor) and Kevin Padian (Subject Editor)  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

Associate Editor Comments to Author (Dr Jake Socha):

Associate Editor: 1

Comments to the Author:

Congratulations on an excellent piece of scholarship. Both reviewers had glowing things to say about the manuscript, and we all agree that it is an exciting contribution to the literature. There are a few small suggestions from both reviewers. I would highly advise you to take those under consideration and revise the manuscript accordingly in the next submission, before final publication.

Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

This is an exciting paper as it describes a novel, and entirely unexpected adaptation in sea snakes. The paper is well written, the methods valid, the illustrations expertly prepared and presented, and valid conclusions reached from the data. I recommend publication.

However, in the abstract, where the authors presumably are trying to minimise the number of words to fit within the prescribed limit, there are several constructions that should be revised. They are:

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Line 41: "sea snake" is a noun, not an adjective. The possessive should be used. I suggest changing "sea snake cutaneous" to "sea snakes' cutaneous"

The body of the paper is relatively free of such constructions.

Reviewer: 2

#### Comments to the Author(s)

This is an interesting and very well-written manuscript that reports a novel feature of vasculature in a sea snake. The approach of this investigation is conceptually sound, and the conclusions of the authors are well supported by the findings of the study. In my opinion, this manuscript represents an important, meritorious paper, and the authors have done a very nice job of reporting their findings. I have no suggestions for major changes, but I do have a number of queries and one editorial emendation that I feel might improve an already excellent paper. (Revisions related to the items below might not be feasible and are optional.)

1. Inasmuch as the vascular structure and its proposed function are not yet demonstrated to be an "adaptation for underwater respiration," I think the title would be more informative if the words "novel adaptation for underwater respiration in" are changed to something like "novel vascular plexus in the head of".
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3. Is there any further information concerning the ecology or behavior of this species that might be relevant to the discussion? This species is euryhaline, and there might be features of habitat where this snake occurs or sojourns that possibly relate to environmental oxygen levels, or reclusive behaviors of snakes that might expose them to variable oxygen levels. If there is any information about this, it would be significant to mention.
4. Is there external indication of the location of the foramen that is visible on the scales of the top of the head? I believe this might be so based on photos I have seen, but I am not sure.
5. Is there indication of anything special about the permeability barrier (mesos layer of epidermis; or arrangement of lipids) in the cephalic scales of this snake? This might be expected if the vascular system is specialized for gaseous exchange or uptake. Otherwise I would not expect the thickened scales of the head to be especially permeable.
6. Both items # 2 and 5 above are relevant to the use of Fick's diffusion equation mentioned on page 8 of the manuscript (both diffusion distance and coefficient).

I do not wish to remain anonymous. - Harvey B. Lillywhite

## Author's Response to Decision Letter for (RSOS-191099.R0)

See Appendix A.

## Decision letter (RSOS-191099.R1)

01-Aug-2019

Dear Dr Palci,

I am pleased to inform you that your manuscript entitled "Novel vascular plexus in the head of a sea snake (Elapidae, Hydrophiinae) revealed by high-resolution computed tomography and histology." is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office ([openscience\\_proofs@royalsociety.org](mailto:openscience_proofs@royalsociety.org) and [openscience@royalsociety.org](mailto:openscience@royalsociety.org)) to let us know if you are likely to be away from e-mail contact. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (<http://bit.ly/cpFAQ>). Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,  
Andrew Dunn  
Royal Society Open Science Editorial Office  
Royal Society Open Science  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

on behalf of Dr Jake Socha (Associate Editor) and Kevin Padian (Subject Editor)  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

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# Appendix A

Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

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Line 41: "sea snake" is a noun, not an adjective. The possessive should be used. I suggest changing "sea snake cutaneous" to "sea snakes' cutaneous"

The body of the paper is relatively free of such constructions.

We agree with all of Reviewer 1's comments and modified the abstract accordingly (edits highlighted in red font).

Reviewer: 2

Comments to the Author(s)

This is an interesting and very well-written manuscript that reports a novel feature of vasculature in a sea snake. The approach of this investigation is conceptually sound, and the conclusions of the authors are well supported by the findings of the study. In my opinion, this manuscript represents an important, meritorious paper, and the authors have done a very nice job of reporting their findings. I have no suggestions for major changes, but I do have a number of queries and one editorial emendation that I feel might improve an already excellent paper. (Revisions related to the items below might not be feasible and are optional.)

1. Inasmuch as the vascular structure and its proposed function are not yet demonstrated to be an "adaptation for underwater respiration," I think the title would be more informative if the words "novel adaptation for underwater respiration in" are changed to something like "novel vascular plexus in the head of".

We agree with the suggestion and modified the title accordingly.

2. It is not clear from the descriptions and figures how far below the skin are the blood vessels of focus. Evidently these are deep in the dermis. Presumably there is a capillary network close to the epidermis, but the evidence for this is not discussed, nor are the

“smaller branches” mentioned in the caption to Fig. 1 distinguishable to my eye (at least, near the epidermis). If the vascular network of larger vessels (MCVN) is indeed respiratory in function, one would expect connecting capillaries that are very close to the epidermis. I suggest the authors might address this issue.

We have added a sentence to the Results sections to better describe the presence and location of the blood vessels (lines 120-123). We have also added an additional supplementary figure, now Fig S3) to illustrate the capillaries located immediately under the epidermis.

3. Is there any further information concerning the ecology or behavior of this species that might be relevant to the discussion? This species is euryhaline, and there might be features of habitat where this snake occurs or sojourns that possibly relate to environmental oxygen levels, or reclusive behaviors of snakes that might expose them to variable oxygen levels. If there is any information about this, it would be significant to mention.

Unfortunately, there is no obvious correlation between ecology or behaviour and presence of a MCVN, as we stated on lines (227-231): “However, despite the functional advantage provided by the MCVN, there is not yet any evident correlation between the presence of large parietal foramina (and likely associated MCVN) and particular ecologies of the snake species that possess them.”

Like lots of *Hydrophis*, *H. cyanocinctus* is found in varied habitats that only sometimes include estuaries, whereas some *Hydrophis* do seem to be restricted to estuarine and inshore habitats (e.g. *H. obscurus*, *H. schistosus*, *H. caeruleus*, *H. donaldi*).

The challenges of hypoxia and cycling hypoxia-reoxygenation are probably common to all sea snakes. While *H. cyanocinctus* shares similar habitat types with other sea snakes, it might perform more strenuous swimming or predatory behaviours in these habitats. They can subdue large aggressive eels in deep water. But again so do several other *Hydrophis*.

4. Is there external indication of the location of the foramen that is visible on the scales of the top of the head? I believe this might be so based on photos I have seen, but I am not sure.

As shown in Fig. 1b, there is no indication of the presence of a foramen through the scales of the top of the head. We have now added a sentence to our Introduction (lines 63-65) to make this clear.

5. Is there indication of anything special about the permeability barrier (mesos layer of epidermis; or arrangement of lipids) in the cephalic scales of this snake? This might be expected if the vascular system is specialized for gaseous exchange or uptake. Otherwise I would not expect the thickened scales of the head to be especially permeable.

The histology sections suggest that the mesos layer in *H. cyanocinctus* is likely very thin or absent. A condition reminiscent of what has been recently documented in the congeneric

sea snake *H. platurus* (Lillywhite and Menon, 2019). We have now added two sentences (lines 116-119 and lines 176-178) to address this point, added the reference in question (ref. 12), and also added a new supplementary figure (Fig. S2) to illustrate the difference between the epidermis of *Oxyuranus* (a terrestrial snake with a well-developed mesos layer) and *H. cyanocinctus* (where the mesos layer is absent or very thin).

6. Both items # 2 and 5 above are relevant to the use of Fick's diffusion equation mentioned on page 8 of the manuscript (both diffusion distance and coefficient).

We have addressed both points (see above).

I do not wish to remain anonymous. – Harvey B. Lillywhite