Ultrastructural evidence of a mechanosensory function of scale organs (sensilla) in sea snakes (Hydrophiinae)

Jenna M. Crowe-Riddell, Ruth Williams, Lucille Chapuis and Kate L. Sanders

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Final acceptance: 15 March 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-182022.R0 (Original submission)

Review form: Reviewer 1 (Duncan Leitch)

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

Is it clear how to access all supporting data? 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)
The paper provides compelling anatomical evidence regarding the specialized mechano-sensory function of cephalic and tail scale organs from two species of sea snake. I appreciate that the authors have used a variety of histological and EM techniques to describe the unique morphology of these organs, including careful measure of adjacent skin layers, suggesting specialized tactile function, similar to the mechano-sensory organs noted in terrestrial snakes.

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Review form: Reviewer 2 (Kurt Schwenk)

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Decision letter (RSOS-182022.R0)

15-Feb-2019

Dear Ms Crowe-Riddell

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-182022 entitled "Ultrastructural evidence of a mechanosensory function of scale ‘sensilla’ in sea snakes (Hydrophiinae)" 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-182022

• 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 24-Feb-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:

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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.

Please note that Royal Society Open Science charge article processing charges for all new submissions that are accepted for publication. Charges will also apply to papers transferred to Royal Society Open Science from other Royal Society Publishing journals, as well as papers submitted as part of our collaboration with the Royal Society of Chemistry (http://rsos.royalsocietypublishing.org/chemistry).

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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

on behalf of Dr Richard Benton (Associate Editor) and Kevin Padian (Subject Editor)
openscience@royalsociety.org

Reviewer comments to Author:
Reviewer: 1

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authors have used a variety of histological and EM techniques to describe the unique morphology of these organs, including careful measure of adjacent skin layers, suggesting specialized tactile function, similar to the mechanosensory organs noted in terrestrial snakes.

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Author’s Response to Decision Letter for (RSOS-182022.R0)

See Appendices A & B.

Decision letter (RSOS-182022.R1)

15-Mar-2019

Dear Ms Crowe-Riddell,

I am pleased to inform you that your manuscript entitled "Ultrastructural evidence of a mechanosensory function of scale organs (‘sensilla’) in sea snakes (Hydrophiinae)" 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 and 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,
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Dr Richard Benton (Associate Editor) and Professor Kevin Padian (Subject Editor)
openscience@royalsociety.org

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Dear Andrew Dunn,

We are delighted to have our paper “Ultrastructural evidence of a mechanosensory function of scale ‘sensilla’ in sea snakes (Hydrophiinae)” accepted for publication in Open Science. We are grateful for the detailed and constructive comments that have enhanced the quality of the manuscript. We have made the recommended changes, all of which are minor, with one exception: unfortunately, we were not able to include a schematic diagram (as suggested by Reviewer 1) at this time. Please see our responses to reviewer comments below.

Kind regards,

Jenna Crowe-Riddell and co-authors

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
The paper provides compelling anatomical evidence regarding the specialized mechanosensory function of cephalic and tail scale organs from two species of sea snake. I appreciate that the authors have used a variety of histological and EM techniques to describe the unique morphology of these organs, including careful measure of adjacent skin layers, suggesting specialized tactile function, similar to the mechanosensory organs noted in terrestrial snakes.

| Reviewer 1 comments                                                                 | Author’s response                                                                                       |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 1) A summary schematic figure would serve this paper well. I suggest two (or more     | This is a great suggestion and would improve the paper. Unfortunately, we have not had time to     |
| panels) representing the specialized cephalic organs, as well as the distinct tail    | make this change.                                                                                    |
| organs. This could be prepared from observations from all the microscopic techniques |                                                                                                        |
| in total (e.g., relative location of putative neuronal bundles from the PGP           |                                                                                                        |
| immunohistochemistry, numbers of center cells as based on the counts from TEM, etc.)|                                                                                                        |
| and would serve the authors well in visually showing the differences in these organs,|                                                                                                        |
| such as locations/numbers of discoid organs, lamellated corpuscles, differences in   |                                                                                                        |
| thickness of the skin strata.                                                        |                                                                                                        |
| 2) The paper could benefit with a little more discussion regarding the potential     | We have incorporated relevant information from the Glenn Northcutt review paper on the comparative   |
| innervation of these organs. The work of comparative neuroanatomists like Glenn      | nerve patterns of lateral line systems in the ‘Tail scale organs’ section in the discussion and      |
| Northcutt have showed elaborate branching patterns that suggest particular sensory   | added the following sentence (lines 413-418): “Future studies should investigate the neural      |
| function/importance in fish, reptile, and amphibian species, and these patterns have   | pathways and compare electrophysiological responses underlying scale mechanoreceptors     |
| often been used to make evolutionary inferences. The authors touch upon this in the   | distributed on the head and body of snakes. Such efforts may discover that sea snakes     |
| "Tail scale organs" section in the discussion but this can be taken a bit further.     | possess specialised nerve pathways                                                                   |
| Innervation to the hydrodynamic sensors of the                                      |                                                                                                        |

Appendix A
The authors could communicate with a little more confidence about the unlikely role of these sense organs in electroreception (as in the last sentence of the Dermal photoreception... section). Their histological sections do not seem to show any kind of canal or pore typical of passive electroreceptors, like ampullary-type organs. Also, based on the sections shown in the paper, it doesn't appear that their are more specialized active electroreceptive organs, like tuberous organs or mormyromasts seen in freshwater weakly-electric fish. We have made this change to paragraph ‘Tail scale organs’ in the discussion, such that (lines 432-435): “An electro-magneto-sense is plausible (5), but our histological sections do not show canals or pores that are indicative of passive electroreceptors (e.g. ampullary-type organs) or specialised active electroreceptive organs (e.g. tuberous organs or mormyromasts of weakly-electric fish) (66,67).”

Other minor correction/comments:

1) The background white balancing looks unusual among panels in Figs. 5 and 6. The white balance has been corrected for Figs. 5 and 6.

2) The labels are difficult to read in Fig. 7, with the black labeling on top of the black and white TEM. Perhaps the brightness or contrast could be adjusted, or the labels themselves colored. Font size has been increased and changed to bold. Scale bars and font have also been increased.

3) The putative myelinated axon looks unusual with the sheathing appear very thin. The authors acknowledge that this is the best resolution available, however. Unfortunately, we were unable to achieve a higher resolution image of the putative myelinated axon.

4) In paragraph 2 of the discussion, the authors state that they can see free nerve axons terminating in the alpha layers in Fig. 3. Free nerve endings are a specific kind of specialized sensory end organ, often described as related to pain sensation. The nerve bundles adjacent to the putative sense organs are visible, but free nerve endings terminating in the the distal epidermis don't seem visible here (maybe just at this resolution). This was a typo- meant to reference Figure 5 (immunohistochemical results), this change has made as well as removing “free” before “nerve axons”

Reviewer: 2

Comments to the Author(s)

In a previous study the authors found that fully marine, hydrophiine snakes had, on average, a higher density of scale organs on cranial scales than their terrestrial relatives. Scale organs also tended to be larger and cover a larger area of the scale, although there was broad overlap in these measures. In the present study the authors consider whether the derived scale organs of marine species have retained the ancestral mechanoreceptive (terrestrial) function, or if they have also changed in function. They use light and transmission electron microscopy, and
immunohistochemistry to identify neuronal tissue to address the question. They show convincingly that the scale receptors retain a mechanoreceptive (touch) function.

The study is sound, the conclusions reasonable and supported by the data. I have only a few general comments and several small, editorial comments.

GENERAL COMMENTS

| Reviewer 2 comments | Author’s response |
|---------------------|------------------|
| (1) [p. 4, 1st paragraph; p. 11, lines 335-336; p. 12, lines 339-340] There is some ambiguity/mischaracterization about the role of mechanoreceptors in sensing ‘touch’ vs. “hydrodynamic stimuli.” I believe that this is a false distinction. The evidence is that the snake scale organs are mechanoreceptors sensitive to pressure. Pressure can take the form of physical touch or a compression wave traveling through water (a hydrodynamic stimulus). Thus there is not a dichotomy between ‘touch receptors’ and ‘hydrodynamic receptors’. The evidence suggests, circumstantially, that the scale organs of snakes are adapted to be more sensitive to pressure/mechanical stimuli than their terrestrial counterparts, presumably because water pressure waves have less energy than a physical touch. To be clear, I believe that this is what the authors mean to say, but as written, it is either unclear or the distinction is overstated. The distinction relates to the nature or source of mechanical, pressure stimuli, not (necessarily) to the nature of the receptors. | We understand the reviewer’s concern and have clarified the relationship between mechanosensitivity to touch versus water motion in the Abstract (lines 19-22), Introduction (lines 76-80; 91-93) and Discussion (lines 340-346; 360-364) However, we are reluctant to use of the term ‘pressure’ for the following reasons. Water movement can consist of mid water 'pressure waves' or water surface waves. But the term 'pressure wave' has a complex and often confused interpretation. A vibrating object in the water, for example, can produce change in pressure (this is what we call 'sound'), and we can measure it in Pascals but will also produce displacement of the particles in the medium (which is NOT pressure). To detect the pressure wave (in Pascal), you need a pressure-sensitive device (pressure - displacement transducer). Pressure detection is mediated by the inner ears of land vertebrates (which can be considered mechanoreceptors), and by the swim bladders of some fishes. It is possible that the inner ears of sea snakes also detect pressure, but this remains to be proven. Based on this, we consider it likely that the mechanoreceptors in the skin of sea snakes cannot detect pressure, but can detect motion displacement, velocity or acceleration, similar to the mechanosensitive neuromasts in the lateral line of fishes and other animals that detect motion, but not pressure. We briefly mention the possibility that scale organs are sensitive to pressure (i.e. baroreception) in the Discussion (lines 436-442). |
| Related to the above, note that the Merkel cell neurite complexes characteristic of crocodilian ISOs are also mechanosensory and hence would not actually play an “alternative sensory role” (p. 4, lines 94-95). | Indeed, Merkel cell neurite complexes are associated with mechanoreception. We have modified text to reflect this (lines 89-95): |
| “We aimed to better understand the evolution of scale organs in sea snakes by describing their ultrastructure in two fully-aquatic species, Aipysurus laevis and Hydrophis stokesii, using immunohistochemistry, and light and electron microscopy. If sea snake scale organs are retained for a mechanosensory role, either close-contact touch or detection of water motion (deflected off objects or prey/predators), we would expect them to have retained the ultrastructure described in terrestrial snakes, |
Finally, it is suggested (p. 4, lines 80-82) that Acrochordus uses its scale organs to sense “water motion.” I do not believe this is true. My understanding is that the snakes respond to physical touch of the fish. This should be checked.

We have checked the original reference (Povel and van der Kooij, 1997) and subsequent refs (Lillywhite 2014), in which the authors confirm mechanosensory function to scale ‘sensillae’ in Acrochordus and speculate that they are sensitive to water motion generated by fish prey given its analogous structure to hair-cells within fish neuromasts. We have modified the text to reflect the uncertainty in function in the literature. In the introduction (lines 80-83):

| “Indeed, two independently aquatic snakes, Erpeton and Acrochordus, are distantly related to hydrophiine sea snakes but have protruding organs that are likely sensitive to water motion generated by the movement of fish prey (5,35).” |

In the Discussion (lines 373-374):

| “Scale ‘sensillae’ in file snakes (Acrochordus) are thought to be sensitive to the hydrodynamic motion generated by the movement of fish prey.” |

| (2) Throughout the paper the scale organs are referred to as “sensilla”. I understand that this term is often used in the literature to refer to similar structures in squamate reptiles. However, it is inaccurate and misleading as a sensillum is a hair-like structure (usually in insects or other invertebrate taxa). It is also true that many lizard scale organs have a central hair-like protrusion that could more accurately be called a sensillum (exclusive of the rest of the receptor). Other terms are used in the literature for the sensory organs described in this paper, including ‘scale organ’ and ‘integumentary sensory organ’ or ‘ISO’. These would seem to me to be far preferable than “sensilla/sensillum”, but obviously this is the choice of the authors. |

| We agree with Review 2 and welcome the opportunity to use a more accurate term. After defining the term in the introduction (lines 15-16), we have replaced ‘sensilla/sensillum’ with ‘scale organ/s’ throughout the ms. |

| (3) Throughout the description of the sensory organs its inner, dermal component is referred to as a “dermal capsule”. In my opinion, this is inaccurate and inconsistent with most anatomical usage. A capsule represents a discrete covering or sheath that surrounds something “[a membranous structure…that envelopes an organ”—Stedman’s Medical Dictionary, 27th ed.], which is not the case here. The dermal protrusion into the epidermis is more accurately described as a ‘dermal capsule’ is anatomically inaccurate term for the underlying organ structure that we describe in the ms. Accordingly, we have replaced all instances of ‘dermal capsule’ with ‘dermal papilla’ throughout the ms. |

Concurring with Reviewers 2 point above (2), ‘dermal capsule’ is anatomically inaccurate term for the underlying organ structure that we describe in the ms. Accordingly, we have replaced all instances of ‘dermal capsule’ with ‘dermal papilla’ throughout the ms.
papilla’, as parenthetically noted on p. 5, line 134. This should be the term used throughout the paper.

| (4) Note that a finding of tactile/mechanoreception does not exclude all other possible functions, e.g., they could still function in modifying flow over the snake’s surface (though unlikely). | We have added the following sentence to paragraph ‘Dermal photoreception and other cutaneous sensory modalities’ in the discussion to address this point (lines 448-450):

“Finally, these sensory hypotheses do not exclude other non-sensory functions for scale organs, e.g. modifying boundary layer of skin, so these roles should be considered in future studies in the scale organs of sea snakes.” |

| EDITORIAL COMMENTS |

| Line 40: I do not believe that there is any evidence to support the assertion that snakes use mechanoreceptors to discriminate prey types. This is pure speculation based on mixed receptor types within the mouth. Evidence suggests that virtually all prey discrimination is chemosensory (gustatory and vomeronasal). | The paper referenced in the text (Nishida et al. 2000) describes ultrastructure of papillae in the mouth of Elaphe snakes, which provides compelling evidence for both chemo and mechanoreceptive functions for these oral organs. Nevertheless, we agree that the evidenced that these mechanoreceptors are used to discriminate prey types is indeed speculative. We have changed the text to clarify that these organs may be used in feeding (as appose to discriminating prey types) (lines 39-41):

“Snakes are likely to use these mechanosensory organs to explore and navigate substrate (7,8), during courtship (11) and feeding behaviours (9,10), but the anatomy and neurophysiology of scale organs are conspicuously understudied in comparison to other sensory organs” |

| Line 45: change “stimulus” to ‘stimuli’ | This change has been made. |

| Line 95: delete hyphen in “Merkel-cell” | This change has been made throughout the ms. |

| Line 104: “collected 1 10 km offshore”; seems to be a typo, not clear what it should be | A hyphen was deleted during the conversion to pdf, I have changes to ‘1 to 10 km offshore’ |

| Line 127: and elsewhere; insert “trichrome after Gomori’s one-step”; also, do not capitalize “One-Step” | This change has been made. |

| Line 130: delete “the height (thickness)” and change to ‘thickness’ | This change has been made. |

| Line 184: It is unclear what is meant by “horizontally arranged” in reference to the ‘central cells’; in the images they are either clustered, vertical or circular—never horizontal | We have removed this term from the text. |

| Line 187: re: the apparently basal taper in H. stokesii—are you sure that this is not simply a plane of section issue? Did you have serial sections | I have re-examined my images of serial sections and have come to the same conclusion as the reviewer: the basal taper is likely the result of the plane of the section. We
across the width of the receptors to confirm this? From other images it appears that the dermal papilla extends outward/laterally toward the scale surface, i.e., the distal part of the papilla is wider than its base. As such, a section that just passes through the point that a lateral extension joins the central core would look tapered toward the base.

have made the following change to the ms (lines 189-190):

“The dermal papilla was occasionally tapered at its basal end in H. stokesii (Figure 3A), but this likely to be an artefact of tissue sectioning.”

| Line 204: | change “skin” to ‘scale’ for clarity | This change has been made. |
| Line 238: | change “present at base of dermal” to ‘present at the base of the dermal’ | This change has been made. |
| Line 247: | replace the comma with a period; start new sentence with “The outer bumps…” | This change has been made. |
| Line 279: | The comment about “cap cells” based on Jackson (1977) seems a bit pointless. There is no histological difference between the keratinocytes covering the dermal papilla and others. Obviously any cells in this position would provide abrasion resistance, but no moreso than anywhere else | We agree with this comment and have removed the term ‘cap cells’ throughout the ms and referred to them simply as ‘the keratinocytes above the dermal papilla’ as necessary. However, we have kept the term ‘cap cells’ as Jackson (1977) is one of the only available previous studies that have described scale organs in snakes and so we believe the terminology should be noted in the ms. |
| Lines 286-287: | The figures do not show any direct evidence of discoid receptor innervation that I can see. Obviously they must be innervated and the nerves get pretty close within the scale organ, but no nerves leading directly to the discoid receptors, particularly the more distant, epidermal receptors, are evident. | Indeed. Previous studies on snake skin have found that nerves originating in the dermis terminate in epidermal ‘discoid’ receptors (Proske 1969). However, we did not observe direct evidence in our serial sections of this, therefore the text has been changed accordingly (lines 214-216):

“Dermal axons travelled to the scale organs (Figure 5C), then meandered through the central dermal papilla before innervating the outer epidermis and presumably terminate as distinct discoid endings in the alpha layer (Figure 5A, B).” |
| Lines 296-299: | It seems implausible to me that the central cells have no sensory function. What’s the point of the whole organ structure then, particularly given that discoid receptors are distributed all over? How confident are you about this, i.e., what is the probability that you would have seen synaptic complexes? There are neurons all over within the dermal papilla and it seems unlikely that they are merely supplying discoid receptors. What else would they be doing? Just free nerve endings? | We concur with Reviewer 2, this sentence now reads as such (lines 301-304):

“We did not find synaptic contacts between axons and central cells, which is consistent with light microscopy studies of other colubroid snakes (e.g. Elaphe) (23). Nevertheless, the presence of discoid receptors superior to the dermal papilla suggest that the central cells have a functional role in transducing mechanical stimuli.” |
Catania (1995), ref. 59, does not provide any evidence for sensitivity to hydrodynamic stimuli in star-nosed moles, nor can I find any other reference to such a thing. They definitely use the star organ for direct touch of food objects while foraging within water, but again (see General Comment 1), this is no different from terrestrial touch. I did not check ref. 60 for the platypus, but I would confirm that they do, indeed, have receptors that are sensitive to water movement AND that they have been “co-opted” from terrestrial cutaneous touch receptors. In fact, I would confirm this for all of them. Direct touch underwater is not the same thing as being used to detect water movement (hydrodynamic stimuli). Pinniped whiskers are a good example for mammals (they use them to detect vortices indicating fish trails) [see review by Dehnhardt & Mauck (2008), pp. 295-314, In Sensory Evolution on the Threshold, JGM Thewissen and S. Nummela (eds.), Univ. of California Press, Berkeley, CA]. also manatee whiskers and body hairs [reviewed in Bauer et al. (2018) The tactile senses of marine mammals. Int. J. Comp. Psychology 31, special issue, M. Botero ed.].

We have deleted these references from the text and included pinniped vibrissae as a prime example of co-option of cutaneous mechanoreceptors.

| Lines 359-362: | Catania (1995), ref. 59, does not provide any evidence for sensitivity to hydrodynamic stimuli in star-nosed moles, nor can I find any other reference to such a thing. They definitely use the star organ for direct touch of food objects while foraging within water, but again (see General Comment 1), this is no different from terrestrial touch. I did not check ref. 60 for the platypus, but I would confirm that they do, indeed, have receptors that are sensitive to water movement AND that they have been “co-opted” from terrestrial cutaneous touch receptors. In fact, I would confirm this for all of them. Direct touch underwater is not the same thing as being used to detect water movement (hydrodynamic stimuli). Pinniped whiskers are a good example for mammals (they use them to detect vortices indicating fish trails) [see review by Dehnhardt & Mauck (2008), pp. 295-314, In Sensory Evolution on the Threshold, JGM Thewissen and S. Nummela (eds.), Univ. of California Press, Berkeley, CA]. also manatee whiskers and body hairs [reviewed in Bauer et al. (2018) The tactile senses of marine mammals. Int. J. Comp. Psychology 31, special issue, M. Botero ed.]. |
|----------------|-------------------------------------------------------------------------------------------------|
| We have deleted these references from the text and included pinniped vibrissae as a prime example of co-option of cutaneous mechanoreceptors. |

| Line 401: | insert ‘compared to a’ after “differential sensitivity” This change has been made. |
| Line 402: | I don’t see why the trigeminal or other cranial nerves that innervate cephalic cutaneous receptors are “specialized”… The sensory organs they innervate might be specialized receptors, but the cranial nerves, themselves, are not specialized. This line has been deleted, and new sentence has been added to add clarity (lines 414-419): “Future studies should investigate the neural pathways and compare electrophysiological responses underlying scale mechanoreceptors distributed on the head and body of snakes. Such efforts may discover that sea snakes possess specialised nerve pathways and/or responsive fields that are analogous to the cranial nerve canals of neuromasts in fish and amphibians, or the vibrissae of secondarily-aquatic systems in mammals (55,61), which would support a hydrodynamic function for cephalic scale organs.” |
| Line 404: | the “dorsal root ganglion” is not a “peripheral nerve of the spinal cord”—it is a part of the spinal nerve within which the sensory nerve bodies lie. For lines 402-404, it is sufficient to note that the cephalic receptors are innervated by cranial nerves while the This line has been deleted (see change in text in previous point). |
Postcranial receptors are innervated by spinal nerves, which is exactly what one would expect.

| Line 406: the receptors are not used “to actively seek”—they are used while he snake actively seeks… |
| --- |
| This line has been deleted (see change in text in previous point). |

| Line 409: do you mean ‘electrophysiological’ rather than ‘electrophysical’? |
| --- |
| Indeed, this change has been made. |

| Lines 429-432: there is also no histological support for a magnetic sense |
| --- |
| We have changes this line to make a stronger assertion in line with Reviewer 1 and Reviewer 2 comments on magnetic sense (lines 432-436): |
| “Several other sensory functions have been tentatively attributed to the scale organs of sea snakes, but these currently lack supporting evidence. An electro-magneto-sense is plausible (5), but our histological sections do not show canals or pores that are indicative of passive electroreceptors (e.g. ampullary-type organs) or specialised active electroreceptive organs (e.g. tuberous organs or mormyromasts of weakly-electric fish) (66,67).” |

| FIGURE LEGENDS: 2A: no plane of section is give for the image; 2B: delete “cross-” (redundant); ‘hematoxylin’ is misspelled “hemotoxylin”; 2C: insert ‘trichrome’ after “Gomori one-step”; 3A: no plane of section given; 3B, C: delete “cross-”; ‘transverse’ is misspelled “traverse”; 4: check for above changes; also, Latin binomial name not italicized; “deeper cross-section” doesn’t make sense to me, please clarify; 7: “keratin filaments tonofilaments (t)”? must be a typo—missing parentheses? |
| --- |
| These changes have been made. |
Ultrastructural evidence of a mechanosensory function of scale organs (‘sensilla’) in sea snakes (Hydrophiinae)

Jenna M. Crowe-Riddell1*, Ruth Williams2, Lucille Chapuis3, Kate L. Sanders1

1 School of Biological Sciences, The University of Adelaide, Adelaide SA 5005, Australia
2 Adelaide Microscopy, the Centre for Advanced Microscopy and Microanalysis, Adelaide SA 5005, Australia
3 College of Life and Environmental Science, University of Exeter, Exeter EX4 4QD, United Kingdom

*Corresponding author: jenna.crowe-riddell@adelaide.edu.au mcroweriddell@gmail.com
Abstract

The evolution of epidermal scales was a major innovation in lepidosaurs, providing a barrier to dehydration and physical stress, while functioning as a sensitive interface for detecting mechanical stimuli in the environment. In snakes, mechanoreception involves tiny scale organs (‘sensilla’) that are concentrated on the surface of the head. The fully marine sea snakes (Hydrophiinae) are closely related to terrestrial hydrophiine snakes but have substantially more protruding (dome-shaped) sensilla scale organs that often cover a larger portion of the scale surface. Various divergent selection pressures in the marine environment could account for this morphological variation, including, relating to enhanced detection of mechanical stimuli (from direct contact with stimuli and/or indirect contact via water motion (i.e. ‘hydrodynamic reception’ either tactile or hydrodynamic), or co-option for alternate sensory or non-sensory functions. We addressed these hypotheses using immunohistochemistry and light- and electron microscopy to describe the cells and nerve connections underlying scale sensilla organs in two sea snakes, Aipysurus laevis and Hydrophis stokessii. Our results show ultrastructural features in the cephalic sensilla scale organs of both marine species that closely resemble the mechanosensitive Meissner-like corpuscles that underlie terrestrial snake sensilla scale organs. We conclude that the sensilla scale organs of marine hydrophiines have retained a mechanosensory function, but future studies are needed to examine whether they are sensitive to hydrodynamic stimuli.

Keywords: sea snake, sensilla scale organs, cutaneous, mechanoreceptor, skin, ultrastructure, transmission electron microscopy, sensilla

Introduction

Hardened epidermal scales are a characteristic trait of snakes (and other lepidosaurs: lizards and tuatara) that facilitate defensive signalling, camouflage, water retention, and locomotion (1–3). The epidermal scales also provide the primary surface for mechanoreception, which is the ability to sense mechanical stimuli that result from pressure or physical displacement (vibration) (4). Scale organs (‘sensilla’ or ‘tubercles’ sensu (5–7) are small mechanoreceptors that protrude from the surface of epidermal scales of the head and body of snakes. Snakes are likely to use these mechanosensory organs to explore and navigate substrate (8,9), discriminate prey types (9,10) and during engagement in courtship behaviours...
(10), and feeding (11,12) behaviors, but the anatomy and neurophysiology of scale sensilla organs are conspicuously understudied in comparison to other sensory organs—for example, eyes (13), auditory structures (14), vomeronasal organ (15) and heat-sensing pits (16).

In terrestrial snakes, scale sensilla organs are concentrated on the head and are highly sensitive to mechanical stimulation, particularly moving stimuli (17–20). The underlying ultrastructure of cephalic scale sensilla organs consists of an innervated cluster of dermal cells (‘dermal papillae’) that displaces the surrounding epidermis to create round skin elevations (21–23). These underlying features of scale sensilla organs have been likened to ‘Meissner corpuscles’, which are low-threshold mechanoreceptors (LTMRs) sensitive to innocuous ‘light touch’ stimuli in the glabrous (hairless) skin of mammals (24,25). Scale sensilla organs on the body-head are less specialised in their underlying ultrastructure than on the body, which lack dermal papillae, and the outer skin elevations are instead caused by a superficial thickening of the epidermis (10,26). These ultrastructural differences between head and body scale sensilla, and the concentration of sensilla on the head, are thought to reflect the role of the head as the primary tactile interface in snakes (17,27).

Snakes exhibit substantial variation in the size, shape, density, and distributions of their scale sensilla organs. Enlarged and/or high densities of sensilla scale organs have been reported in fossorial snakes (e.g. Leptotyphlopidae) and some sea snakes (Hydrophiinae), whereas in other colubroid snakes sensilla organs are small and/or sparse (e.g. Dipsadinae) or even absent in some species (e.g. Viperidae) (22,27–29). Interspecific differences in the traits of these sensilla organs likely relate to various aspects of species’ environment, ecology, and phylogeny. However, our understanding of the adaptive diversity functional of snake sensilla scale organs is hindered by a lack of comparative data describing differences in the external traits of scale sensilla organs and their underlying ultrastructure.

Hydrophiine snakes (Elapidae) provide a useful comparative framework to investigate the evolution of squamate scale sensilla organs in response to major ecological transitions (7). The fully marine, viviparous sea snakes comprise a clade of more than 60 species that evolved within the terrestrial Australian hydrophiine radiation (tiger snakes, death adders, taipans) approximately 9 to 18 million years ago (30). Previous work has found that the cephalic scale sensilla organs of sea snakes are substantially more protruding (dome-shaped) compared to their terrestrial counterparts, and in some lineages cover a much larger proportion of the scale surface (> 6% versus < 2.5% in sampled taxa) (7). This divergence in
external sensilla morphology might reflect divergent selection pressures in the marine environment. However, the hitherto lack of data on the ultrastructure of scale sensilla organs in sea snakes precludes meaningful comparisons with terrestrial snakes.

In their external appearance, the dome-shaped scale organs of sea snakes closely resemble the integumentary scale organs (ISOs) of crocodilians, which are cephalic mechanoreceptors with elaborate Merkel cell neurite complexes and sensitivity to water motion (i.e. hydrodynamic reception) (31–33). A dome-shaped scale organ provides increased surface area for stimuli to be received from multiple directions, possibly enhancing hydrodynamic mechanoreception sensitivity in an aquatic habitat whereby water motion can be detected from both biotic sources, e.g. conspecifics, prey and predators, and abiotic sources, e.g. turbulence caused by water currents deflected past objects (4). Indeed, two independently aquatic snake genera, Erpeton and Acrochordus, are distantly related to hydrophiine sea snakes but have protruding organs that are likely to be sensitive to water motion generated by the movement of fish prey (i.e. hydrodynamic stimuli) (5,34). It is also plausible that scale sensilla organs have been co-opted in sea snakes for a different sensory modality, such as dermal photoreception phototaxis (found in Aipysurus sea snakes (35,36), or electromagnetic sensing for navigation (15). Alternatively, scale sensilla organs may have been co-opted for a non-sensory function such as enhanced friction for gripping during mating, or disruption of the skin boundary layer to increase swimming performance (analogous to the denticles of shark skin or tubercles on the fins of whales (37–39)).

We aimed to better understand the evolution of scale sensilla organs in sea snakes by describing their ultrastructure in two fully-aquatic species, (Aipysurus laevis and Hydrophis stokesii) using immunohistochemistry, and light and electron microscopy. If sea snake scale sensilla organs are retained for a modified for enhanced mechanosensory roles, either tactile close-contact touch or detection of hydrodynamic water motion (deflected off objects or prey/predators), we would expect them to have retained the ultrastructure described in terrestrial snakes, and possibly contain other sensory cells such as the Merkel cell neurite complexes of crocodilian ISOs. Co-option for alternative sensory roles would be implicated if different cell types are present, such as the Merkel cell neurite complexes of crocodilian ISOs. Finally, if dome-shaped scale organs provide a non-sensory (e.g. structural) function, we would expect their elevation from the skin surface to be created by superficial thickening of the epidermis with no associated neuronal or receptive cells.
Materials and methods

Specimens and tissue sampling

Two museum specimens of the sea snake species *Aipysurus laevis* (one individual) and *Hydrophis stokesii* (one individual) were used for gross morphological observations. Fresh specimens of these species (two individuals of *A. laevis*; one individual of *H. stokesii*) were collected 1–10 km offshore from the coast of Broome, Western Australia, in June 2015 and September 2016.

Immediately after euthanasia, cephalic scales were sampled from all three sea snakes, and tail scales were sampled from the posterior dorsal surface and ventral tip of the tail in a single *A. laevis* because this species exhibits tail phototaxis linked to dermal photoreception (36). Entire scales were dissected to sample the whole skin from epidermis to subcutaneous tissue. The specimen details and locations of sampled scales are shown in Table 1 and Figure 1.

A single specimen of *Oxyuranus scutellatus* (the Australian taipan) was sourced from a captive breeding population (Venom Supplies Pty Ltd, South Australia) to sample brain tissue for antibody controls (see below) because this species is closely related to viviparous sea snakes (30). All samples were fixed by immersion in either 4% paraformaldehyde for immunohistochemistry, or 1.5% glutaraldehyde and 4% paraformaldehyde for electron-microscopy. After immersion in fixative for 24 hours, samples were washed and stored in phosphate buffered saline (PBS; pH 7.4) with sucrose, before being transferred into phosphate buffer with 0.05% sodium azide.

Stereo and light microscopy

The outer skin morphology of museum specimens was examined using a stereomicroscope with a mounted camera (SMZ25, Nikon Inc., Japan). Specimens were submerged in water and illuminated by a ring of light-emitting diodes (P2-FIRL LED Ring Illumination Unit, Nikon Inc., Japan) to reduce specular reflections from the scales. A high-depth-of-field photographic image was composed using imaging software (NIS-Elements Advanced Research v5.10, Nikon Inc., Japan).

The general cellular morphology of the skin samples was examined using light microscopy. Samples were dehydrated by successive immersion in alcohol, then paraffin-embedded for serial sectioning (10 µm). Slides were stained with hematoxylin-eosin or Gomori’s One-Step (40), scanned using a digital slide scanner (Nanozoomer, Hamamatsu Photonics, Japan) and measurements taken using imaging software (Nanozoomer Digital...
We measured the height (thickness) of the epidermis located above scale sensilla organs, and at adjacent areas of skin that did not contain sensilla organs. Because the outer layer of hardened skin (beta layer) sometimes became artificially separated from surrounding layers during tissue processing, we measured only the living (nucleated) epidermal layer (stratum germinativum). The diameter and height of dermal capsule papillae (papilla) and other dermal structures were measured and the ratio of diameter:height calculated.

Statistics

We used the two-sample t-test (unpaired) to examine differences in epidermal thickness between scale organs and adjacent skin that did not contain scale organs. Before statistical analyses, we checked that data were normally distributed using Bartlett’s test. Statistical analyses were performed using base packages in R v3.5.1 (R Core Team, 2017).

Immunohistochemistry

Immunohistochemistry was performed on paraffin-embedded serial sections (10 µm) for a neuronal marker, protein gene product 9.5 (PgP9.5). Briefly, slides were blocked for endogenous peroxidase with 0.5% hydrogen peroxide in methanol at room temperature for 30 minutes (min). Slides were rinsed in PBS and processed in 10 mM sodium citrate (pH 6.0) for heat-induced epitope retrieval. Slides were washed twice in PBS, before blocking in 3% normal horse serum (NHS) in PBS for 30 min. Sections were incubated with mouse monoclonal anti-PgP9.5 antibody (dilution 1:2000 with 3% NHS) at room temperature overnight. Sections were then washed twice in PBS and incubated with a peroxidase-conjugated secondary antibody (IgG anti-mouse, 1:500 diluted in PBS with 3% NHS) for 30 min, then incubated with streptavidin peroxide (dilution 1:1000 with 3% NHS) for 1 hour. Binding sites were revealed using a red chromogen (NovaRed Peroxidase Substrate Kit, Vector, USA) according to manufacturer instructions and incubated for 2 to 3 min. Slides were washed in distilled water for 5 min before counterstaining in Harris hematoxylin for 30 to 60 seconds and allowed to air dry. A primary antibody control was performed using the above protocol on snake (taipan) brain tissue; a secondary antibody control was performed using the above protocol, with the primary antibody incubation step omitted, on snake brain and cephalic skin tissue. Slides were imaged using an optical microscope (BX51, Olympus, Australia) and the saturation and hue of images was adjusted using imaging software (Adobe Photoshop v2017.1.1, Adobe Systems Inc., USA). Unfortunately, due to preservation issues.
we were unable to perform immunohistochemistry on these cephalic skin sections in *A. laevis*.

**Electron microscopy**

To view ultrastructure, skin samples were prepared for electron microscopy. Samples were post-fixed in 2% osmium tetroxide solution, then dehydrated in ascending series of ethanol and infiltrated in epoxy resin. Resin blocks were then polymerised overnight at 70 degrees Celsius. Semi-thin (1 µm) sections were cut and stained with toluidine blue to locate an individual scale *sensillum organ* under light microscopy. Ultra-thin (70 nm) sections were cut and stained with uranyl acetate and lead citrate. Sections were placed on nickel coated mesh grids and viewed at 100 kV under a transmission electron microscope (*Tecnai G2 Spirit TEM, FEI Company, USA*).

**Results**

Several epidermal layers were identified using light microscopy: the nucleated layer (*stratum germinativum*) was non-keratinised corneous and consisted of a basal layer of elongate or columnar cells and one to three layers of round, loosely arrange keratinocytes; the non-nucleated layer (*stratum corneum*) consisted of keratinised corneous α and β cells. According to definitions from (1,3), skin samples that were viewed under light microscopy (Table 1) were in the resting phase of epidermal shedding cycle; skin samples viewed under the electron microscope (Table 3.1) appeared to be in pre-renewal phase.

**Cephalic scale organs**

Observed under a stereomicroscope, the cephalic scale *sensilla organs* appeared as unpigmented external elevations (‘bumps’) of outer skin (Figure 1). Observed under light microscopy, the cephalic scale *sensilla organs* of *A. laevis* (Figure 2) and *H. stokesii* (Figure 3) shared a similar structure that consisted of a cluster of 9 to 11 cells (‘central cells’), which were horizontally arranged, originating in the dermis and evaginating the epidermis to create a dermal *capsulepapilla* (‘papilla’). The ratio of length to diameter of the dermal *capsulepapilla* was approximately 1:1 for both *A. laevis* and *H. stokesii* (Table S1).

The dermal *capsulepapilla* was occasionally tapered at its basal end in *H. stokesii* (Figure 3A), but this likely to be an artefact of tissue sectioning, remained expanded in *A. laevis* (Figure 2B, C). In some dermal *capsulepapillae* we were able to identify a blood vessel leading to (and thus presumably vascularising) the central cells (Figure 2B). In *H. stokesii*,
The Gomori’s One Step Gomori’s one step trichrome stain revealed collagen fibres interspersed between central cells and often separated the dermal capsule from keratinocytes within the epidermis (Figure 3C). In both species, the dermal capsule was approximately 50% thinner than the epidermis of the surrounding regions of skin that did not contain sensilla organs (17 µm; t = −11.16, 110 d.f., P < 0.001) and in H. stokesii, it was approximately 15% thinner than the adjacent flat epidermis (28 µm; t = −2.19, 67 d.f., P = 0.03).

There was a second type of dermal capsule on the cephalic scales in H. stokesii that contained approximately 10 central cells and displaced the surrounding epidermis but, in contrast to the cephalic scale sensilla organs, did not result in a distinctive bumps in the outer skin surface (Figure 3.4). These smaller scale sensilla organs were more variable in shape compared to typical sensilla organs (ratio length:diameter 1.7; Table S1) and often located at the base of depressions on the outer surface of the scale (Figure 4). The epidermis above the dermal capsule was 25% thinner than adjacent flat epidermis (25 µm, t = −2.76, 26 d.f., P = 0.01; approximately same height as cap cells in the epidermis above papilla of other cephalic sensilla organs, t = 0.85, 12 d.f., P = 0.41).

The cephalic dermis and epidermis of H. stokesii were immunoreactive for PGP9.5 (Figure 3.5). Specificity of immunoreactions were confirmed by antibody controls (Figure S1) and by the localised staining of nerve bundles that had previously been identified under light microscopy (Figure 2A; Figure 3A). Dermal axons travelled to the scale sensilla organs (Figure 5C), then meandered through the central dermal capsule before innervating the outer epidermis and often (presumably) terminating as distinct discoid endings in the alpha layer (Figure 5A, B). These discoid endings were primarily located above the dermal capsule, but were also present in flat epidermis that did not contain sensilla organs (Figure 6A). Unfortunately, the second type of dermal capsule in H. stokesii (described above; Figure 4) were not present in the sections stained for immunohistochemistry.

Immunoreactions were also localised to ovoid structures within the cephalic dermis of H. stokesii (Figure 3.6). These structures corresponded to lamellar cells that were ovoid in shape and resembled small Pacinian-like corpuscles (mean length 29 ± 15 µm and mean diameter of 22 ± 12 µm; Table S1). The location of these ‘lamellar corpuscles’ in H. stokesii...
ranged from 61 to 124 µm (mean 93 µm) depth from the basal layer of the epidermis. Lamellar corpuscles were also identified in the cephalic dermis of *A. laevis* that were similar ovoid shape (mean length 37 ± 26 µm and mean diameter 25 ± 5 µm; Figure 2C) to those found on the cephalic dermis of *H. stokesii*. The location of the lamellar corpuscles in *A. laevis* ranged from 53 to 168 µm (mean 118 µm; Table S1) depth from the basal layer of the epidermis. Although the lamellar corpuscles were dispersed throughout the dermis (stratum laxum), they were often subjacent to scale sensilla organs (Figure 2C; Figure 3B,C). Unfortunately, due to preservation issues we were unable to perform immunohistochemistry on these cephalic skin sections in *A. laevis*.

The dermal capsule papilla of a scale sensillum organ was observed in *A. laevis* using electron microscopy (Figure 7). High magnification images showed a cluster of central cells within the dermal capsule papilla (Figure 7B). These central cells were distinguished from surrounding keratinocytes by their round shape and lack of tonofilaments (Figure 7B inset two). Tonofilaments were present in the intracellular space of keratinocytes throughout the epidermis (Figure 7B). Tight junctions (desmosomes) and associated tonofibrils can be seen between central cells and keratinocytes (Figure 7B inset two). In the intercellular domain, small bundles of transverse collagen fibres and a single, small putative nerve axon were present at base of the dermal capsule papilla (closer to dermis; Figure 7B inset one). Small phospholipid inclusions were also present (Figure 7B inset one). Unfortunately, we were unable to image the putative axon at higher magnification so could not confirm the presence of neuronal elements (*e.g.* lamellar arrangement of Schwann cells, neurofilaments).

**Scale organs on the tail of *Aipysurus laevis***

Two scale structures were identified in the tail skin of *A. laevis*. Although, we were unable to discern bumps in the outer tail skin surface using a stereomicroscope (Figure 1B), several skin elevations were identified in cross-sections of the skin under light microscopy (Figure 8). The epidermal elevations of the tail (*tail scale sensilla organs*) lacked the dermal capsule papillae associated with the cephalic scale sensilla organs. The outer bumps were instead created by thickening of the epidermis (Figure 8A), which was 57% thicker than adjacent flat epidermis (47 µm, t = 14.18, 86 d.f., P < 0.001) and 17 µm (65%) thicker than epidermis above of cephalic scale sensilla organs (t = −14.26, 18 d.f., P < 0.001). Tail scale sensilla organs also lacked the collagen fibres and blood vessels that were associated with cephalic scale sensilla organs. A second scale structure identified in the tail skin of *A. laevis* consisted of a small dermal capsule papilla of approximately 10 central cells.
with a ratio of length and diameter of 1:1 (Table S1; Figure 8B). Although the dermal capapillation displaced the surrounding epidermal layer (including the columnar cells of the *stratum germinativum*) this did not result in elevations of the outer epidermis (Figure 6B). The epidermis above the dermal capapillation was 42% thinner than adjacent epidermis that did not contain dermal capapillae (11 µm; t = −4.65, 86 d.f., P < 0.001) and slightly thinner (5.5 µm) than the cap cell epidermis above of cephalic capapillae (t = 2.59, 18 d.f., P = 0.02). Subjacent to these tail dermal capapillae, collagen fibres in the dermis (*stratum laxum*) were dispersed and melanosomes could not be seen (Figure 8B). Unfortunately, due to preservation issues we were unable to perform immunohistochemistry on these tail sections.

Discussion

Cephalic scale organs

Scale capapillae and dermal capapillae

Previous work found that the cephalic capcapillae of sea snakes are substantially more protruding and often cover a larger proportion of the scale surface than the capcapillae of terrestrial hydrophiine snakes (7). The present study shows that, despite these differences, capcapillae in sea snakes have retained a similar underlying ultrastructure to their terrestrial counterparts. The capcapillae examined in *Aipysurus laevis* and *Hydrophis stokesii* are characterised by a dermal capapillation that consists of an aggregation of central cells with collagen fibres, blood vessels and nerve axons in the intercellular domain that together displace the surrounding epidermis (Figure 2-7). A similar underlying structure has been reported for the cephalic capapillae of ten terrestrial species representing the several major phylogenetic groups of snakes (agamids, xenopsids, iguanids, scolecophidians and colubroids, ) and lizards (agamids, iguanids and varanids) (10,21–23,42–45). In terrestrial snakes and sea snakes, the epidermis above the dermal capapillation is comprised of columnar keratinocytes (i.e. *stratum germinativum*) that form a layer that is 15% to 50% thinner than the epidermis of adjacent flat skin. The columnar keratinocytes above the dermal capapillation have been described as ‘cap cells’ in snakes and suggested to provide protection against abrasion or aid in transducing mechanosensory stimuli (22). We discovered that sea snake skin contained free nerve axons that extend from the dermis and terminate within the alpha layer (epidermis) as distinct discoid structures (Figure #5). In

Commented [JC3]: ‘Squamates’ as snakes and lizards are not monophyletic groups.
terrestrial colubroid snakes, these structures have variously been described as ‘discoid receptors’ (21), ‘end bulbs’ (20) and ‘button-like’ (10) nerve endings. In the sea snake skin, we found discoid receptors distributed throughout the epidermis, but aggregated above the dermal capsulepapilla (Figure 5A, B) deriving from axons at the base of the dermal capsulepapilla (Figure 3.5C). This adds evidence for a sensory function of scale sensillaorgans in sea snakes.

Our images from transmission electron microscopy provide the first high resolution ultrastructure data of a cephalic scale sensillum organ in a snake. Inspection of Figure 3.7B shows that the central cells within dermal capsulepapilla are clearly differentiated from surrounding keratinocytes by their lack of tonofilaments. Tonofilaments are keratin formations of keratin-like proteins that provide structural integrity to the squamate epidermis (46). Although lacking in tonofilaments, central cells maintain contact elements with surrounding keratinocytes via multiple tight junctions (desmosomes) (Figure 7B, inset two). A putative axon was also identified in the intercellular domain of the dermal capsulepapilla (Figure 7B, inset one), which may represent the ‘terminal receptors’ or myelinated axons previously identified in lizards (45). We did not find synaptic contacts between axons and central cells, which is consistent with light microscopy studies of other colubroid snakes (e.g. Elaphe) (23). Nevertheless, the presence of discoid receptors superior to the dermal papilla suggest that the and suggests that the central cells (and associated dermal capsule) have a functional role in transducing mechanical stimuli, have a structural role rather than functioning as a direct transducer of stimuli.

In addition to dermal capsulepapillae associated with cephalic scale sensillaorgans, we detected capsulepapillae typically (but not always) located at the bottom base of depressions in the outer skin in H. stokesii (Figure 4). These dermal capsulepapillae consisted of approximately 10 central cells (Figure 5) and displaced surrounding keratinocytes but, in contrast to the ultrastructure we describe for cephalic scale sensillaorgans, did not result in a skin elevation (bump). Putative nerve structures leading to the dermal capsulepapilla were identified using Gomori’s One Step Gomori’s one step trichrome stain under light microscopy (Figure 4B), but we were unable to conduct antibody staining of neuronal markers. It is unclear whether these dermal capsulepapillae are distinct scale structures or merely undeveloped or damaged scale sensillaorgans.

Lamellar corpuscles
We detected lamellated, ovoid cells in the deeper dermis of cephalic skin in both species examined and demonstrated that these lamellar corpuscles were neuronal-positive in *H. stokesii* (Figure 6). These structures resemble the ‘non-encapsulated lamellated receptors’ identified in other squamates such as *Boa, Elaphe, Iguana*, and *Agama* genera (11,12,21,47). These structures resemble the ‘non-encapsulated lamellated receptors’ identified in other squamates such as *Boa*, *Elaphe*, *Iguana*, and *Agama* genera (11,12,21,47). These structures are neuronal-positive in *H. stokesii* (Figure 6). These structures resemble the ‘non-encapsulated lamellated receptors’ identified in other squamates such as *Boa, Elaphe, Iguana*, and *Agama* genera (11,12,21,47). These structures are neuronal-positive in *H. stokesii*.

Pacinian corpuscles consist of connective tissue and fibroblasts lined by flat neuronal ‘Schwann’ cells; the lamellar structures identified in sea snakes tested immuno-positive for the neuronal maker PgP9.5 suggesting that these are indeed modified neuronal cells. The sensitivity of these receptors has not been targeted examined in previous electrophysiological tests of sea snakes skin.

**Ancestral and derived sensory functions for cephalic scale organs**

The ultrastructural features described above for cephalic scale organs of terrestrial and marine snakes represent all of the components of Meissner-like corpuscles. Meissner corpuscles are rapidly adapting LTMRs present in the dermal papillae of mammal glabrous skin (48). Electrophysiological experiments of cranial nerves in colubroid snakes found that they are rapidly adapting LTMRs with receptive fields that overlap with Meissner corpuscles (i.e. 12 mm² (17). Our finding that the cephalic scale organs of sea snakes share a very similar ultrastructure with their terrestrial relatives (and appear to lack novel or specialised cell types) provides evidence that marine lineages have retained the ancestral mechanosensory role for these organs.

The dome-shape and often high scale coverage of scale mechanoreceptors in sea snakes suggests divergent selection on these organs in marine environments, either for retained (ancestral) enhanced sensitivity to tactile stimuli or a derived sensitivity to hydrodynamic stimuli. Sea snakes forage in benthic habitats, frequently probing burrows and crevices as do terrestrial snakes on land (49), but there is no obvious reason why sea snakes should require a heightened tactile sense compared to terrestrial species. Sea snakes forage in benthic habitats, frequently probing burrows and crevices as do terrestrial snakes on land (51). It seems more likely that sea snakes have experienced selection pressures for sensitivity to hydrodynamic stimuli (7). Observations of the sea snake *Hydrophis (Pelamis) platurus* approaching and biting a vibrating object (50) provides some behavioural evidence that sea
snakes are responsive to hydrodynamic stimuli. Evoked potentials have been recorded from the midbrain of the sea snake *Hydrophis* (*Lapemis*) *curtus* in response to a vibrating sphere (50 to 200 Hz, peak sensitivity at 100 Hz), but no nervous response was successfully recorded directly from a scale *sensillum organ* of this species. However, more recently, auditory evoked potentials were recorded (from the midbrain) of *A. laevis* and *H. stokesii* in response to tone bursts from 40 to 600 Hz (peak sensitivity at 60 Hz) (Chapuis et al., unpublished data under review). These preliminary investigations showed that some species of sea snakes are capable of detecting low amplitude water motion, and/or particle motion caused by sound stimuli. Moreover, although these studies were not able to discern whether hydrodynamic stimuli were being received by mechanoreceptive scale organs in the skin or hair-cells in the inner ear, however, the peak sensitivities to the mechanical stimuli broadly overlap with peak sensitivities of Meissner (10 to 50 Hz) and Pacinian (200 to 300 Hz) corpuscles.

Hydrodynamic reception allows the detection of water motion, usually caused by water disturbances or animal movement, and is characterised by very low frequency components (peak at 10 Hz with a maximum of 50 Hz). This sensory ability has evolved repeatedly in aquatic organisms (e.g. the lateral line systems in fish, cephalopods and amphibians) wherein hydrodynamic stimuli are transduced by cutaneous mechanoreceptors (e.g. the lateral line systems in fish, cephalopods and amphibians). Cutaneous mechanoreceptors have also been co-opted for hydrodynamic reception in aquatically foraging animals, secondarily aquatic including mammals, a well-studied example of which are the vibrissae (whiskers) of pinnipeds (54–56), (e.g. star-nosed moles (58), platypus (59), birds (e.g. ducks, geese, ibis (60) and reptiles (61)).

Among snakes, two independently aquatic taxa (that are distantly related to hydrophiines) have evolved highly derived scale mechanoreceptors that putatively function to sense the water motions generated by the movement of prey. Tentacled snakes (*Erypeton tentaculum*) have the largest mechanoreceptors among vertebrates with two cephalic tentacles (measuring two to three 4 millimetres) and made up of dermis, epidermis and free nerve endings (34,57). Scale ‘sensilla’ in file snakes (*Acrochordus*) are thought to be sensitive to the hydrodynamic motion generated by the movement of fish prey. These small organs are vascularised like sea snake *sensilla organs* but instead of a dermal *capsule papilla* they consist of specialised epidermal cells that underlie highly-derived bristles that protrude from the skin (5,15). Tentacled snakes (*Erypeton tentaculum*) have the largest mechanoreceptors among vertebrates with two cephalic tentacles (2 to 3 millimetres) made up of dermis.
epidermis and free nerve endings (35,59). *Erpeton* and *Acrochordus* these snake lineages represent older aquatic transitions in snake phylogeny, and their mechanoreception is linked to specialised ambush predator strategies for hunting ambush fish prey in turbid freshwater habitats of low visibility (58). In contrast, sea snakes have recent marine origins and are an ecologically very diverse clade comprising species that variably occupy blue water reefs or turbid inshore habitats, are diurnal or nocturnal, and specialise on active or sedentary prey. The turtle-headed sea snake, *Emydocephalus annulatus*, is notable in having the second highest scale coverage of *sensilla scale organs* (3.8%) while being diurnally active and specialising on sessile fish eggs in clear water reefs (7,59). These results suggest a complex evolutionary history of scale organs and further research should aim to link selection pressures on hydrodynamic reception with particular ecologies (e.g. that optimal foraging strategies, water turbidity) may not be the primary selection pressure for hydrodynamic sense in among sea snakes.

**Tail scale organs**

Based on cellular morphology, there is a clear distinction between cephalic and posteriorly located scale *sensilla organs* in sea snakes. Scale *sensilla organs* present on the tail skin of *A. laevis* do not contain dermal *capsules papillae*; skin elevations are instead created by a thickening of the epidermis (Figure 8A). These structurally ‘simplified’ *sensilla structures scale organs* have been reported in studies of the body skin of the sea snake *E. annulatus* (37) and the tail skin of some terrestrial snakes (10). Many functional roles have been proposed for body scale *sensilla organs* in sea snakes, including mechanoreception, sex mate recognition, and enhanced friction for improved swimming performance, gripping and/or ecdysis (7,26,37). We were unable to stain for the presence of free nerve endings in tail scale *sensilla organs*, however, nerve staining of ‘supracloacal tubercles’ in the snakes *Thamnophis sirtalis* and *Nerodia rhombifer* (formally *Natrix rhombifera*) found that they were innervated in a similar pattern to cephalic scale *sensilla organs*, and thought to be important for sensory feedback to aid alignment of the cloacae position during copulation (10,60). Although these posteriorly located scale *sensilla organs* exhibit clearly ultrastructural differences compared to cephalic scale *sensilla organs*, it is by their ultrastructure and likely that they have a mechanoreceptive and/or structural function in sea snakes.
The ultrastructural differences in cephalic scale sensilla organs versus posteriorly located scale organs may reflect variation in mechanoreceptor sensitivity in the head compared to those on the rest of the body. Research in mammals suggests that the structure of the skin organ may be just as important as the neurons that carry the electrical impulse--collagen can provide physical tethering, structural integrity, or aid in propagating or modulating the sensation of force (25). Thus, the absence of a dermal capsule papilla for scale sensilla organs on the body and tail skin might indicate a less specialised mechanoreceptor with differential sensitivity compared to a cephalic mechanoreceptor. Thus, our results support previous studies on terrestrial snakes that suggest that the head of sea snakes is the prime exploratory organ for actively seeking mechanical stimulation (17,27). Future studies should investigate the neural pathways and compare electrophysiological responses underlying scale mechanoreceptors distributed on the head and body of snakes. Such efforts may discover that sea snakes possess specialised nerve pathways and/or responsive fields that are analogous to the cranial nerve canals of neuromasts in fish and amphibians, or the vibrissae of secondarily-aquatic systems in mammals (55,61), which would support a hydrodynamic function for cephalic scale organs.

Furthermore, cephalic cutaneous receptors are innervated by specialised cranial nerves (e.g. trigeminal ganglion), while the rest of the body is innervated by peripheral nerves of the spinal cord (i.e. dorsal root ganglion) (4). These neural pathways are thought to reflect differences in somatosensory processing wherein the head harbours specialised tactile receptors that are used to actively seek stimuli in the surrounding environment, in contrast to the body, which passively receives information (4,63). Thus, our results suggest that the head of sea snakes is the prime exploratory organ for actively seeking mechanical stimulation. Future studies should investigate the neural pathways and compare electrophysiological responses underlying scale mechanoreceptors distributed on the head and body of snakes.

Dermal photoreception and other cutaneous sensory modalities
The skin provides a primary interface for receiving multiple stimuli, creating an opportunity for multi-modal cutaneous receptors. Indeed, molecular and electrophysiological studies of ISOs in crocodiles indicate multi-modal sensitivity to mechanical stimuli and thermal and pH gradients (62,63). Dermal photoreceptors in the tail skin of *Aipysurus* sea snakes mediate phototactic behaviour in these species (36); we did not detect candidate photoreceptive structures (e.g. photoreceptors, stacked membranes) in the tail skin of *A. laevis*, but we did...
find structurally simplified scale organs (described above) and other small dermal papilla
(Figure 8B). Given that cutaneous receptors have been linked with both mechano- and
photoreception in amphibians (64) and marine invertebrates (65), these scale organs merit
further investigation for their putative role in photoreception. Our study (and previous studies
using electron microscopy (Chapter 2, Crowe Riddell et al., 2016; Povel and VanDerKooij,
1997) demonstrate that scale sensilla are devoid of pores and so a chemosensory
function is highly unlikely. Given that cutaneous receptors have been linked with both mechano-
and photoreception in amphibians (64) and marine invertebrates (65), these scale organs merit
further investigation for their putative role in photoreception. Our study (and previous studies
using electron microscopy (Chapter 2, Crowe Riddell et al., 2016; Povel and VanDerKooij,
1997) demonstrate that scale sensilla are devoid of pores and so a chemosensory
function is highly unlikely.

Several other sensory functions have been tentatively attributed to the scale organs of
sea snakes, but these currently lack supporting evidence. An electro-magneto-sense is plausible (5), but our histological sections do not show canals or pores that are indicative of
passive electrorceptors (e.g. ampullary-type organs) or specialised active electrorceptive
organs (e.g. tuberous organs or mormyromasts of weakly-electric fish) (66,67). Similarly, in
addition to previous studies using electron microscopy (5,7), our study demonstrates that
scale organs in sea snakes are devoid of pores and so a chemosensory function is highly
unlikely. Baroreception of the changes in air pressure that precede extreme weather events
has been attributed to sea kraits, which are an independently marine clade of hydrophiines;
however, it is unclear whether sea snakes react in a similar way and how cutaneous
mechanoreceptors might transduce pressure information in sea snakes or sea kraits. Salinity is
an important predictor of sea snake distribution (68) because many species require
access to freshwater for hydration (69–71), but pH receptors are more likely to be located in
papillae in the mouth (11,72). The thermal sensitivity of scale sensilla organs has been
investigated in Elaphe colubroid (Elaphe) snakes with results indicating, which found, that
although some cutaneous nerves are exclusively sensitive to heat, mechanoreceptive fibres
are not responsive to either heating or cooling (17,73). Dermal photoreceptors in the tail skin
of Aipysurus sea snakes mediate phototactic behaviour in these species. Although we did not
detect candidate photoreceptive structures (e.g. photoreceptors, lenses) in the tail skin of A.
laevis, we did find ‘simplified’ scale sensilla (described above) and other small dermal
capsule (Figure 8B). Given that cutaneous receptors have been linked with both mechano-
and photoreception in amphibians (69) and marine invertebrates (70), these scale organs
merit further investigation for their putative role in photoreception. Finally, these sensory
hypotheses do not exclude other non-sensory functions for scale organs, e.g. modifying
boundary layer of skin, so these roles should be considered in future studies in the scale
organs of sea snakes.

Finally, an electro-magneto-sense has been proposed for scale sensilla in snakes (5),

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but our understanding of the spatial ecology, and thus long-range navigation abilities, of sea snakes is limited.

Conclusions

Our study shows that the ultrastructure of cephalic sensilla scale organs of sea snakes closely resembles the mechanosensitive Meissner-like corpuscles that underlie the scale sensilla organs in terrestrial snakes. This provides evidence that the sensilla scale organs of marine hydrophiine lineages have retained an ancestral mechanosensory function. Our findings provide the basis for future research into the sensitivity of cutaneous receptors in sea snakes including mechan-, hydro- and photo-sensory modalities. Our study highlights that snakes are an important group for understanding the evolution of mechanoreception in vertebrates, particularly in response to shifting sensory landscapes.

Ethics statement: Animals were collected in accordance with the Western Australian Department of Biodiversity, Conservation and Attractions Department of Parks and Wildlife of Western Australia licence to take fauna for scientific purposes (Permit #SF010002). Euthanasia was carried out in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes [73], under Animal Ethics Committee protocols from the University of Adelaide (S-2015-119) and the University of Western Australia (RA/3/100/1369).

Data accessibility: Supplementary data are interactive digital scans of slides (ndp.view files) and skin measurements (.xlsx) for A. laevis and H. stokesii. These are available with the supplementary Figure S1s attached separately. Available at Figshare, DOI: 10.25909/5c5bb6777f249; https://figshare.com/s/f029519e4f6304b34565

Competing interests: The authors declare no competing interests.

Author contributions: J.M.C.-R. and K.L.S. conceived of the study. J.M.C.-R., L.C. and K.L.S collected samples, J.M.C.-R. and R.W. prepared samples for electron-carried out microscopy analysis and interpretation with input from L.C.; J.M.C.-R. and K.L.S wrote the manuscript. The manuscript was written by J.M.C.-R. with significant input from all co-authors, L.C. and R.W.

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Supplementary Material

Figures

**Figure S1** Primary and secondary antibody controls for PGP9.5 in taipan (*Oxyuranus scutellatus*) brain tissue and sea snake (*Hydrophis stokesii*) cephalic skin tissue.

Electronic files

**File ES1** Interactive digital scans of histology slides (ndp.view, zipped)

**File ES2** Skin measurements for *A. laevis* and *H. stokesii* (.xlsx).
Table 3.1. Taxonomy, life stage, museum accession or field numbers and sample size of two species of sea snakes (Hydrophiinae) used in this study. Time until last shed was deduced for captive specimens by the presence of shed skins. Tissue samples were collected from captive specimens for various microscopy analyses: stereomicroscopy (SM), light microscopy (LM), transmission electron microscopy (TEM) and immunohistochemistry (IHC). Museum specimens were sourced from the Western Australian Museum (WAM) and the Field Museum of National History, Chicago (FMNH).

| Genus          | Species       | Museum or Field numbers | Sex   | Life stage | Time to last shed (days) | Scale type & location          | Microscopy analyses |
|----------------|---------------|-------------------------|-------|------------|--------------------------|--------------------------------|---------------------|
| Aipysurus      | laevis        | WAMR17426               | M     | Adult      | 18                        | 6th supralabial (right side) | LM                  |
|                | laevis        | KLS0590                 | M     | Adult      | > 128                     | Nasal scale (right side)      | LM                  |
| Hydrophis      | stokesii      | HS270916                | M     | Adult      | 107-128                   | Nasal scale (right side)      | LM & IHC            |
|                | stokesii      | FMNH202826              | Unknown | Juvenile | Unknown                  | Gross morphology of skin      | TEM & SM            |

Figure 1. Gross morphology of the skin of sea snakes illustrating small, unpigmented scale organs (‘sensilla’). Line drawing of sea snake indicates sampling region of available skin sampled for this study: nasal scales from the head of A. laevis and H. stokesii, and supralabial scales from the head and caudal scales from the tail in A. laevis only. A) Gross morphology of scale organs on the nasal scale of A. laevis. B) Gross morphology of the caudal scales of A. laevis illustrating sparse scale organs. C) Gross morphology of scale organs on the nasal scale of H. stokesii. Stereomicroscope images were taken from museum specimens: A) WAMR174260 and C) FMNH202826. Scale bars represent 1 millimetre. Line drawing based on image of A. laevis from (74) and modified with permission.

Figure 2. Light micrographs of a transverse section of cephalic skin (supralabial scale) from Aipysurus laevis. A) Transverse section shows that scale organs (*) are skin elevations (bumps) created by dermal papillae (dental capsule), other features of the dermis are clearly visible including nerve bundles, blood vessels and collagen; note that the beta layer has artificially separated from alpha layer. B-C) Higher magnification of transverse section of scale organs (*) that show central cells within the dermal capillae, which displace the stratum germinativum of the epidermis; dermal capillae are vascularised by blood vessels; note the red blood cells (rbc), lamellar corpuscles (lc) and melanophores (m) within the dermis. Slides were stained with haematoxylin and eosin and magnified at A) ×5.5, B) ×20 and C) ×30.

Figure 3. Light micrographs of a transverse section of cephalic skin (nasal scale) from Hydrophis stokesii. A) Transverse section shows that scale organs (*) are skin elevations (bumps) created by dermal papillae (dental capsule), other features of the dermis are clearly visible including nerve bundles, blood vessels and collagen fibres, and hinge region of the scale. B) Higher magnification of transverse section of scale organs, the central cells within the dermal capillae displace the stratum germinativum of the epidermis. C) Transverse section of edge of scale shows a small bundle of collagen fibres.
surrounded by central cells. Note the lamellar corpuscles (lc) within the dermis. Slides were stained with
gomori’s one-step and magnified at A) \( \times 6.2 \), B) \( \times 22.8 \) and C) \( \times \).

**Figure 4.** Light micrographs of a transverse section of cephalic skin (nasal scale) of *Hydrophis stokesii* showing that dermal capsulapapillae are not associated with external skin elevations (bumps). A-B)

Central cells of a dermal capsulapapillae (*) displace surrounding stratum germinativum of the epidermis, but do not result in skin elevations. Nerve bundle are closely associated with base of the dermal capsulapapilla.

Slide was stained with Gomori’s one-step and magnified at A) \( \times 20 \) and B) \( \times 40 \).

**Figure 5.** Immuno-reactivity of a neuron specific protein (PGP9.5) on cephalic skin (nasal scale) of *Hydrophis stokesii*; reactive protein appears dark pink. A) Transverse cross-section of scale lamellae showing transverse section of s崭nillaeorgan (*) with neuronal-positive stain within the dermal capsulapapillae, as well as within the epidermis and alpha layer above the dermal capsulapapillae. Several neuronal-positive, discoid endings (arrow) are present within the stratum germinativum and alpha layers of the epidermis. Lamellar corpuscles (lc) within the dermis are also immuno-positive and can be distinguished from melanocytes (me) and dispersed melanophores (m), which have a dark brown colouration. B) Deeper cross-sections of scale lamellaeorgan showing neuronal-positive discoid endings (arrows). C) A trail of neuronal-positive stain (arrow heads) leading to a forming scale lamellaeorgan (*). Negative control was conducted by omitting primary antibody. Slides were counter stained with Harris hematoxylin and magnified at A) \( \times 30 \), B) \( \times 50 \), C) \( \times 50 \).

**Figure 6.** Immuno-reactivity of a neuron specific protein (PGP9.5) of lamellar corpuscles (lc) in the cephalic dermis (nasal scale) of *Hydrophis stokesii*. The location within the dermis, and co-localisation of immuno-staining with lamellar structures suggests that they are Pacinian-like corpuscles. A) Immuno-reactivity of PGP9.5, reactive protein appears dark pink, showing immuno-positive stain localised to lamellar corpuscles (lc) in the dermis and discoid endings (arrows) in the epidermis. These structures can be distinguished from melanocytes (me) and dispersed melanophores (m), which have a dark brown colouration. B) Transverse cross-sections of the skin showing structure of lamellar corpuscles and an associated blood vessel (bv) and nerve bundle (n). Slides were stained and magnified: A) Harris hematoxylin, \( \times 30 \), and B) Gomori’s one step trichrome, \( \times 50 \).

**Figure 7.** Light micrograph and transmission electron micrographs (TEM) of cross sections of cephalic scale lamellaeorgan in sea snakes. A) Transverse cross-section of scale lamellaeorgan (*) in *Aipysurus laevis* showing the dermal capsulapapillae within the epidermis. B) Higher magnification of dermal capsulapapillae (*) in *A. laevis*. First inset shows nuclei of central cells (c) and epidermal cells (keratinocytes; k), and collagen fibres (coll), a structure typically found within the dermis, in the intercellular domain of the dermal capsulapapillae. A putative myelinated axon (arrow heads) is present in the intercellular domain of the central cells; small phospholipid (p) inclusions are also present. Inset two shows intercellular junctions (desmosomes; d) at the membrane of central cells (c) and the keratinocytes (k). Note the fine keratin-like tonofilaments keratin filaments (tonofilaments; t), associated with the desmosomes and large aggregations of keratin-like filaments (tonofilaments; e) in the intracellular domain of the keratinocytes. Light micrograph slide was stained with hematoxylin-eosin and magnified at A) \( \times 34.1 \); TEM: B) \( \times 1900, 1a) \times 4800, 1b) \times 8800; 3a) \times 9300 and 3b) \( \times 18500 \).
Figure 8. Light micrographs of transverse cross-sections of tail skin (posterior caudal scales) of *Aipysurus laevis*. A) Scale sensilla organs (*) in the tail are skin elevations created by a thickening of underlying epidermis. B) Unknown dermal epidermal papilla (*) consists of central cells that displaces surrounding *stratum germinativum* of the epidermis, but does not result in skin elevations. Note that the dermis immediately underlying dermal epidermal papilla consists of loosely arranged collagen fibres devoid of melanophores (m).

Slides were stained with hematoxylin-eosin and magnified at A) ×16, B) ×17.2
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