

A phylogenomic rodent tree reveals the repeated evolution of masseter architectures

Mark T. Swanson, Carl H. Oliveros and Jacob A. Esselstyn

Article citation details

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Review timeline

Original submission: 9 January 2019

1st revised submission: 21 March 2019

2nd revised submission: 15 April 2019

Final acceptance: 16 April 2019

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

Review History

RSPB-2019-0066.R0 (Original submission)

Review form: Reviewer 1 (Philip Cox)

Recommendation

Accept with minor revision (please list in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This paper addresses the long-standing question in rodent morphology of how many times each of the masticatory muscle morphotypes has evolved across the order. It is very pleasing to see this question being explicitly addressed and I think this study will be of wide appeal, not only to those of us who work on rodents, but also researchers with interests in evolutionary convergence.

I should state at the outset of this review that I am not sufficiently expert to comment on the methodological details of DNA sequencing and phylogenetic reconstruction, so I will restrict my comments to the morphological aspects of this manuscript.

On the whole, I think this is a very interesting paper and I don't have any major concerns about the study or the conclusions that have been drawn. I think it's a real shame that you didn't include more glirids or bathyergids in your sample, but you have acknowledged and discussed this limitation. There are just some inaccurate details here and there, particularly in relation to the muscle anatomy, which lead me to suspect that the authors haven't quite grasped some of the finer nuances. Partly, this may be down to the utterly confusing double nomenclature that exists for rodent chewing muscles. If it's any help, I summarise the morphological and nomenclatural differences in Cox & Jeffery, 2011, *J Morph*, 294: 915-928.

Line 39: 'affects' should be 'effects'

Line 42: insert 'have' before 'evolved'

Line 50-54: It is stated here that modifications have increased the efficiency of gnawing (at the incisors), but don't forget about chewing (at the molars). Molar chewing becomes much more efficient when the ZM muscle passes through the infraorbital foramen (as shown in Cox, 2017, *PeerJ* 5: 3741).

Lines 56-59: You've mixed up the deep masseter and the ZM here. In sciuromorphy, it is the deep masseter that originates on the rostrum and runs to the mandible ventral to the zygomatic arch, whereas in hystricomorphy it is part of the ZM that originates on the rostrum and passes through the enlarged infraorbital foramen. You've mixed these up in figure 1 as well.

Line 57: I wouldn't describe the infraorbital foramen of sciuromorphs as 'reduced'. It's the usual

condition in mammals to have a small IO foramen that only has the IO nerve passing through it. Lines 59-61: I find your description of myomorphy a bit confusing, particularly 'the medial portion' – the medial portion of what? Myomorphy is best explained as the combination of sciuromorphy and hystricomorphy: the deep masseter has extended on to the rostrum underneath the zygomatic arch and the ZM has also extended on to the rostrum through an enlarged zygomatic arch.

Line 61: It's true that we did suggest that both gnawing and chewing efficiency are maximised in myomorphous rodents, but it might be useful context to note that we also suggested that sciuromorphy improves gnawing efficiency and hystricomorphy improves chewing efficiency – that's why myomorphy is good for both feeding activities.

Line 73: It would be helpful to note that the mountain beaver is found in Sciuromorpha alongside squirrels and dormice.

Lines 94-95: The deep masseter has never moved forward through the infraorbital foramen. In fact, I'm a bit confused by this sentence – what has four origins in crown rodents?

Line 164: If you're going to exclude direct transitions from protrogomorphy to myomorphy, then surely you should also exclude direct transitions from sciuromorphy to hystricomorphy and vice versa as these would also require two character state changes – the expansion of the ZM to the rostrum and the loss of the deep masseter from the rostrum or the reverse. Why were these possibilities not excluded in your model?

Line 252: Reference 34 is misplaced. It is about dormice and so should be a reference for the preceding sentence. Instead, here you may wish to reference Maier & Schrenk 1987 *Zeitschrift für Säugetierkunde* 52: 156-164.

Discussion overall: I think what's missing from the discussion is an acknowledgement that coding rodent muscles as sciuromorph, hystricomorph, myomorph or protrogomorph misses an awful lot of detail, and may group species together that aren't in fact that similar. For instance, whilst the bathyergid in your analysis, *Fukomys*, is technically hystricomorphous, it is only a very small part of the ZM that pushes through the IO foramen in this species (see Van Daele et al 2009 *Physiol Biochem Zool* 82: 40-50). This is a very different morphology to the enormous infraorbital portion of the ZM found in *Pedetes* for instance. I think a sentence or two acknowledging the variation present within the morphotypes would be a valuable addition here (see Hautier et al 2015 *Grade and clades among rodents: the promise of geometric morphometrics*. In: *Evolution of the Rodents* [eds Cox & Hautier]).

Figure 1. As mentioned above, you've mixed up the ZM and the deep masseter in this figure. It also misses the fact that the ZM muscle actually extends all the way along the medial surface of the zygomatic arch. What the diagram shows is technically only the infraorbital portion of the ZM. To be honest, as it's so central to your paper, I think you'd be better off putting the diagram of the four muscle morphotypes as a separate figure. At the moment, it's squashed up in the corner of your phylogeny and it's difficult to see properly. Make it bigger and label it clearly.

Philip Cox
University of York

Review form: Reviewer 2

Recommendation

Accept with minor revision (please list in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

The article addresses long-standing evidence that the masseter muscle patterns of rodents are convergently derived. A basic description of the masseteric states is given, both in the text and Fig. 1, but details on how species were assigned to one or another state were given. The focus of masseteric shift on gnawing functionality is perhaps misplaced, as Cox and others have argued that gnawing and chewing are directly affected by these shifts. It might help general readers to point out that when the incisors are occluded in gnawing, the cheekteeth are DISENGAGED for chewing, and vice versa.

Although the taxon sampling in the phylogenetic analysis is excellent, it is curious that the 3 sciuriforms (which include tree, flying and ground squirrels) should all be late Miocene derivatives of a single tribe. Otherwise, the tree looks good and consistent with other reconstructions (Blanga-Kanfi et al, Fabre et al, Upham et al).

The authors do not consider the implied homoplasy of character states inferred to be convergently derived. How heterogeneous are the morphological conditions assigned to hystricomorphy, etc? The chief novelty of the contribution seems to be in providing a minimum count (7, limited by taxon sampling; unsampled species might also demonstrate convergence) of character state transitions.

Review form: Reviewer 3

Recommendation

Reject – article is not of sufficient interest (we will consider a transfer to another journal)

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

Good

General interest: Is the paper of sufficient general interest?

Acceptable

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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Swanson and co-authors provide a new phylogenetic tree of rodents including all families and use this tree to explore the evolution of the masseter muscle architecture. The paper is generally well written and the analyses appear solid. I am not an expert on phylogenetic methods and as such am unable to comment on the actual tree and how it was built. However, I was somewhat disappointed by the lack of data on masseter muscle architecture. Nowhere in the paper do the authors describe where the data on masseter muscle architecture came from or whether it was critically evaluated. We now know that the classic categories of sciuromorphy, hystricomorphy and myomorphy are much more variable than previously described with many variations existing. As such an essential element of any analysis attempting to investigate the evolution of the architecture of the masseter muscle must first re-evaluate the anatomy of this muscle in all the taxa included in the analyses.

I also had some further comments:

How is the number of times a trait evolved indicative of the evolutionary success of a group? This is stated in the abstract but I do not understand the rationale for this statement.

The authors say that no one has quantified the forward shift of the masseter in rodents, but they themselves do also not quantify this.

An analysis of rate shifts associated with changes in the anatomy of the masseter muscle would have been an interesting additional analysis. Does the forward shift of the masseter result in an increase in the evolutionary rate in this trait? And does it lead to an increase in the diversification rate of the group?

Decision letter (RSPB-2019-0066.R0)

04-Mar-2019

Dear Mr Swanson:

I am writing to inform you that your manuscript RSPB-2019-0066 entitled "A phylogenomic rodent tree reveals the repeated evolution of masseter architectures" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance. Indeed, as the paper was close to complete rejection it is pivotal that you convince the 3rd referee much better if you do resubmit.

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

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

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

In your revision process, please take a second look at how open your science is; our policy is that all data involved with the study should be made openly accessible-- see: <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/>
Insufficient sharing of data can delay or even cause rejection of a paper.

To upload a resubmitted manuscript, log into <http://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor John Hutchinson, Editor

Associate Editor
Comments to Author:
Dear Dr Swanson,

Your manuscript has been reviewed by three leading scientists in the field, and while two reviewers have identified only minor points the third one has identified major weakness. Considering the comments of the third reviewer I cannot recommend the MS for publication.
Best wishes,
Roberto Feuda

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

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Philip Cox
University of York

Referee: 2

Comments to the Author(s)

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Author's Response to Decision Letter for (RSPB-2019-0066.R0)

See Appendix A.

RSPB-2019-0672.R0

Review form: Reviewer 1 (Philip Cox)

Recommendation

Accept with minor revision (please list in comments)

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

Good

General interest: Is the paper of sufficient general interest?

Good

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

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

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

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

I am satisfied that the authors have addressed all the issues that I raised when I first reviewed this manuscript. I now just have a few tiny typographical comments:

line 22: add 'extant' before 'mammalian species'

line 58: sentence doesn't quite read correctly - maybe 'the deep masseter extends underneath the zygomatic arch on to the rostrum'

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Philip Cox
University of York

Review form: Reviewer 2

Recommendation

Accept as is

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

Excellent

General interest: Is the paper of sufficient general interest?

Good

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

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

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Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

The authors have substantially clarified and improved the manuscript. It represents a fine contribution

Decision letter (RSPB-2019-0672.R0)

09-Apr-2019

Dear Mr Swanson

I am pleased to inform you that your manuscript RSPB-2019-0672 entitled "A phylogenomic rodent tree reveals the repeated evolution of masseter architectures" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

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

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

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- 1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".
- 2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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

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

5) Data accessibility section and data citation

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

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

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

NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (<http://datadryad.org/>) and have not already done so you can submit your data via this link

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

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

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

Sincerely,

Professor John R. Hutchinson, Editor
 Proceedings B
 mailto: proceedingsb@royalsociety.org

Associate Editor
 Comments to Author:
 Dear Mr Swanson:

Thank you for resubmitting you MS to Proceeding of the Royal Society B. I think that you have thoroughly addressed the previous comments. However, before I can recommend this for publication, some minor typographical changes are required.

Best wishes,
 Roberto Feuda

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

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Referee: 2

Comments to the Author(s).

The authors have substantially clarified and improved the manuscript. It represents a fine contribution

Author's Response to Decision Letter for (RSPB-2019-0672.R0)

See Appendix B.

Decision letter (RSPB-2019-0672.R1)

16-Apr-2019

Dear Mr Swanson

I am pleased to inform you that your manuscript entitled "A phylogenomic rodent tree reveals the repeated evolution of masseter architectures" has been accepted for publication in Proceedings B.

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

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

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Electronic supplementary material:

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

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

Sincerely,

Proceedings B

<mailto:proceedingsb@royalsociety.org>

Appendix A

Associate Editor

Comments to Author:

Dear Dr Swanson,

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Best wishes,

Roberto Feuda

Thank you very much for the constructive reviews. We have adopted the vast majority of the recommended changes including justifying the assignment of character states, clarifying the terminology, and acknowledging variation within character states. These modifications have greatly improved the quality of the manuscript and can be found in the tracked changes version below. We believe this paper will become a valuable contribution to the understanding of rodent phylogenetics and evolution and hope that you will find this revised manuscript suitable for publication in Proceedings B.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

This paper addresses the long-standing question in rodent morphology of how many times each of the masticatory muscle morphotypes has evolved across the order. It is very pleasing to see this question being explicitly addressed and I think this study will be of wide appeal, not only to those of us who work on rodents, but also researchers with interests in evolutionary convergence.

I should state at the outset of this review that I am not sufficiently expert to comment on the methodological details of DNA sequencing and phylogenetic reconstruction, so I will restrict my comments to the morphological aspects of this manuscript.

On the whole, I think this is a very interesting paper and I don't have any major concerns about the study or the conclusions that have been drawn. I think it's a real shame that you didn't include more glirids or bathyergids in your sample, but you have acknowledged and discussed this limitation. There are just some inaccurate details here and there, particularly in relation to the muscle anatomy, which lead me to suspect that the authors haven't quite grasped some of the finer nuances. Partly, this may be down to the utterly confusing double nomenclature that exists for rodent chewing muscles. If it's any help, I summarise the morphological and nomenclatural differences in Cox & Jeffery, 2011, J Morph, 294: 915-928.

Line 39: 'affects' should be 'effects'

We removed this word to clarify the general point.

Line 42: insert 'have' before 'evolved'

Done.

Line 50-54: It is stated here that modifications have increased the efficiency of gnawing (at the incisors), but don't forget about chewing (at the molars). Molar chewing becomes much more efficient when the ZM muscle passes through the infraorbital foramen (as shown in Cox, 2017, PeerJ 5: 3741).

We modified the first two paragraphs of the introduction to better incorporate chewing and molar related feeding activities.

Lines 56-59: You've mixed up the deep masseter and the ZM here. In sciuromorphy, it is the deep masseter that originates on the rostrum and runs to the mandible ventral to the zygomatic arch, whereas in hystricomorphy it is part of the ZM that originates on the rostrum and passes through the enlarged infraorbital foramen. You've mixed these up in figure 1 as well.

Fixed.

Line 57: I wouldn't describe the infraorbital foramen of sciuromorphs as 'reduced'. It's the usual condition in mammals to have a small IO foramen that only has the IO nerve passing through it.

We replaced 'reduced' with 'small' in the text to avoid implying directionality in the evolution of this character.

Lines 59-61: I find your description of myomorphy a bit confusing, particularly 'the medial portion' – the medial portion of what? Myomorphy is best explained as the combination of sciuromorphy and hystricomorphy: the deep masseter has extended on to the rostrum underneath the zygomatic arch and the ZM has also extended on to the rostrum through an enlarged zygomatic arch.

Thanks, we have modified the text to better describe myomorphy. See new lines 66-69.

Line 61: It's true that we did suggest that both gnawing and chewing efficiency are maximised in myomorphous rodents, but it might be useful context to note that we also suggested that sciuromorphy improves gnawing efficiency and hystricomorphy improves chewing efficiency – that's why myomorphy is good for both feeding activities.

Thanks for the suggestion. We have added this context.

Line 73: It would be helpful to note that the mountain beaver is found in Sciuromorpha alongside squirrels and dormice.

Added.

Lines 94-95: The deep masseter has never moved forward through the infraorbital foramen. In fact, I'm a bit confused by this sentence – what has four origins in crown rodents?

Our apologies, this was a result of our confusion over the proper terminology. We changed 'deep masseter' here to 'a portion of the zygomatico-mandibularis' to clarify that references 34 and 35 present speculation or hypotheses for the number of origins of hystricomorphy in crown rodents and Ctenohystrica respectively.

Line 164: If you're going to exclude direct transitions from protrogomorphy to myomorphy, then surely you should also exclude direct transitions from sciuromorphy to hystricomorphy and vice versa as these would also require two character state changes – the expansion of the ZM to the rostrum and the loss of the deep masseter from the rostrum or the reverse. Why were these possibilities not excluded in your model?

We felt it was unfair to exclude the possibility of direct transitions between sciuromorphy and hystricomorphy because Wood, in his 1965 Evolution paper (p. 120), suggested a close relationship between the sciuromorphic beaver and a hystricomorphous taxon. Although we allowed for the possibility of direct transitions between sciuromorphy and hystricomorphy and vice versa, the model did not infer any such events to have occurred. Therefore, adding this constraint would not have any effect on our results.

Line 252: Reference 34 is misplaced. It is about dormice and so should be a reference for the preceding sentence. Instead, here you may wish to reference Maier & Schrenk 1987 Zeitschrift fur Säugetierkunde 52: 156-164.

Done

Discussion overall: I think what's missing from the discussion is an acknowledgement that coding rodent muscles as sciuromorph, hystricomorph, myomorph or protrogomorph misses an awful lot of detail, and may group species together that aren't in fact that similar. For instance, whilst the bathyergid in your analysis, Fukomys, is technically hystricomorphous, it is only a very small part of the ZM that pushes through the IO foramen in this species (see Van Daele et al 2009 Physiol Biochem Zool 82: 40-50). This is a very different morphology to the enormous infraorbital portion of the ZM found in Pedetes for instance. I think a sentence or two acknowledging the variation present within the morphotypes would be a valuable addition here (see Hautier et al 2015 Grade and clades among rodents: the promise of geometric morphometrics. In: Evolution of the Rodents [eds Cox & Hautier]).

We added a paragraph to the discussion noting variation in the classic masseter morphotypes and that convergently derived character states might not be biomechanical equivalents.

Figure 1. As mentioned above, you've mixed up the ZM and the deep masseter in this figure. It also misses the fact that the ZM muscle actually extends all the way along the medial surface of the zygomatic arch. What the diagram shows is technically only the infraorbital portion of the ZM. To be honest, as it's so central to your paper, I think you'd be better off putting the diagram of the four muscle morphotypes as a separate figure. At the moment, it's squashed up in the corner of your phylogeny and it's difficult to see properly. Make it bigger and label it clearly.

We made the masseter illustrations a separate figure and relabeled the muscle highlighted in blue as the "Infraorbital portion of the zygomatico-mandibularis".

*Philip Cox
University of York*

Referee: 2

Comments to the Author(s)

The article addresses long-standing evidence that the masseter muscle patterns of rodents are convergently derived. A basic description of the masseteric states is given, both in the text and Fig. 1, but details on how species were assigned to one or another state were given. The focus of masseteric shift on gnawing functionality is perhaps misplaced, as Cox and others have argued that gnawing and chewing are directly affected by these shifts. It might help general readers to point out that when the incisors are occluded in gnawing, the cheekteeth are DISENGAGED for chewing, and vice versa.

Thank you for the suggestion. We added text to the methods section and an appendix which clarifies the literature sources we used to assign character states. We also incorporated more information on chewing into the introduction and noted the molars are disengaged during gnawing and vice versa. The added context should improve the appeal of our manuscript to the general reader.

Although the taxon sampling in the phylogenetic analysis is excellent, it is curious that the 3 sciuriforms (which include tree, flying and ground squirrels) should all be late Miocene derivatives of a single tribe. Otherwise, the tree looks good and consistent with other reconstructions (Blanga-Kanfi et al, Fabre et al, Upham et al).

The authors do not consider the implied homoplasy of character states inferred to be convergently derived. How heterogeneous are the morphological conditions assigned to hystricomorphy, etc? The chief novelty of the contribution seems to be in providing a minimum count (7, limited by taxon sampling; unsampled species might also demonstrate convergence) of character state transitions.

This is a good point, also noted by the other Referees, and we added a paragraph in the discussion to address variation within and across convergently derived origins of hystricomorphy.

Referee: 3

Comments to the Author(s)

Swanson and co-authors provide a new phylogenetic tree of rodents including all families and use this tree to explore the evolution of the masseter muscle architecture. The paper is generally well written and the analyses appear solid. I am not an expert on phylogenetic methods and as such am unable to comment on the actual tree and how it was built. However, I was somewhat disappointed by the lack of data on masseter muscle architecture. Nowhere in the paper do the authors describe where the data on masseter muscle architecture came from or whether it was critically evaluated. We now know that the classic categories of sciuromorphy, hystricomorphy and myomorphy are much more variable than previously described with many variations existing. As such an essential element of any analysis attempting to investigate the evolution of the architecture of the masseter muscle must first re-evaluate the anatomy of this muscle in all the taxa included in the analyses.

We followed tradition in the literature and used classically defined masseter morphotypes because a lack of confidence in relationships within Rodentia rendered knowledge of the number of independent origins of these morphotypes inaccessible. For instance, it was previously uncertain whether Dipodoidea and Anomaluroomorpha are descendants of the same or independent transitions from protrogomorphy to hystricomorphy. Our results will facilitate further research into morphological and biomechanical variation across independent origins of these morphotypes.

We have added text in the methods section to identify the literature sources we used to assign the classic masseter morphology character states to each species and that we examined skulls of specimens from each family to confirm the assignment of those character states. This work allowed us to assess the variation in masseter morphology among species described as protrogomorphous, sciuromorphous, hystricomorphous, and myomorphous. For example, while *Fukomys damarensis* and *Pedetes capensis* represent extremes in the size of the infraorbital portion of the zygomatico-mandibularis, while all other hystricomorphous taxa are intermediates. Despite this variation, we chose to assign the classic masseter states in an effort to be objective, to keep the number of states manageable, and to provide the framework needed for future investigations of the morphological variation within each of these categories. As noted above, we have added text in the discussion acknowledging the variation in classic masseter morphotypes.

I also had some further comments:

How is the number of times a trait evolved indicative of the evolutionary success of a group? This is stated in the abstract but I do not understand the rationale for this statement.

Good point. This was unclear in the submitted draft. We have edited the first sentence of the abstract to explain that understanding the number of times a trait has evolved is necessary to determine if the trait has contributed to evolutionary success by fostering diversification.

The authors say that no one has quantified the forward shift of the masseter in

rodents, but they themselves do also not quantify this.

We were interested in the number of times the origin of the masseter muscle has shifted forward over the course of rodent evolution. We state in the abstract and final paragraph of the discussion that this has occurred at least 7 times with 1 reversal from hystricomorphy to protrogomorphy. Quantifying the degree to which the masseter has shifted forward across all of rodent diversity is a much more complicated question. Future investigations of this issue will be aided by the framework our study provides.

An analysis of rate shifts associated with changes in the anatomy of the masseter muscle would have been an interesting additional analysis. Does the forward shift of the masseter result in an increase in the evolutionary rate in this trait ? And does it lead to an increase in the diversification rate of the group?

While the taxon sampling in our study is too sparse to attempt an analysis of rate shifts, recent species-rich analyses of Rodentia (Fabre et al. 2012) and Mammalia (Upham et al. 2019) did not identify any rate shifts at nodes with character state changes. We added text to reflect this point.

Appendix B

Comments to Author:

Dear Mr Swanson:

Thank you for resubmitting you MS to Proceeding of the Royal Society B. I think that you have thoroughly addressed the previous comments. However, before I can recommend this for publication, some minor typographical changes are required.

*Best wishes,
Roberto Feuda*

Thank you for bringing these errors to our attention. We have adopted Referee 1's recommended changes and uploaded our alignments and trees to Dryad. We are grateful for your efforts hope you will find this revised manuscript suitable for publication in Proceedings B.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

I am satisfied that the authors have addressed all the issues that I raised when I first reviewed this manuscript. I now just have a few tiny typographical comments:

line 22: add 'extant' before 'mammalian species'

Done.

line 58: sentence doesn't quite read correctly - maybe 'the deep masseter extends underneath the zygomatic arch on to the rostrum'

We rephrased the sentence as suggested.

line 74: hystricomorphy also evolved at least twice as well as sciuromorphy and myomorphy (in fact, three times - in Ctenohystrica, Anomaluroomorpha and Dipodidae)

We have removed this part of the sentence since it is redundant with the beginning.

*Philip Cox
University of York*

Referee: 2

Comments to the Author(s).

The authors have substantially clarified and improved the manuscript. It represents a fine contribution