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Architectural instability, inverted skews and mitochondrial phylogenomics of Isopoda: outgroup choice affects the long-branch attraction artefacts

Hong Zou, Ivan Jakovlić, Dong Zhang, Cong-Jie Hua, Rong Chen, Wen-Xiang Li, Ming Li and Gui-Tang Wang

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R. Soc. open sci. **7**: 191887.
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Review timeline

Original submission:	13 November 2019	Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.
Revised submission:	9 January 2020	
Final acceptance:	14 January 2020	

Review History

RSOS-191887.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

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

Comments to the Author(s)
Zou et al. sequenced the complete mitogenome of Asotana magnifica, and conducted comparative mitogenomic architecture analyses, with focus on skew patterns, and conducted phylogenetic analyses using several different datasets. The work on mitochondrial skews description is thorough, and the paper is well written.
Some minor points the author should address in the revised version are listed as below.

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Page 4 Line 50: What is"ORI"？

Page 10 Line 32: Here you use "only 12 non-coding regions", but before and after the article did not explain clearly, how many non-coding areas are considered normal?

Page 13 Line 42-48: "As mitochondrial genes……the third codon position (synonymous mutations)". It's better to have the reference behind it.

Review form: Reviewer 2 (Jianmei An)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
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Recommendation?
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The manuscript is perfect and suitable for publication in your journal. But there are minor revision need to be done.
1. Page 4, Line 3-39; The known exception is not only one species, but two species, They are Asellus aquaticus (GC-Skew=-0.122) and Janira maculosa (GC-Skew=-0.026);
2. Page 17, Line 59; Reference 63 is 2018; should use the newest literature (2019).

Decision letter (RSOS-191887.R0)

02-Jan-2020

Dear Dr Jakovlic

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-191887 entitled "Architectural instability, inverted skews, and mitochondrial phylogenomics of Isopoda: outgroup choice affects the long-branch attraction artefacts" 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:
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- 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.

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Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 11-Jan-2020. 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.

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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:

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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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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 David Ferrier (Associate Editor) and Kevin Padian (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author (Dr David Ferrier):

Both reviewers have made a handful of suggestions for some minor revisions, which should be accommodated in order to make this manuscript acceptable for publication.

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
Zou et al. sequenced the complete mitogenome of Asotana magnifica, and conducted comparative mitogenomic architecture analyses, with focus on skew patterns, and conducted phylogenetic analyses using several different datasets. The work on mitochondrial skews description is thorough, and the paper is well written.
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Reviewer: 2

Comments to the Author(s)
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2. Page 17, Line 59; Reference 63 is 2018; should use the newest literature (2019).

Author's Response to Decision Letter for (RSOS-191887.R0)

See Appendix A.

Decision letter (RSOS-191887.R1)

14-Jan-2020

Dear Dr Jakovlic,

It is a pleasure to accept your manuscript entitled "Architectural instability, inverted skews, and mitochondrial phylogenomics of Isopoda: outgroup choice affects the long-branch attraction artefacts" in its current form for publication in Royal Society Open Science.

Please ensure that you send to the editorial office an editable version of your accepted manuscript, and individual files for each figure and table included in your manuscript. You can send these in a zip folder if more convenient. Failure to provide these files may delay the processing of your proof. You may disregard this request if you have already provided these files to the editorial office.

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Thank you for your fine contribution. 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
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on behalf of Dr David Ferrier (Associate Editor) and Kevin Padian (Subject Editor)
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Appendix A

Associate Editor Comments to Author (Dr David Ferrier):

Both reviewers have made a handful of suggestions for some minor revisions, which should be accommodated in order to make this manuscript acceptable for publication.

R: We appreciate the time and expertise that you and reviewers invested into our manuscript. The comments and critiques were constructive and helpful. We addressed all problems as best as the available literature allowed us. We also proofread the entire manuscript once again and made a few changes in places where we felt that there is room for improved clarity. All changes can be reviewed in the 'track-change' version of the manuscript.

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
Zou et al. sequenced the complete mitogenome of Asotana magnifica, and conducted comparative mitogenomic architecture analyses, with focus on skew patterns, and conducted phylogenetic analyses using several different datasets. The work on mitochondrial skews description is thorough, and the paper is well written.
Some minor points the author should address in the revised version are listed as below.

Page 4 Line10-11: you say "different datasets……often producing starkly contradictory phylogenetic hypotheses". It's better to have the reference behind it.

R: We discuss specific problems in the text immediately following the statement, and support it with a large number of references. Following your objection, we added three references that present a partial, but relatively good introduction into the multitude of phylogenetic hypotheses and topologies produced by different studies: "…often producing starkly contradictory phylogenetic hypotheses [1–3]."

Page 4 Line 18: "This is to", how does "this" refer to? In this section, you use many "this" word. If used incorrectly, it can confuse the reader.

R: Following your comment, we edited the entire section for clarity.

Page 4 Line 50: What is"ORI"？

R: We apologise for this mistake. We initially used ORI (origin of replication inversion) abbreviation, but then decided to change it to ROI (replication origin inversion) later. We fixed it.

Page 10 Line 32: Here you use "only 12 non-coding regions", but before and after the article did not explain clearly, how many non-coding areas are considered normal?

R: Unlike the nuclear genome, which is mostly comprised of non-coding DNA, mitogenomes are usually highly compact, with very little non-coding DNA [4,5]. With 36-37 genes as the standard gene number, theoretically there could be 36-37 intergenic regions. The existence of only 12 intergenic regions therefore indicates that there are no intergenic regions between 2/3 of adjacent genes. However, some lineages possess mitogenomes comprised mostly of noncoding DNA, with much larger numbers of intergenic spaces. For example, many nonbilaterian lineages possess large noncoding regions comprised of repetitive elements [6]. Expansion of noncoding regions has also been observed in isolated metazoan lineages, such as some nematodes, and even vertebrates [7–9]. Unfortunately, we could not identify a comprehensive review of the number of NCRs (nor overlapping genes) in metazoan mitogenomes, so many of these observations stem from our own studies of mitogenomes of invertebrates. Therefore, we added several references where suitable studies are available, and elsewhere modified the text in a way to avoid reliance on our personal observations: "As standard for mitogenomes of most animals [10], including isopods [11,12], the mitogenome of *A. magnifica* is also highly compact: we identified 12 non-coding regions (NCR; also referred to as intergenic spacers), only four of which were larger than 10 bp, and 14 gene overlaps. Although exceptionally large overlaps were identified in mitogenomes of some isopod lineages [13], there is no indication of such overlaps in *A. magnifica*. The largest (both 7bp) were found between *atp6*/*8* and *nad4*/*4L* genes, but the overlap between these genes is conserved in many metazoan lineages [14], including the isopods [12]."

In the last paragraph of this section, where we discuss rearrangement mechanisms, we added a few references that exemplify mitogenomes with highly expanded NCRs (in number and size). We also added a bit of discussion to explain better why this is unusual and important, and why we can use this to reject the TDLR mechanism: "The high compactness of isopod mitogenomes is very intriguing in the light of the rapid gene order evolution in this lineage. Often, taxa that exhibit high rates of mitochondrial architecture evolution also exhibit multiple expanded non-coding regions, sometimes spanning hundreds and even thousands of bases [7–9]. In some cases, these are believed to be a consequence of the tandem-duplication-random-loss (TDRL) rearrangement mechanism, wherein pseudogenes and non-coding DNA are generated and then again lost over the evolutionary time [9,10,15–17]. Therefore, high number of gene order rearrangements between closely related taxa and high compactness of their mitogenomes strongly suggest that TDRL mechanism is not the most parsimonious explanation for the high rate of gene order rearrangements in isopods. "

Page 13 Line 42-48: "As mitochondrial genes……the third codon position (synonymous mutations)". It's better to have the reference behind it.

R: We added a reference for each part of the sentence, and moved it a bit downwards in the paragraph, as it is a bit better logical fit: "As mitochondrial genes generally evolve under a strong purifying selection [18], nonadaptive hydrolytic deamination of bases generally has far more pronounced impact on the third codon position (synonymous mutations) of protein coding genes than on the first two codon positions [19]."

Reviewer: 2

Comments to the Author(s)
The manuscript is perfect and suitable for publication in your journal. But there are minor revision need to be done.
1. Page 4, Line 3-39; The known exception is not only one species, but two species, They are Asellus aquaticus (GC-Skew=-0.122) and Janira maculosa (GC-Skew=-0.026);

R: Initially we chose not to mention *Janira maculosa* as it is incomplete (<10,000 bp), but we can infer with some confidence that the entire Asellota clade exhibits negative skews, so we followed your advice and mentioned both species in the corrected manuscript.

2. Page 17, Line 59; Reference 63 is 2018; should use the newest literature (2019).

R: We updated the reference in the corrected version according to the instructions at the WORMS database (i.e., we refer specifically to the Isopoda web page).

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3. Wilson GDF. 2009 The phylogenetic position of the Isopoda in the Peracarida (Crustacea: Malacostraca). *Arthropod Syst. Phylogeny* **67**, 159–198.

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