InCoB2013 <incob2013@easychair.org> Jun 22

to me

Dear Devendra Biswal

We have evaluated all the comments received and concluded that your paper

Submission: 81
Title: An integrated pipeline for next-generation sequencing and annotation of the complete mitochondrial genome of the giant intestinal fluke, Fasciolopsis buski (Lankester, 1857) Looss, 1899 (Digenea: Fasciolidae).

requires substantial revisions (for details see reviewers' comments) with regards your methodology that will most likely require additional experiments before it might be suitable for publication in BMC Genomics InCoB2013 Supplement Issue. If you are willing to carry out these revisions you are requested to submit a revised version by July 13. The revised version will undergo a second review before a 'accept' or 'reject' decision is reached.

1) Adhere to BMC authors' guidelines to ensure that the manuscript is accurate, complete, and optimally formatted. Upload the manuscript as one (1) PDF file containing text plus tables and figures. Changes in text should be visible in red color (use in Word under "Review" functions the "compare two versions of document" option)

The attachment (1 zip-compressed folder) should contain:

Word file of revised manuscript
Figures as separate files (format see below)
Response letter (PDF)
Supplementary files, if applicable

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2) Figures

Each figure should include a single illustration and should fit on a single page in portrait format. If a figure consists of separate parts, it is important that a single composite illustration file be submitted which contains all parts of the figure.

Please read our figure preparation guidelines for detailed instructions on maximising the quality of your figures.
(http://www.biomedcentral.com/ifora/figures)

Formats

The following file formats can be accepted:

PDF (preferred format for diagrams)
DOCX/DOC (single page only)
PPTX/PPT (single slide only)
EPS
PNG (preferred format for photos or images)
TIFF
JPEG
BMP

Figure legends

The legends should be included in the main manuscript text file at the end of the document, rather than being a part of the figure file. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals - i.e. Figure 1, 2, 3 etc); short title of figure (maximum 15 words); detailed legend, up to 300 words.

Regards,

InCoB2013 Publication Co-chairs
Christian Schoenbach, Shoba Ranganathan and Bairong Shen

---------- REVIEW 1 ----------
PAPER: 81
TITLE: An integrated pipeline for next-generation sequencing and annotation of the complete mitochondrial genome of the giant intestinal fluke, Fasciolopsis buski (Lankester, 1857) Looss, 1899 (Digenea: Fasciolidae).
AUTHORS: Devendra Biswal, Sudip Ghatani, Jollene Shylla, Ranjana Sahu, Nandita Mullapudi, Alok Bhattacharya and Veena Tandon

OVERALL EVALUATION: 2 (accept)
REVIEWER'S CONFIDENCE: 3 (medium)

---------- REVIEW ----------
The article by Biswal et al details the process used to sequence the mt genome of F. buski and the associated findings. This article is appropriate for BMC Genomics.

I have several comments on the article:

1. It would benefit from being corrected for English and typos.
2. Figure and Table legends are minimal, and do not provide all the details required to understand them. e.g Figure 4 - what does the horizontal axis represent? i.e scale. Plus details of the coloured boxes is not given in the legend, only in the text.
3. I don't think the table numbering in the text matches the actual tables, e.g. nucleotide composition across species doesn't appear in Table 1 (see page 11 in text). The authors should consider the use of supplementary tables for some of their results tables.
4. Reference 12 appears to be incomplete.
5. Given the primer design and sequencing strategy, is it really surprising that the mt genome of F. buski is "almost" similar to that of F. hepatica? How can we be sure that this isn't an artefact of design but really is the biological truth?

---------- REVIEW 2 ----------
PAPER: 81
TITLE: An integrated pipeline for next-generation sequencing and annotation of the complete mitochondrial genome of the giant intestinal fluke, Fasciolopsis buski (Lankester, 1857) Looss, 1899 (Digenea: Fasciolidae).
AUTHORS: Devendra Biswal, Sudip Ghatani, Jollene Shylla, Ranjana Sahu, Nandita Mullapudi, Alok Bhattacharya and Veena Tandon

OVERALL EVALUATION: -2 (reject)
REVIEWER'S CONFIDENCE: 3 (medium)

---------- REVIEW ----------
The authors make use of data that are not yet publicly available (F buski WGS not published so far). If submission #81 is published, interested parties would not be able to reproduce the authors' work. In addition, the authors should make ALL data publicly availabe, deposit it an an approptiate repository and obtain accession numbers and/or provide data sets as additional files.

Primer design/PCR: based on the authors's writing this reviewer is not convinced of the results. It

seems the entire results are based on one (1) DNA sample (FD-2) without appropriate replicates.

Sanger-sequncing cofirmed region: specify in the manuscript text which region was confirmed. Why only one region and not two regions from replicate samples.

- novelty/originality: yes

- importance to field: limited

- appropriatness for this journal: yes

- sound methodology: partially

- quality of data or experimental results: partially

- support of discussion/conclusions by results: partially

- references to prior work: yes

- length, organization and clarity (language): no; the manucript requires major editing

- quality of display items: partially acceptable; manuscript appeared to be prepared in a hurry; the majority of tables would suit additional data (supplement) but do not fit as display items in the manuscript. It would help if the authors glean from similar published papers what has been shown and how it was displayed.

- compliance with standards (e.g. MIAME) etc.(if applicable): partially

- accessibility of data/software/websites: partially

Response to reviewers' comments on Paper 81 submitted to BMC Genomics through INCOB 2013 easychair

Dear Sir

Re: Submission Paper 81

Title: **An integrated pipeline for next-generation sequencing and annotation of the complete mitochondrial genome of the giant intestinal fluke, Fasciolopsis buski (Lankester, 1857) Looss, 1899 (Digenea: Fasciolidae)**

Please find attached a revised version of our manuscript "An integrated pipeline for next-generation sequencing and annotation of the complete mitochondrial genome of the giant intestinal fluke, Fasciolopsis buski (Lankester, 1857) Looss, 1899 (Digenea: Fasciolidae).

The attachment includes Word file and also a pdf file of revised manuscript with tables and figures

- Figures as separate files
- Response letter (PDF)

We would like to thank the reviewers for their time and their valuable comments.

The reviewers' comments were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following lines are our point-by-point responses to each of the comments of the reviewers.

Response to comments of reviewers

Reviewer 1

1. It would benefit from being corrected for English and typos.

Response: The manuscript is revised with the help of a language expert addressing typo and grammatical errors.

2. Figure and Table legends are minimal, and do not provide all the details required to understand them. e.g Figure 4 - what does the horizontal axis represent? i.e scale. Plus details of the coloured boxes is not given in the legend, only in the text.

Response: Figure legends have been enhanced with appropriate modifications.

3. I don't think the table numbering in the text matches the actual tables, e.g. nucleotide composition across species doesn't appear in Table 1 (see page 11 in text). The authors should consider the use of supplementary tables for some of their results tables.

Response: Tables have been arranged properly with correct numbering throughout the text.

4. Reference 12 appears to be incomplete.

Response: reference 12 is corrected as detailed below:

Jex AR, Littlewood DTJ, Gasser RB: Toward next-generation sequencing of mitochondrial genomes—focus on parasitic worms of animals and biotechnological implications. Biotechnol. Adv 2010, 28:151-159.

5. Given the primer design and sequencing strategy, is it really surprising that the mt genome of F. buski is "almost" similar to that of F. hepatica? How can we be sure that this isn't an artefact of design but really is the biological truth?

The results indeed are really surprising as Fasciola hepatica is a common liver fluke, while F. buski is an intestinal fluke. However, the outcome is not an artifact of design as we went for Sanger validation of another biological replicate for the two regions, which we had validated for the original sample; this has been elaborated in the revised manuscript. Besides, F. buski and Fasciola hepatica belong to the same family (Fascioloidae) and hence, a striking similarity may not be ruled out.

Reviewer 2:

Query: The authors make use of data that are not yet publicly available (F buski WGS not published so far). If submission #81 is published, interested parties would not be able to reproduce the authors' work. In addition, the authors should make ALL data publicly availabe, deposit it an an approptiate repository and obtain accession numbers and/or provide data sets as additional files.

Response: As suggested, raw data were uploaded for the mtDNA seq part (Illumina FastQ files for now) to SRA. The data pertaining to this study is available in the National Centre for Biotechnology Information (NCBI) Bioproject database with Accession: ***PRJNA210017 and ID: 210017****. The contig assembly files are deposited in NCBI Sequence Read Archive (SRA) with* ***Accession: SRR924085****.*

Query: Primer design/PCR: based on the authors' writing this reviewer is not convinced of the results. It seems the entire results are based on one (1) DNA sample (FD-2) without appropriate replicates.

Response: Typically NGS experiments being cost-prohibitive are conducted on single specimens. Validation (of a subset) is done on replicates. But as per the reviewer's

suggestions we are happy to inform you that we confirmed the findings by carrying out experiments on another reported whole genomic DNA from an independent F. buski sample (Sample FD3). Sanger sequencing was performed on two separate regions SAMPLE FD3-Region C24-C26 and SAMPLE FD3-Region C2-C16 as described in the manuscript. Two separate regions from two independent biological samples showed 98-99% identity.

Query: Sanger-sequencing confirmed region: specify in the manuscript text which region was confirmed. Why only one region and not two regions from replicate samples.

Response: To confirm our findings reported whole genomic DNA from an independent F. buski sample replicate (Sample FD3) was used and Sanger sequencing was performed on two separate regions (Sample FD3-Region C24-C26 and Sample FD3-Region C2-C16) as described above.

Query: manuscript appeared to be prepared in a hurry; the majority of tables would suit additional data (supplement) but do not fit as display items in the manuscript. It would help if the authors glean from similar published papers what has been shown and how it was displayed.

Response: The manuscript is greatly enhanced with error corrections and proper display of figures and tables throughout the manuscript taking cue from other publications on similar notes.

We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in BMC Genomics.

We shall look forward to hearing from you in a positive note at your earliest convenience.

Sincerely,

Veena Tandon

and Alok Bhattacharya

(Corresponding authors)