

Opponent's review on the thesis

Reviewer: Mgr. Jan Pačes, Ph. D.

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Title: Genome assembly of Mechow's mole-rat (*Fukomys mechowii*) as a prerequisite for the analysis of transposon expression in mole-rat oocytes

Author: Bc. Nikolas Tolar

Supervisor: Dr. Lenka Gahurova, Ph.D.

I had the privilege of reviewing the thesis titled "Genome Assembly of Mechow's Mole-Rat (*Fukomys mechowii*) as a Prerequisite for the Analysis of Transposon Expression in Mole-Rat Oocytes" authored by Bc. Nikolas Tolar under the guidance of Dr. Lenka Gahurova, Ph.D. I am pleased to provide a positive review of this exceptional work.

Tolar's thesis addresses an important gap in the field of mole-rat genomics and transposon biology. The study successfully accomplished the challenging task of assembling the genome of Mechow's mole-rat using a hybrid approach that combines long ONT reads and short Illumina reads. This achievement highlights Tolar's expertise in genomics and bioinformatics, as well as their ability to handle complex datasets and overcome technical challenges.

The introduction is well written and demonstrates excellent readability, effectively engaging the reader by providing a clear and concise overview of the research objectives, the significance of the study, and the relevant background information.

The thesis also provides valuable insights into transposon expression in mole-rat oocytes, a previously unexplored area of research. Tolar's analysis of transposon expression levels in oocytes from various mammalian species, including mice, rats, and mole-rats, is thorough and well-executed. The findings challenge existing hypotheses by demonstrating that mole-rat oocytes do not exhibit reduced transposon activity compared to other species, except for mice and rats. These results open new avenues for understanding the unique characteristics of transposon biology in mole-rats.

One notable strength of Tolar's work is the comprehensive annotation of transposons and genes within the assembled genome. The inclusion of repeat and preliminary gene annotations adds significant value to the research community by providing a valuable resource for future studies on Mechow's mole-rat and related species.

Throughout the thesis, Tolar demonstrates a meticulous approach to data analysis and a deep understanding of the underlying biological processes. The comparisons made between transposon expression levels in different species, the breakdown of expression by transposon families, and the examination of the impact of repeat annotation methods on perceived expression levels all showcase Tolar's attention to detail and scientific rigor.

Furthermore, Tolar's thesis emphasizes the importance of using up-to-date repeat annotation tools, such as RepeatModeller2 and RepeatMasker, and recommends validation of results obtained using representative repeat libraries. These methodological insights are valuable contributions to the field and will guide future studies in accurately assessing transposon expression.

In summary, Bc. Nikolas Tolar's thesis represents an outstanding contribution to the field of bioinformatics and mole-rat genomics. The successful genome assembly, combined with the analysis of transposon expression in mole-rat oocytes, provides valuable insights into the biology of these fascinating creatures. Tolar's meticulous approach, under the guidance of Dr. Lenka Gahurova, demonstrates their exceptional skills in bioinformatics and their ability to tackle complex biological questions. I highly recommend this thesis for its scientific rigor, thorough analysis, and potential impact on the field of transposon biology and genomics research.

The presented work is a proof of the author's ability to work in a team on complex problems of contemporary genomics. The methodology used includes not only the routine use of the latest bioinformatics procedures and software, but also the creation of custom software, prepared for individual projects. The results are many and clearly of high standard. The defined objectives of the thesis have been met.

I did not come across any signs of plagiarism during my reading.

Overall Rating: Highly recommended

in Prague, May 10, 2022

A handwritten signature in blue ink, appearing to read 'Jan Pačes', written over a light blue grid background.

Mgr. Jan Pačes, Ph.D.

Questions and comments

- Regarding Figure 1 on page 5, I would like to point out that the figure, which is taken from Katzourakis' excellent publication, does not mention the existence of non-autonomous parasitic elements in both LTR retrotransposons and DNA transposon classes. This parallels

the relationship between Alu elements and L1 retroelements, where Alu acts as a parasitic element on L1.

- In the "Materials and methods" chapter, I found the inclusion of the exact code used to run the programs to be very commendable. However, I would recommend the use of a monospaced font to improve the clarity and readability of the code in future cases.
- Why was Fragments Per Kilobase of Transcript per Million Mapped Reads (FPKM) chosen over Transcripts Per Million (TPM), when TPM has the ability to maintain consistent totals across different experiments for more accurate and comparable quantification of gene expression?
- For the analysis of transposable elements (TEs) in transcriptomics, I would recommend considering Tetrascripts, a specialized tool designed specifically for this purpose. (Jin Y, Tam OH, Paniagua E, Hammell M. Tetrascripts: a package for incorporating transposable elements into differential expression analysis of RNA-seq datasets. *Bioinformatics*. 2015 Nov 15;31(22):3593-9. doi: 10.1093/bioinformatics/btv422. Epub 2015 Jul 23. PMID: 26206304; PMCID: PMC4757950.)