1st June 2020

Reviewer´s Report PhD Thesis Pavlina Vechtova

In the thesis titled "Comparative transcriptomics of *Ixodes ricinus* tick life stages" the author explores data obtained from different groups of ticks. Overall, I was impressed, it has very good quality with a high scientific level of international standard. It is also clear, well-structured and contains essential references in the topic. It represents a valuable contribution to our knowledge in the subject and the methods used are adequate. The candidate has performed a large amount of insightful research and obtained new original results. All important results presented in the thesis are published in peer-review journals or accepted for publication. Therefore, I believe the candidate merits the PhD degree.

Below I listed some points that may help improving the thesis. These are as follows:

General comments:

1. The title focuses solely in the transcriptomics aspects, but the thesis also deals with epigenetic processes and PTM such as methylation and glycosylation. Perhaps the title should reflect this?
1. Although it is generally clear and very well written, I found a few mistakes, especially in the introduction:
    a. It should be carefully revised, there are some spelling mistakes and grammatical errors (minor).
    b. In page 7, near the end of the page "analogical research" is mentioned. I believe it should read "analogous".
    c. In page 10 this part of the sentence "that Prokaryotes had compared to Eukaryotes" does not read well. I would change it to "than Prokaryotes compared to Eukaryotes"
    d. In page 23 "owing" should be replaced by "owning"
2. Have you considered publishing the introduction? It is obvious you have done a considerable amount of work and after some polishing, I think it would be worth publishing.
3. The subject of the thesis are *Ixodes ricinus* ticks, but they are hardly mentioned in the introduction. Why is that?
4. In Figure 1, page 57, a and b are mentioned in the legend, but they are missing in the main figure, it´s not clear what you are referring to.
5. Figure 2, page 71, a and b are missing again but this time in the legend.
6. Shouldn´t supplementary material be included in the thesis?

Specific questions:

Introduction

7. Why do you think orthologous genes in distant invertebrate species show a high degree of homology?
8. In figure 19, page 53, how do you explain the absence of some of the genes involved in methylation in the genome of *I. scapularis* compared to *R. microplus* and the closely related *I. ricinus*?

Research paper 1. A bite so sweet

9. Why do you think the study of the glycobiology in ticks deserves more attention?
10. In page 57 you mention four possible routes that may facilitate pathogen survival and transmission. One of them is the attachment of carbohydrate-binding proteins of the arthropod to the pathogen as part of its innate immunity. How does this help survival of the pathogen?
11. Is it possible to maintain Borrelia in cell free culture? In that case, do you know if the glycosylation patterns are different compared to Borrelia grown in cells?
12. For some tick proteins, the role of glycosylation appears to be masking antigenic epitopes to minimize the immune response. What is the relevance of this for the development of vaccines containing glycosylated recombinant proteins?
13. In relation to *A. phagocytophilum OmpA*, it is transcriptionally induced during tick feeding on mice and during invasion of mammalian but not tick cells. Why do you think this is?
14. The presence of *A. phagocytophilum* affects the midgut microbiota of ticks and biofilm composition. Do you know how, is it beneficial or detrimental?

Research paper 2. Newly identified DNA methyltransferases

15. In page 85, materials and methods, where did the ticks come from? If they were lab reared ticks, do you think the results would have been different with ticks collected from the field?
16. What was the criteria for the selection of the different tick stages for the study? Why not partially fed larvae or unfed nymphs? Do you think it would be interesting to include male ticks as well?
17. In page 90 you mention that antibody staining in fed females showed low DNA methylation. Could it be partially due to the difficulty of obtaining good quality material from these ticks?
18. Do you think methyltransferases could be a good target for the development of anti-tick vaccines?

Research paper 3. Catalogue of stage-specific transcripts

19. In this study the same stages of ticks are used. What is the reason for not including fed larvae or unfed nymphs?
20. In page 99, you mention that the samples were a mixture of three different cohorts to cover genetic variability. If these were lab reared ticks from the same facility, how can you ensure such variability?
21. In page 118, Table 4. How were the transcripts ranked? Why did you limit the results to only 5 of the highest-ranking transcripts? Is it possible that lower ranked transcripts could have more biological relevance?
22. How would you extrapolate your results to studies on tick cells? Do you think it would be interesting to compare transcriptomics data from eggs vs tick cells? How would your data help to develop new methods in tick cell cultures?

23. If you could start over, what would you do differently?
24. Based on your findings, what are your recommendations for future studies? In your opinion, what areas need more research?

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The thesis 'Comparative transcriptomics of *Ixodes ricinus* tick life stages' presented by the PhD candidate Pavlína Věchtová has three main chapters including a general introduction, a chapter of published manuscripts with one review and two research manuscripts and a general conclusion. The introduction of 53 pages presents a revision of glycosylation and DNA methylation in prokaryotes and eukaryotes. The review manuscript 'A bite so sweet: the glycobiology interface of tick-host-pathogen interactions', in which the candidate is the first author, presents in details the topic of glycosylation in ticks and tick-borne pathogens and was published in the journal Parasites and Vectors. The first research manuscript 'Newly identified DNA methyltransferases of *I. ricinus* ticks', in which the candidate is the second author, presents the characterization of three tick DNA methyltransferases, IrDNMT1, IrDNMT3, and IrDAMT, and was published in the journal Ticks and Tick-borne Diseases. The second research manuscript 'Catalogue of stage-specific transcripts in *I. ricinus* and their potential functions during tick life-cycle', in which the candidate is the first author, presents the transcriptome sequence and analysis of four tick stages egg, larva, nymph, and adult female including unfed and partially fed ticks and was accepted for publication in Parasites and Vectors. Finally, the general conclusions and perspectives cover the concluding remarks of the whole thesis. The number of pages of each chapter is appropriate. The thesis covers a diversity of methodologies, mainly DNA electrophoresis, quantitative and qualitative PCRs, transcriptome sequencing and extensive bioinformatics analysis. The methods in the thesis are sound and appropriate to address the questions posed by the candidate. Results and discussions are well-written. The thesis is an important contribution to our knowledge on tick biology.

I recommend the defense of the thesis.

Points to be improved

General point

1_ The introduction lacks a proper use of the literature. Several claims go without needed references. Adding references in the introduction would have helped to back some of the claims. During my revision, I will mention some of the needed references, but my revision of this issue is far from exhaustive.

2_ Page 1, 1.1: In the heading of the subsection, the sentence reads as if 'methylation and glycosylation' are in the 'transcriptome'. While some mRNAs in a transcriptome can be methylated (Nature Reviews Molecular Cell Biology. 2019. 20: 608–624), no transcript has been found to be glycosylated yet. Alternatively, while the transcriptome can contain mRNA encoding for DNA methyltransferases and/or glycosyltransferases, it does contain 'Methylation' and 'glycosylation' which refer to molecular processes. Please, reword.

3_ Page 2, 1.1.2: The heading does not resume what is written in this subsection. The subsection mentions very briefly some tick-borne pathogens, but do not cover the literature on tick stage-specific transcriptomic studies. Instead, this section refers the reader to table 1, but again not comparison between transcriptomic studies is included in the text or the table. As the first sentence of the subsection mentions specifically 'central Europe' and not all 'Ixodes ticks' are 'well adapted to the climatic conditions of central Europe.', instead of 'Ixodes ticks', a mention to the particular tick(s) species would be more accurate.

4_ Page 3, 1.1.3: This subsection would have benefited from a general introduction to 'methylation' and 'glycosylation' which roles go beyond 'gene expression regulation'. Line 6 of the subsection, 'among others' refers to what? Change 'employed' to 'involved'

5_ Page 4, figure 1. The arrow shows processes influenced by methylation, no by glycosylation. Also the legend of the figure should be more explicative, self-sufficient.

6_ Page 5, the first sentence seems incomplete and needs revision. For the second sentence, a reference is needed. Also, while the text mentions 'other parasitic organisms', table 1 only present transcriptome studies in *I. ricinus*. Change 'Tab' to 'Table', and please, revise this in the whole document.

7_ Page 5, second paragraph, first sentence, it is not clear whether 'mechanisms' refers to 'glycosylation' and 'motivations' to 'research'. Please, make clearer. Fourth sentence, the capacity of glycans to be 'immunogenic' (i.e. relating to or denoting substances able to produce an immune response.) is not exclusively associated with them being in the surface. Not all glycans or proteins in the surface are immunogenic. Please, correct this sentence. Fifth sentence, reference needed and 'molecular mimicry' does not refer exclusively to 'glycans', but to any 'foreign antigen (proteins or glycans) that shares sequence or structural similarities with self-antigens'.

8_ Page 7, this subsection should explain more clearly, at least briefly, the significance of methylation and glycosylation for tick cell cycle. What is known about it and add the necessary references. Also, the level of analysis: cell vs. tissue vs organ and whether it is *in vitro* or *in vivo* should also be specified. While the title specifies 'tick cell', most studies on tick cells are in vitro, single-cell studies (e.g. Single-cell RNA sequencing) have not been done yet in ticks *in vivo*. Most studies in ticks reach only the organ level, and those are mainly focus in guts and salivary glands.

9_ Page 7, first line, delete 'Sugars or' and change 'omnipotent' to 'important' or 'ubiquitous'. In the third and fourth sentences, 'lots of' and 'most obvious' are not appropriate expressions for a thesis. Please, check this in the whole document.

10_ Page 8, how do we know that 'Glycans, were not an indispensable part of the non-currently existing cellular life'? Please, reword. The syntax of the first sentence of that subsection is wrong, reword. Provide a more complete definition of ' glycomics': 'a scientific field that describes an array of glycoconjugates' is a very limited definition. I suggest, 'Glycomics is the comprehensive study of glycomes (the entire complement of sugars, whether free or present in more complex molecules of an organism), including genetic, physiologic, pathologic, and other aspects' (source, Wikipedia).

11_ Page 9, figure 2. The legend of the figure should be explicative and self-sufficient. For example, what are the squares, circles and the numbers inside brackets representing? Spell the abbreviations.

12_ Page 10, the first sentence after the figure needs a reference and argumentation. This comparison, is based on what? Please, provide numbers to support the statement that 'bacteria have a higher diversity of glycans structures compared with Eukaryotes'. A statement in page 16, 'N-glycosylation reached the highest degree of its diversity and specialization in Eukaryotes.', contradict this.

13_ Page 11, 1.2.2: this subsection does not cover the 'Glycan functions', but a very general presentation of about glycan synthesis and transport. Please, rewrite or re-name the section. In the first sentence, a glycan cannot be fully functional before the protein is completely synthetized and folded. For the sake of precision, please, reword.

14_ Page 12, 1.2.3: This section could have benefited from a figure showing the main structural features of major glycosyltransferases.

15_ Page 17, 1.2.6: Third sentence, please change 'in different direction' for a more specific phrase.

16_ Page 18, second sentence, this is the first time 'Ca. elegans' is mentioned in the text. Same for ' D. melanogaster '. Spell the full name of the species and then used the abbreviation. Also, choose only a way to abbreviate species names, 'two first letters of the genus' (e.g. 'Ca. elegans') or 'only first letter of the genus' (e.g. ' D. melanogaster '), but do not include the two forms in the same document. In the second paragraph, correct the spelling of 'Gůycosylation' 'heterogenosu'.

17_ Page 22, 1.2.8: It is confusing to find 'Conclusions' in the middle of the document. Except for those in the published manuscripts, please, reserve 'conclusions' only for the end of the thesis.

18_ Page 23, 1.3: The first sentence suits better a definition of 'DNA modifications' in general than one of 'DNA methylation' in particular. Please, be more precise, for example by mentioning that 'DNA methylation' refers to the covalent and reversible addition of methyl groups to genomic DNA without alteration of its sequence. In the fourth sentence, the statement that 'methylation' is 'the most well documented' is misleading. There is abundant literature on histone modifications and non-coding RNA as epigenetic modulators of gene expression.

19_ Page 24, table 2: This definition does not allow differentiating 'global' from 'methylated'. Please, provide more precise definitions for each of them. Otherwise, name both 'global' from 'methylated' as 'methylated'. Also, it is not clear in the table what 'mosaic' means. Trimmed the name of the table and move additional information to the foot of the table.

20_ Page 25, 1.3.1: This section refers to 'Eukaryotic genome methylation'. I wonder whether table 2 will be more purposeful here than in the previous section.

21_ Page 25, 1.3.2.1: Define 'illegitimate transcription' in the first paragraph. In the second paragraph, this may not be the most complete definition of the bimodal methylation

pattern. It could be improved to 'Bimodal distribution among functional categories of genes refers to the fact that gene body methylation is high in ubiquitously expressed housekeeping genes and low in genes that are regulated, depending on the context' (Curr Opin Plant Biol. 2017. 36:103-110; Mol Biol Evol. 2012. 29:1907–1916 & Proc Natl Acad Sci USA. 2018; 115(52):13342-13346).

Manuscripts.

In the figure 4 of the manuscript 'Catalogue of stage-specific transcripts in *I. ricinus* and their potential functions during tick life-cycle', all letters to the right of the panels are upside-down. In addition, to correcting this, better to move the names of the clusters to the left and order from cluster 1 to 11.

Questions

1_ Considering previous (Carbohydr Res. 2014; 389:93-9 & J Insect Physiol. 2012; 58(9):1277-87) and recently published research (Vaccines (Basel). 2020; 8(1):18 & Front Immunol. 2019. 10:1056) on the glycans and glycosyltransferases identified in ticks, could you present a detailed view of what specific glycan structures can be synthetized by the ticks and which ones may originate in the host and acquired by the tick during feeding?

2_ In the manuscript 'A bite so sweet: the glycobiology interface of tick-host-pathogen interactions', the importance of the α-Gal epitope is mentioned. Considering the discovery of galactosyltransferases (Sci Rep. 2018; 8(1):14224) and other N-glycosylation enzymes in ticks (Vaccines (Basel). 2020; 8(1):18), could you propose a possible pathway of α-Gal glycosylation in ticks?