

## REVIEW ON A DISSERTATION (DOCTORAL) THESIS

### *Virome of Honey Bee*

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Tutor: **doc. RNDr. Ruth Tachezy, Ph.D.**

Reviewer: **prof. Ing. Jaroslav Hrabák, Ph.D.**

#### General Comments:

Dissertation thesis of Mgr. Dominika Kadlečková is focused on the virome of a honeybee (*Apis mellifera*) identified in colonies from 9 geographical areas of the Czech Republic. Honeybee viruses represent a significant threat to beekeeping as they can be responsible for major colony loss in apiaries infected by *Varroa destructor*. It is hypothesized that in the outcome of viral infections of honeybees, several factors contribute, such as stress, contamination of the environment by pesticides, presence of acaricides in the beehives, lack of diversity in pollen, etc. Therefore, the studies of honeybee virome are essential to understanding the reason for colony loss and its prevention.

The thesis aimed to comprehensively explore the honeybee virome, especially its diversity, dynamics, and implications for bee health. There were three main objectives – characterization of virome within the bee colony, study of virome dynamics, and study of factors that can impact honeybee virome. Interestingly, there was very high variability within a single bee colony. In the first project, the Author collected nine bee workers per colony, and using NetovIR method and short read sequencing, she analyzed the virome and its diversity within a colony. For sequencing data, several bioinformatic tools and precise virus identification have been used.

Interestingly, there was a very high virome diversity even within a colony. The second part of the study focused on five geographical locations and three bee hives per apiary for three years of sampling. Within the study, novel viruses have also been described (four picornaviruses). This is also an essential result of the thesis.

The dissertation thesis is written in English and has a classic division into introduction, objectives, methodology, and discussion. The introductory part provides a very nice review of current knowledge on viruses identified in *Apis mellifera*. The references cover the current knowledge in the field as well.

I only have a few minor critical comments about the text:

- In the results, more epizootiology data should be analyzed, especially the infestation of the colonies by the *Varroa destructor* mite. The Author mentioned that there was no significant manifestation. It is, however, evident that most of the colonies in the Czech Republic are infested. It should also be nice to provide data about the use of acaricides (see question below).
- The paragraph 5.3. – Other results – proteomics and qPCR: this paragraph looks inappropriate to include in the results section as there was no detailed description of the methods in the methodology part.
- The Czech abstract contains some language errors.

- In the text, the author should follow the same writing style, i.e., the passive voice in the plural. It is advisable to avoid the singular number or literary inappropriate forms (e.g., "We are slowly moving...", p. 22).


### Questions:

1. I know that the study was focused solely on honeybee viruses. Have you, however, identified bacteriophages specific to significant bacterial pathogens of *Apis mellifera* (e.g., bacteriophages of *Paenibacillus larvae*) in your sequencing data?
2. Do you think the stress of bee colonies can play a significant role in the sensitivity of *Apis mellifera* to viruses, especially of the colony collapse disorder in some countries?
3. Recently, based on *in vitro* data, some studies demonstrated a significant increase in the sensitivity of *Apis mellifera* to viruses by amitraz degradation products. Those products (intermediates N-2,4-dimethylphenyl-N-methylformamidine, N-2,4-dimethylphenylformamide, and the final product 2,4-dimethylaniline) can persist and accumulate in beeswax for a long time. As we know, amitraz has been used to control *Varroa destructor* in the Czech Republic for 40 years. Can this phenomenon also be important in colony loss in the Czech Republic?
4. What is the role of Rhabdoviridae in bee colonies? Which tissues/cells are mostly infected in *Apis mellifera* by the viruses of this family?

### RECOMMENDATION

The dissertation fulfills the conditions set for its level in terms of scope and content. Therefore, I **recommend** this thesis for defense.

In Pilsen, 9<sup>th</sup> September 2024



prof. Ing. Jaroslav Hrabák, Ph.D.