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Review of the doctoral dissertation of Mgr. Martin Těšický

The topic of Martin Těšický's PhD research was the intestinal microbiota, immunity, and senescence addressed from evolutionary and ecological perspectives in wild birds from two related taxons *Passeriformes* and *Psittaciformes*. I find the topic very appealing and unique as the evolution of the immune system and related topics in feral birds is a neglected topic. The research builds upon previous pioneering work of the supervisor and the hosting research group, which characterized key immune-related genes in birds, such as Toll-like receptors.

The dissertation is written in a short form with attached publications. It has a typical structure consisting of the introduction, definition of the research questions, general methods, results and discussion, and conclusions. It is written in very good English in a clear way with an acceptable amount of errors and typos (although the missing labels of the x axis in the graphs in Figure 4 are on the borderline). Overall, it was my pleasure to read it.

The dissertation is based on 11 publications in peer-reviewed journals and 4 manuscripts submitted to peer-reviewed journals. The applicant is the first author of three publications and one manuscript. Overall, the publication activity is considerable and the number of publications and manuscripts is much higher than is typical for PhD graduates in my field (Immunology). I am not familiar with many of the journals. However, based on the fact that most of them are in the first quartile based on the impact factor, it seems these journals belong to the respected ones in the field.

Although the results are fragmented in multiple publications, the author managed to summarize them in a very concise and comprehensive way. The individual studies are clustered into three areas, focusing on the intra- and interspecies diversity of bacteria colonizing wild birds, evolution and diversity of innate immune receptors in wild passerines, and monitoring of the markers of senescence. For the sake of

space, I will summarize only the major findings in the first author publication of the applicant. They documented that the unhatched egg and fetus of the great tit are (nearly) sterile, which contradicts the previous studies in chicken. The second study used several bioinformatics and computational biology methods to uncover convergent evolution of TLR4 and TLR5 genes in *Paridae*. The last two publications identified the changes in testosterone levels in males during aging and increasing levels of granulocytes and leukotriene B4 (LTB4), a marker for chronic inflammation, during aging in the great tit, which documented signs of (immuno)senescence in birds.

I find the research directions and results of the dissertation compelling, provoking further research, and having broad implications for multiple disciplines including evolutionary biology, biology of aging, immunology, veterinary science, and human health considering the global threat of the bird influenza. It is clear that the applicant obtained broad skills and expertise in the field work, laboratory experiments, as well as bioinformatics and computational analyses.

The overall quality of the dissertation is very high. Although I could find some imperfections in the parts referring to general immunology, these do not represent a major issue. Overall, I recommend the dissertation of Martin Těšický to be accepted for the defense and graduation.

The dissertation inspired me to ask the following questions.

1. The applicant identified that bird eggs are (almost) sterile before hatching and the GIT of birds is colonized mostly after hatching, which is supposedly different from mammals (mentioned on the pages xiii and 37, but not on the page 25). In mammals, the bacterial colonization of the placenta and fetus is still a matter of debate, although the recent studies suggest that the previously identified bacteria in the fetus are probably contaminants (Kennedy et al. *Nature* 2023 Jan;613(7945):639-649). On the other hand, newborn mammals are probably colonized perinatally as they pass through the female reproductive tract). Could the applicant comment on the similarities and differences between the bacterial colonization of birds and mammals (time and route), if the fetuses are (nearly) sterile in both cases?
2. Is it possible to resolve the question whether the unhatched eggs are completely sterile or just nearly sterile? If there were only few bacteria in the fetal GIT, why wouldn't they colonize the whole GIT niche during the incubation period before hatching?
3. In the study focusing on the evolution of TLR4 and TLR5 receptors, the positively selected sites were predicted *in silico* based on the putative ability to alter the function of these receptors. Did the applicant try to identify functional differences between some particular variants of TLR4 and TLR5 in a wet experiment? If not, what would be the proper experimental approach?
4. One of the measured features of senescence were decreased testosterone levels (hormonal senescence). The decrease in testosterone levels in aged individuals was observed in males, but not in females. Does it mean that there are physiological differences in the senescence between males and females in birds? Could the applicant show a graph with the measured values of testosterone vs. age (i.e, not the residuals from the minimal adequate model) in males and females? If I understood the methodology, the testosterone levels were measured in birds after mating (during taking care of the nestlings). What would be the expected testosterone levels and the role of aging if samples were collected in the mating period? Are there known or expected differences in the senescence between males of (quasi-)monogamous and polygamous bird species? I am asking

because of the discussed roles of potential trade-off choices between reproduction and self-maintenance in the senescence.

5. In the publication focusing on inflammageing, LTB4 was used as a sole proxy for chronic inflammation. Which additional markers of chronic inflammation would the applicant chose, if he had unlimited resources and access to the biological material.
6. Four of the manuscripts were not accepted for publication yet when the dissertation was written. In my opinion, it is not required or relevant to name journals where the publications were submitted to in such cases. It might be better to publish these manuscripts as preprints and include it in the dissertation as such. Could the applicant briefly comment on the current status of these manuscripts?

Mgr. Ondřej Štěpánek, Ph.D.