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**Anna Mácova thesis '*Apodemus* vs. *Eimeria*: Evolutionary factors of speciation and genomic diversification in host-parasite system'**

The thesis submitted by Anna Mácova features a series of five published and two unpublished manuscripts, two as first author. The work forms a logical portfolio focused on the description of *Eimeria* populations in wild mammals and an assessment of population-level evolution. The work is innovative and internationally competitive. **Based on these papers I recommend that the thesis is suitable for defence.** Manuscripts 1 and 5, and draft 1, are likely to have the greatest scientific impact, re-exploring the host-parasite co-speciation paradigm and recommending improved approaches for genetic/phylogenetic characterisation. Manuscript 4 and draft 2 explore the occurrence and characterisation of parasites in previously undefined host populations. Manuscript 2 provides an interesting evaluation of (pseudo) 'parasites' recovered from bank voles, with particular relevance to the other studies that can be brought out in the defence. Manuscript 3 is a little removed from the other work, but adds to the literature surrounding the host populations. The phylogenetic analyses are detailed and well executed. The oocyst images are high quality and very impressive.

Questions

1. You have presented a series of very clear objectives. Did you have one or more overarching hypothesis(es)? What were they?

2. The concept of a parasite adapting to expand its host range is logical and consistent with Darwinian evolution, but why might a parasite 'abandon' a host (thesis page 25), and how might this happen in the field?

3. What does a parasite need to be able to host switch?

4. Beyond co-speciation, what else might drive a parasite to evolve?

5. How would you define a 'pseudo-parasite'?

6. You have provided a detailed and compelling case of 'pseudo-parasitism', isolating parasites that appear to have been transiting through the digestive tract of non-infected (colonised) hosts. How did you exclude this phenomenon from complicating your population-level analyses with *Apodemus* and other hosts? How can we resolve this problem for studies with foraging species?

7. Your work supports several accounts that the 18S rDNA sequence is inappropriate for discrimination between many *Eimeria* species. Evidence that cytochrome oxidase c subunit 1 (COI) is similarly uninformative for *Eimeria* that infect rodents is more novel. Do you think the ORF 470 sequence is likely to be similarly informative for *Eimeria* from other host populations, for example poultry? How might you have tested this?

8. How do you select an outgroup when you are developing a sequence set for phylogenetic analysis? What characteristics are you looking for?

9. Genetic evaluation of parasite populations using a single or small number of markers limits capacity to assess the occurrence of hybridisation. In your work with multiple markers, especially the panel of 35 Fluidigm Access Array markers, did you see any hint of hybridisation between parasite sequence types? Do you think this might happen in *Eimeria* field populations? [If not, why not?]

10. Did you have the chance to work with sequences from the Fluidigm Access Array? If you did, how did you handle sequencing errors?

11. Can you explain what a high $F_{ST}$ value means and its relevance to diversification?

12. You have aligned COI sequences using amino acid mode, but analysed them in nucleotide mode. What are the benefits of doing this?

13. Are you aware of any host environmental conditions that can change the appearance of the sporulated oocyst? [e.g. sub-lethal exposure to clopidol and methyl benzoquate resulted in a heritable bisporocystic form of *E. maxima*]. How might we account for this when relying on morphological characterisation?

14. You have commented on differences between *Isospora* and *Cystoisospora*. Where do you consider *Atoxoplasma* fits into the picture? How does this compare to *Eimeria* species such as *E. stiedae* in rabbits (extra-intestinal infection) or *Eimeria* that infect migratory birds such as crane (cause of the chronic disease disseminated visceral coccidiosis) – should these be considered as true eimerians?

15. Given that *Caryospora*, *Cyclospora* and some *Isospora* cluster within *Eimeria*, should we subdivide the genus? Why/why not?

16. How do you feel manuscript 3 fits into the thesis portfolio?

17. I was interested to note that a bootstrap value of 100 was used in manuscript 4 for comparison of *Cryptosporidium* sequences. I recognise that you were not the lead on this project, but can you suggest why this value might have been chosen? Is this a problem?

18. It is mentioned in manuscript 4 that the detection of *Toxocara canis* in Svalbard arctic foxes was striking, making an association with ability to survive at low temperatures. Do you think this report might be due to the lack of previous sampling, or might it be associated with climate change? If so, what might the repercussions be?

19. How confident can we be that the species *E. falciformis*, *E. ferrisi* and *E. vermiformis* are 'real'? Are they stable?

Damer Blake.
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