ONTARIO VETERINARY COLLEGE
Department of Pathobiology

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Dr. Jana Kvičerová
Department of Parasitology, Faculty of Science,
University of South Bohemia,
České Budějovice, Czech Republic

Re. PhD Thesis Review – Mgr. Anna Mácová

Dear Dr. Kvičerová:

It was a pleasure to read the compilation of work by Mgr. Anna Mácová assembled in her PhD thesis. Phylogenetic analyses of coccidia are difficult enough without the complexities associated with attempting to correlate host usage on top of the phylogeny of the parasites. Mgr. Mácová reports on an impressive collection of studies, all aimed at finally understanding the flow of eimerian parasites (and their genomes) within and among rodent hosts. Although there are certainly questions that remain to be addressed, she has greatly expanded our understanding of this complex parasitic ecosystem and generated valuable data and analyses that will guide all future studies.

In summary, I heartily and unreservedly recommend the thesis for defense.

Comments:

I am personally well acquainted with the difficulties of field-based research and the complexities of specimen collection and analyses as outlined in her work. I was impressed by both the quantity and quality of material that was collected, and the evident care that was taken in the subsequent molecular biology and bioinformatics activities.

Minor Comments:

Although it would increase the length of an already substantial thesis, I think that the supplementary material cited within the published papers (where feasible) should be included as Appendices to the thesis. It makes the work complete and permits the reader to understand the included papers without having to consult external literature. Alternately, either the links within the enclose papers should remain active or a list of direct links to the supplementary material should be provided.

I have made a number of minor corrections/suggestion directly on the PDF document sent for my review (e.g. Figure 5 of Draft 2 is rooted strangely within the eimeriid coccidia rather than the included member of the Sarcocystidae and should be corrected). Ms. Mácová can incorporate these changes as she sees fit. None are substantial and the majority are style or idiom issues. Her writing in what is very likely a second (or third?) language is highly skilled!

Sincerely yours,

John R. Barta, Ph.D.

Professor (Parasitology),
H. B. Ward Medalist, American Society of Parasitologists
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Questions for consideration/discussion during the defense:

More important, general questions:

1) In your introduction, you introduced the widely accepted concept of a parasite-host 'evolutionary arms race' in which changes in one partner in this parasitic relationship lead to changes in the other partner. You generally discussed this concept from the perspective of a single host and stated that parasites generally do not 'want' to kill their host. Can you expand your discussion to the population level? Can you explain how "overdispersion" may play a role at the population level in maintaining a host-parasite "détente"?
2) One of the key features of your work was the finding that there was little variation in the mt COI among some of the parasites obtained from divergent hosts. Could you please provide your opinion on whether this lack of COI diversity results from relatively slow rate of change in that locus in these parasites or from the more recent colonization of multiple divergent hosts by a 'generalist' coccidium? Or, both?
3) Can you discuss some general issues related to doing haplotype analyses on nuclear targets versus plastid (or equally mitochondrial) targets as you did in MS5? Do you think that the diploid versus haploid nature of the nuclear versus organellar targets contributed to your observations? Would the structure of the plastid haploid genome (presumably circular) or mitochondrial genome (presumably linear concatemeric) suggest issues that you might have to address in your sequence data analyses?

Less important, more specific questions:

4) You work on the isosporan pseudoparasites of bank voles, you used live-trapped, parasite-negative individuals as hosts to attempt experimental infections. Although I do not disagree with your conclusion regarding the pseudoparasitic nature of the isosporan oocysts you recovered, can you critique this work in any regard? Is there any scenario that you can conceive in which your results (no apparent infections in oocyst-negative hosts) were misleading? (i.e. the parasite could actually use the bank vole and you got a false-negative in your experiments)
5) In one of your works, you mentioned that a plastid-based locus (ORF470) would be desirable because of its relatively high copy number. Although certainly true for single copy nuclear loci, the relative advantage may not apply to the commonly targeted nu 18S rDNA or mt COI/COIII loci. Could you compare pl ORF470 copy number per parasite to nu 18S rDNA or mt COI/COIII??
6) Use of nu 18S rDNA sequences for inferring phylogenetic relationships (at least at the genus level with coccidia) generally works fairly well but tends to break down when examining closely related species. Although a lack of interspecific genetic diversity at this locus certainly contributes to this failure to resolve closely related parasites, intraspecific variation certainly contributes to this as well, sometimes in the extreme. Did you see any evidence of intraspecific variation within your nu 18S rDNA sequencing attempts (e.g. evidence of indels producing reads that go from high quality to near-unreadable or SNP's with the chromatograms? If so, how did you resolve this?