

Referee's Report

Mgr. Ondřej Korábek

**PhD thesis: Diversity of the land snail genus *Helix* and its relatives
(Gastropoda: Pulmonata: Helicidae) from Mediterranean
origins to postglacial Central Europe**

General comments

The application of cladistic methods in systematics, coupled with the use of DNA sequences, has radically transformed our understanding of phylogeny at all levels in the systematic hierarchy. In the specific case of stylommatophoran mollusc systematics, it has revealed many inadequacies of phylogeny and taxonomy based on either or both conchological characters and details of anatomy; shell characters in particular are subject to repeated homoplasies. Contradictory results create a degree of taxonomic confusion that requires a wide range of skills to sort out.

Nevertheless, the analysis of molecular sequences has proved to be far from straightforward. Both the source of DNA (mitochondrial or nuclear) and the methods of cladistic analysis can influence interpretation and the level of confidence attached to any particular set of conclusions. Many propositions have been challenged on methodological grounds, or because of an increasing awareness of the complications caused by introgression in mtDNA.

Korábek's thesis sets out to apply modern methods to examine diversity in a single, iconic genus of pulmonate land snails. It goes beyond phylogeny and taxonomic revision to consider examples where we can start to trace the historical phylogeography of a selected species (*Helix pomatia*), and where we can gain useful insights into the extent to which morphology varies within well-defined clades, often with parallels in others. I note that the ambitious scope of the thesis has required proficiency not only in molecular systematics, but also in the interpretation of Pleistocene/Holocene fossils, and in the complex and often legalistic world of taxonomic nomenclature.

In making a judgment on the thesis, I have paid particular attention not only to the originality of the work, but to the degree to which the candidate has shown the ability to think critically about the limitations imposed on conclusions by the methods and data available.

Specific comments

The thesis contains an introduction and four substantive chapters, three of which consist of papers in respected peer-reviewed journals. The fourth is a paper prepared for publication. In addition, there is a further published paper included as an appendix since the candidate is not the senior author, but which relies on the other work presented in which he is in the lead.

The introduction places the conclusions drawn in later chapters in context by relating them to the general problems of understanding regional patterns in land mollusc diversity. In particular, it examines the historical context and the evidence for the roles of refugia and dispersal in determining those patterns. It also reflects analytically on the difficulties involved in dealing with the numerous species concepts in use, and the manifest differences in the way they have been applied in different cases, even among pulmonate land snails alone. It serves

well to demonstrate that the detailed studies that follow lead to conclusions of wider interest than the history and phylogeny of a single genus. I have a minor criticism, perhaps a matter of taste rather than substance about the structure and the informal language used here, but I appreciate that the introduction is not designed as a free-standing publication

The chapters containing published work need little further comment. Having accomplished the thorough revision of the genus *Helix* in Chapter 1, its benefits in distinguishing cryptic species and their phylogeography are explored in the two subsequent chapters, as also in the appendix. The whole comes together in the final substantive chapter in which the fruits of thorough revision and detailed sampling of many populations enable us to look at the speed and pattern of dispersal in the classic *Helix pomatia*. I would have liked some more information about the ages of fossil shells found outside the Czech Republic: there may be no information, and this is a factual question for the candidate to answer.

I notice a few linguistic infelicities in the as yet unpublished portions of the thesis. I regard these as trivial, and I have not attempted to provide a list. I would be happy to advise if asked when the material is about to be submitted.

Conclusion

This is a thorough, thoughtful and well referenced thesis. It shows originality and an awareness of the broader context, not only in relating its conclusions to other work, but also in seeing how methods used could be extended to improve our knowledge elsewhere. I have no doubt that it merits the award of a PhD. I would add that it is refreshing to see so much interesting and original material dealt with in a mercifully small space!

I have three possible questions for the candidate, which should be given to him in advance. One is a matter of fact:

Outside of Bohemia and Moravia, are there reliably dated fossils of *Helix pomatia*? If so, how old are they? Is there any evidence of wider range in interglacials, corresponding to finds of *Drobia banatica*? If not, would you be prepared to speculate on why *H. pomatia* has expanded its range in this cycle, while *D. banatica* remains restricted?

The next is more hypothetical:

While I appreciate that obtaining nuclear DNA from enough species within *Helix* is difficult, I wonder in what way your conclusions might change if we had a phylogeny based on nuclear genes? I am conscious that some work I did with Greek colleagues on *Albinaria* species on Crete, using mtDNA has been informally criticised because of the well-known problem of introgression.

The final question (outside my capacity to pass judgment!):

In general, you have very few sequences from any one population. One criticism of many phylogenetic studies at this level, and using mtDNA is that haplotype polymorphism can confound interpretation. I know that some populations of *Cepaea nemoralis* have sets of haplotypes that suggest that they derive from several ancestral populations. Do you have any evidence that might suggest the same in *Helix*?

Robert A. D. Cameron. 23 July 2017.