

Prof. Peter Trontelj
Department of Biology
University of Ljubljana, Biotechnical Faculty
Jamnikarjeva 101
1000 Ljubljana, Slovenia
peter.trontelj@bf.uni-lj.si

Prof. David Storch
Chair of the Board of Examiners
Charles University
Faculty of Science
Albertov 6
128 43 Praha 2
Czech Republic

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Report on the PhD thesis of Mr. Denis COPILAȘ-CIOCIANU under the title

**Biogeography, phylogeny, ecology and systematics of epigeal freshwater
Amphipoda in the Carpathian arc and beyond**

Dear Prof. Storch,

You kindly invited me to write a report on the thesis submitted by Mr. Denis Copilaș-Ciocianu, which I am delivering in this letter. I have read the thesis with great interest, as its subject is quite close to my own area of work.

Before I go into details, I would like to stress that this is truly an exceptional thesis. Rarely have I read papers that well written by young non-English authors; rarely have I seen such a great body of data collected and analyzed within the framework of a single regular-time PhD thesis; and rarely have I seen such mastery of a wide variety of computational and statistical methods in students whose main subject was organismal biology. Virtually never have I seen all these things combined in a single thesis. The publications that constitute the thesis speak for themselves. All seven papers are first-authored by the candidate. They are published in esteemed journals such as *Molecular Ecology* and *Journal of Biogeography*. Besides, the thesis has got an informative general introduction and a succinct overview of the seven individual contributions. This is much appreciated as it eases the reading and understanding of the common and complementary aspects of seven different papers. I think Denis has done an excellent job in this respect, too, as he managed to provide a common basis for a new biogeographical and eco-evolutionary understanding of the diverse amphipod fauna of the eastern and central Balkans.

Broadly, the majority of the thesis consists of in-depth analyses of phylogenetic and phylogeographic patterns of molecular diversity of Amphipods from the Carpathians and central and lower parts of the Danube basin. Denis sequenced several mitochondrial and nuclear loci thus providing independent support for his phylogenetic hypotheses. Sampling

was comprehensive and covered large ranges, sample sizes being usually several tens to over a hundred. He used a wide range of contemporary analytical methods to reconstruct and date phylogenies, to infer demography, and to test hypotheses of geographical dispersal and range fragmentation. Among his most interesting discoveries is the finding that widely distributed nominal species of the Carpathians, *Gammarus fossarum* and *G. balcanicus*, are genetically deeply subdivided into lineages that are much older than the beginning of the Pleistocene. This implies that they must have evolved during the Mio-/Pliocene in association with events related to Carpathian orogeny and fluctuations of the Paratethys Sea, and they survived glaciation in numerous refugia relatively far to the north. Conversely, two other amphipod species, *Niphargus valachicus* and *N. hrabei* from alluvial plains of the Danube basin, are substantially less structured over large geographic distances; their phylogeographic subdivisions date back to the second half of the Pleistocene. Paradoxically, these two species are closely related to groundwater species that as a rule have tiny and ranges and are genetically deeply structured. Denis went on and studied differences in ecology, life history, and eco-morphological traits with the aim to understand the biology behind the observed patterns as well as the functioning of amphipod communities. With this part of his thesis, Denis demonstrated that he is capable of working on various questions of organismal biology using methods and approaches that go way beyond the usual “molecular evo-eco-syst” toolbox. While this part seemingly contains less novelty and striking discoveries, it is scientifically no less important, as it opens new questions about the evolution of amphipod diversity and rejects obsolete taxonomic views.

Since there is no doubt that this thesis exceeds all standards, and that it is by all means sufficient to grant a PhD degree, I see no need to further evaluate it chapter by chapter, especially as all but one of them have already undergone regular peer review. Because of the sheer amount of processed data as well the variety of addressed points it is understandable that several of them could not be pursued in full depth. Furthermore, the results were partly unexpected and as such inevitably left some uncertainty to the conclusions. I propose the candidate to elaborate on a few of these points for his thesis defense.

The first one concerns the mutual reproductive status of the numerous lineages discovered in the nominal species *G. fossarum* and *G. balcanicus*. It was a wise decision not to enter a debate about whether they should be formally named as species or not. Nevertheless, the question is important also for the conducted downstream analyses. These largely rely on the assumption that no migration/gene-flow is taking place between lineages. Because they constitute a-posteriori groupings, further tests would be required, but would be hampered by small sample size, low resolution of nuclear markers, and inappropriate sampling design. The obvious questions are what happens after lineages come into secondary contact, how often has this happened, can we detect such events, what could possibly prevent them, and how would they affect the outcome of the applied demographic reconstructions?

The second point has to do with the unexpected contrast in diversity patterns of *Gammarus* and *Niphargus*. The candidate attributes it to a “secondary colonization of the epigeal environment that ... promoted their-large scale dispersal” and concludes that “the ecological barrier between the surface and the subterranean environment might not be as impenetrable as previously thought”. There are several inconsistencies in this explanation and conclusion. I would like to ask the candidate to critically read Ch. 5 of his thesis and try to address the claims that he finds problematic and/or inconsistent upon thorough re-consideration.

Finally, I have a rather minor remark regarding the importance of subsidence for the diversification of freshwater biota as if this were something new. I would like the candidate to elaborate on this hypothesis and its possible alternatives in both his specific and the general case.

I am confident that Denis will excel in responding to these questions. I thus have the pleasure of concluding this report by formally stating that the PhD thesis submitted by Mr. Denis Copilaş-Ciocianu is an outstanding scientific achievement that I most highly recommend for acceptance and defense.

Sincerely,

Peter Trontelj