



Review of a PhD dissertation by MSc Hedvig Kriszta Csapó
***"Phylogeography and population genomics of boreal marine
macroinvertebrates colonising High Arctic"***

The review has been prepared based on the Polish Academy of Sciences, Institute of Oceanology Scientific Council's nomination as a reviewer under a procedure for conferring a doctoral degree of PhD candidate Hedvig Kriszta Csapó.

The PhD dissertation focuses on the phylogeography of marine macroinvertebrates, contemporary atlantification, and the impact of thermal optima in Holocene on the colonization of European High Arctic by marine species from lower latitudes. The dissertation includes theoretical background and novel research data which were carefully tested and discussed. In general, the colonization, dispersal and historical demography of Arctic invertebrates are recognized to a small extent, and each attempt towards understanding the recent and past biogeography is highly appreciated. I have had the great pleasure of reading the PhD thesis of Hedvig Kriszta Csapó, who presented novel data generated through challenging field campaigns and by using a time-consuming laboratory procedure of DNA extraction and amplification followed by bioinformatic and demographic analysis.

The dissertation is divided into *Summary* in English and Polish, three *Chapters*, and *Author contribution statements*. Additionally, before the *Summary* there is an *Acknowledgments* section included in the thesis. Two chapters have already been published as articles in prestigious, scientific journals. The last chapter is presented as an unpublished manuscript of a scientific paper. The contribution statements indicate that the main author had a leading role in the design of the study, its analysis, and the writing of the manuscripts which proves a significant intellectual contribution to the main outcomes of the chapters. The main goals of the thesis are important in understanding the drivers of the intertidal marine macroinvertebrate distribution along the gradient spanning from Iberian Peninsula to European High Arctic latitudes. The author aims at answering a valid question: does recent Atlantification influence the dispersal of marine macrofauna to the European Arctic? In light of the contemporary discussion about the impact of global warming on the dispersal and distribution of organisms in general, finding the answer is fundamental.

The PhD candidate is the first and corresponding author in two chapters and first author of the last chapter. The fact that other colleagues have already evaluated the papers, makes the role of a reviewer even more challenging.



I. Summary

The Summary consists of a background and the results of the PhD study in a nutshell. The author of the thesis introduces the reader to some basic information about atlantification, and presents the main problems in recognising recent colonisation of High Arctic marine waters by species from lower latitudes. Next, PhD candidate discusses climate fluctuations in the Holocene, and proposes hypotheses of the thesis, which are followed by the results and a short summary of each chapter. Additionally, the reader can find some interesting (yet well-supported later in the thesis) information about multiple Atlantification events, and learn about the returning of neonatives to the Arctic and about true borders of marine Arctic regions.

II. Chapter I (Research paper no 1)

The first part of the PhD dissertation has already been published in an international and prestigious STOTEN journal. The paper was published in 2021 and, according to Scopus, has been cited 39 times until now. Such a good score indicates that the paper is in the scope of a broad audience of scientists and it has drawn considerable attention. The paper is a review of Atlantification literature. The authors define what Atlantification is, along with sharing a physical and biological characterisation of the process. The chapter provides a robust review with interesting outcomes. I decided to skip the description of the whole summary of the article. In my opinion, the most important and new concepts presented in the chapter are: i) the potential impact of mobile anthropogenic substratum like a plastic platform on the speed of dispersal processes of boreal fauna towards the Arctic, ii) increasing the interest in the process of the Atlantification in scientific literature, and finally iii) the hypothesis (tested in later chapters) that recent colonisers in the Arctic are the so-called neonatives coming home after ice retreat.

The chapter makes me ask a question. During the Marine Isotope Stage 11C the GrIS collapsed, and during Eemian interglacial, ice completely disappeared in Scandinavia and almost completely in Svalbard, but both boreal and Arctic animal groups survived these events, at least limno-terrestrial invertebrates. Does the author consider the existing of warm and cold marine refugia for Arctic and boreal fauna depending on the climate fluctuation? Cold refugia during warm periods and vice versa? What if we changed the perspective and considered the scenario that through both natural cycles and a human impact the climate is on the trajectory of warming without a way back. What fate awaits the Arctic and boreal marine fauna according to the author? Will the Arctic taxa disappear completely and will the Arctic waters be dominated by “neonatives”?

III. Chapter II (Research paper no 2)

The second part of the PhD thesis has been published as an original article in a prestigious, international journal *Frontiers in Marine Science*. The author's objective is to discover the





phylogeography and historical demography of three intertidal species: amphipod crustacean *Gammarus oceanicus*, rough periwinkle *Littorina saxatilis* and barnacle *Semibalanus balanoides* in the Northern Atlantic. The paper is of great value. Among numerous distinctive assets of the study, I would emphasize the use of three species differing in dispersal capabilities and life histories, a broad sampling area, literature and new data application, and finally the analysis of intraspecific diversity, population structure and historical demography. The authors used COI sequences from 14 regions and generated hundreds of barcodes from collected individuals. Everything is of good quality and it is evident that the author spared no effort while working on the thesis (sampling, generating 498 new COI sequences, searching of GenBank and Bold, bioinformatic analyses).

I appreciate the sampling strategy, relatively big datasets, analysis, graphics and finally a results followed by the neat discussion. The main outcome of the study shows that the colonisation of the High Arctic Svalbard archipelago by target animals begun during the previous warmer periods in Holocene and the current distribution is not an effect of recent atlantification. Moreover, the authors found a homogenous population structure of *S. balanoides*; at the same time the species formed a distant group in the Arctic. Svalbard populations of *G. oceanicus* were closest to other Arctic populations. The Arctic groups started to expand 2500 ya. Arctic demographic unit was identified for this amphipod species for the first time. Alike *G. oceanicus*, *L. saxatilis* from Svalbard was genetically closer to an Eastern Atlantic population with similar Arctic expansion.

The discussion on anthropogenic transport of *L. saxatilis*, the existence of hypothetical refugia and postglacial expansion to the Arctic is both very interesting and significant. Although the idea and results are great, drawing conclusions based on one genetic marker is tricky, which I can tell from my own experience. The results for *S. balanoides*, for instance, sound reasonable due to highly dispersive larvae, but the last chapter (Research paper no. 3) showed it is not fully valid when genome analysis is applied. What I missed in the manuscript are some basic information such as the intraspecific genetic distance in analysed species and the threshold for the species delimitation. For example, in rotifers or tardigrades 3% in COI is usually used as the threshold. Could the PhD candidate say that in more isolated populations, the COI distance is big enough to talk about observation of speciation?

IV. Chapter III (Research paper no 3)

The last part is presented as an unpublished manuscript of the scientific papers. The document includes all important parts of a classical scientific paper to be published in international journals. Although I found numerous, yet minor editorial issues, this part is definitely my favourite one. The PhD candidate used a low coverage whole genome sequence of *Semibalanus balanoides* for the investigation of the demographic history and origin of the species in the High Arctic Svalbard archipelago. As the author rightly noticed in the Introduction, the genetic resolution of the single marker was not sufficient to infer demographic parameters with precision in *S. balanoides*. To my



delight, the author proved that high coverage whole genome sequencing is not required in phylogeography, and low coverage genome sequencing provides sufficient resolution for biogeographic studies. Contrary to the previous paper (Chapter II), the authors disprove the assumption that the population of *S. balanoides* is closely related and likely panmictic. Although the larva of *S. balanoides* is considered highly dispersive, the different, precise approach showed that its populations are geographically structured into four distinct groups. The main conclusion of the analyses indicates that the divergence of the High Arctic population is dated to Holocene Thermal Optimum instead of Anthropocene.

The only, minor concern I have recognized in all chapters, is the author's assumption that the presence of boreal taxa in the Arctic is commonly thought to be the effect of the recent atlantification. First of all, it would be great to define what "contemporary atlantification" is and when it started. It varies between scientific perspectives. Secondly, Atlantification increase the abundance of some "boreal" species in the Arctic, but without a long-term monitoring and faunistic records dating back to the end of LIA, the settling of "new" species in the Arctic through contemporary Atlantification is vague and virtually a speculation. However, I am glad to observe that the author is struggling with the unsupported opinions through the means of modern, scientific proofs.

V. Overall evaluation

I rate the scientific value of the thesis as high. The 1st chapter has already been used by other scientists. To be honest, from the very moment the chapter was available online, I have been showing it to my students during lectures. I am convinced the chapters II and III will be used by both the current and next generation of scientists in their studies on Atlantification, Arctic borders and species migration.

The PhD of Hedvig Kriszta Csapó significantly contributes to the knowledge on the phylogeography of intertidal marine boreal species with an important discussion on the effects of warming periods on the expanding of the species into the Arctic.

The thesis meets all the requirements imposed on PhD theses as specified in art. 13, section 1 of the Act on academic degrees and academic title and degrees and title from 14 March 2023. I am therefore asking The Scientific Council of the Polish Academy of Sciences to admit Hedvig Kriszta Csapó to further stages of the doctoral dissertation process.

In view of the innovative nature and high professional level of the PhD thesis, I am asking for recognizing thesis with honour.