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Kraków, dn. 23.08.2024

Review of the PhD thesis by M.Sc. **Hedvig Kriszta Csapó**, entitled

**Phylogeography and population genomics of boreal marine
macroinvertebrates colonising High Arctic**

**(Filogeografia i genomika populacji borealnych morskich makrobezkręgowców
kolonizujących Wysoką Arktykę)**

supervised by
prof. dr hab. Michał Grabowski
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The reviewed PhD thesis focuses on processes related to “Atlantification”, a process of increased influence of Atlantic waters on Arctic observed in recent years. This phenomenon influences phylogeography and population genetics of various groups of organisms from different trophic levels, including some invertebrates inhabiting the Svalbard archipelago, which are the subject of the M.Sc. Hedvig Csapó’s research interests.

M.Sc. Hedvig Csapó’s PhD thesis consists of three main parts: two scientific articles already published, and one manuscript looking ready to be submitted for review in a scientific journal. These three articles are preceded by a short summary of the dissertation content written in two versions – in Polish and English. The summary presents in a concise and clear manner the most important assumptions and results of the studies.

The first of presented articles (hereinafter referred to as Article 1) is entitled “**Coming home - Boreal ecosystem claims Atlantic sector of the Arctic**” and was published in a renowned journal *Science of The Total Environment* in 2021 (IF in the year of publication =

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10.75). Hedvig Kriszta Csapó is both the first and corresponding author, which confirms her leading role in preparing the publication. It is worth noting that this article, although published relatively recently, has already been cited many times (38 citations according to Scopus database). This paper is an extended review article, discussing the current state of knowledge on the biogeographical and ecological aspects of the “Atlantification” phenomenon in the European Arctic. 73 publications (regarding both fauna and flora) were the subject of this review. What is very important is that in addition to the summary of the most important data from the mentioned publications, the article also contains a more in-depth general analysis and conclusions are drawn. The Authors’ key conclusion is that they question the currently dominant concept of “Atlantification” as a process resulting solely from the impact of human activity on climate change. Instead, the Authors suggest that the currently observed changes (generally a northward shift in the ranges of many taxa) are in fact recolonizations, i.e. returns to areas where these organisms lived in the past, during warmer climatic periods. Many species are thus neonatives, and distinguishing them from aggressively spreading invasive species is a challenge for marine biologists.

These are not the only conclusions drawn from this review. The Authors confirmed that the rate of primary production in open seas of the Arctic shelf increases. There is also the tendency to decrease energy content of zooplankton in Arctic waters. Important changes are visible also on higher levels of trophic level, for example fishes, where increase of body size and growth rate were observed. These and other conclusions clearly indicate, that currently observed changes in biogeography and genetic structure of fauna and flora of Arctic cannot be simply explained by possible human-induced climatic changes but must be analysed also against the backdrop of historical climate change.

The second of the three articles that make up Ms. Csapó's doctoral thesis (hereinafter Article 2) is entitled “**mtDNA data reveal disparate population structures and High Arctic colonization patterns in three intertidal invertebrates with contrasting life history traits**”. It was published in December 2023 in journal *Frontiers in Marine Science* (IF=2.8, no citations so far). In this article, M.Sc. Hedvig Csapó is also both the first and corresponding author. It presents research on phylogeography and historical demography in Arctic of three boreal invertebrate species with different life histories and dispersal mode: an amphipod *Gammarus oceanicus*, a gastropod *Littorina saxatilis* and a barnacle *Semibalanus balanoides*. Methodology of the study based on large amount of mitochondrial DNA barcode data, including both newly obtained and reference sequences of the mitochondrial cytochrome oxidase I (COI). The work employs a wide range of modern molecular data analysis techniques, allowing for drawing reliable conclusions regarding intraspecific genetic variability, population structure, demography and phylogeography. The analyses were conducted separately for the three studied species, finding both similarities and differences in the genetic structure of their populations and the most probable scenarios for the expansion to Svalbard. Mentioning only the most important conclusions of the work, in the case of *G. oceanicus* and *L. saxatilis* (both directly developing) decrease of haplotype richness towards higher latitude regions was detected. Such pattern was not observed for *S. balanoides*, a species with pelagic larvae. The Authors conclude that all three species expanded their range northwards over past 20,000 years, while stating the lack of evidence that it was a contemporary process related to “Atlantification”.



The third part of PhD thesis reviewed here is a manuscript (hereinafter Article 3) entitled “**Distinct population structure and demographic history of the northern acorn barnacle argue against its spread in the High Arctic as a result of recent Atlantification**”. This work is a continuation of research on acorn barnacle (*S. balanoides*) included in Article 2, but this time using low-coverage whole genome sequence data. The authors tested two alternative hypotheses considering the origin of Svalbard populations of this species. The first hypothesis assumed that the presence of acorn barnacle in Svalbard is an effect of current “Atlantification” of the Arctic, while the second assumed that the source of genetic diversity of the species in Svalbard is dated on the last glacial maximum (LGM). Analysis of genetic structure of populations of *S. balanoides* from Svalbard and other geographic regions (Iceland, Faroe Islands, UK and Scandinavia) revealed high level of inter-population diversity. This is surprising for two reasons. Firstly, the acorn barnacle possesses pelagic larvae in its life cycle, which should allow a high dispersion rate. Secondly, research on this species described in Article 2, conducted on single mitochondrial marker, provided conclusions that were quite the opposite, suggesting existing rather panmictic, large population of this species in North Atlantic. This provides strong evidence that in the case of marine species with large range, single genetic markers may not provide sufficient information in studies focusing on population structure and biogeography. Overall, the research results presented in this article provide evidence to support the second hypotheses tested. The distinctiveness of Svalbard population and lack of genetic footprint of a recent bottleneck support the scenario of colonisation of the archipelago not currently, but during Holocene Thermal Optimum.

While reading Articles 2 and 3, one question arises. Is there any other than genetic evidence of expansion of studied species northwards after LGM? It is to be expected that paleontology may provide interesting data, especially in the case of animals with hard body parts (like *Littorina* or *Semibalanus*). In Article 1, an example of the North Atlantic mussel, *Mytilus edulis*, is mentioned as detected in fossils of Svalbard dated at ca. 12–13 kya. Since Svalbard is an area of intense biological research, I would expect that such data might also exist for *Littorina* or *Semibalanus*. However, there is no information about this in Articles 2 and 3. Even the absence of such data should be noted.

My second question to the Author concerns the taxonomy of *S. balanoides*. Table 1 in Article 3 presents the intragroup nucleotide diversity. Could the degree of genetic differentiation between populations/groups, combined with the reconstructed phylogeny and possible morphological and anatomical data, lead to a future taxonomic revision of this species?

The three papers presented by M.Sc. Hedvig Csapó as a doctoral dissertation are closely related thematically. Although Article 1 is not strictly research work, as a review work it is based on a large number of analyzed publications (73). The review contains important conclusions, as mentioned above, and serves as an excellent basis for further research of a strictly empirical nature. It also demonstrates M.Sc. Csapó's impressive knowledge and expertise in the field. Articles 2 and 3 are strictly research works, aligning perfectly with current scientific trends. The PhD student mastered and utilized a wide range of molecular techniques and, what is particularly important in this type of research, methods of modern



data analysis. Presented studies provide interesting information on the phylogeny and biogeography of the selected invertebrates, but they also have a much broader significance. The obtained results contribute new knowledge and allows for a better understanding of the currently observed phenomenon of shifting the ranges of many boreal marine species towards the north. While it seems quite attractive to claim that these processes are a very recent phenomenon and result from climate warming caused by human activity, the picture that emerges from the presented studies is much nuanced. The review analysis (Article 1) and the empirical studies presented in Articles 2 and 3 indicate that these processes are rather related to climate changes occurring on a broader time scale and resulting from alternating cycles of glaciations and interglacials.

The editorial quality of the discussed doctoral thesis does not raise any significant objections. Articles 1 and 2 are presented in their original journal format, having undergone meticulous editing and copyediting processes. The Summary and Article 3 are presented as manuscripts and they contain only a very few editorial errors. For example, the Polish summary uses both "lat p.n.e." and "BP" simultaneously, which is inconsistent. It should be noted that "lat p.n.e." are dated from the year 0 AD, while BP is traditionally counted from the year 1950. The English version of the Summary does not contain this error. I also noticed that in the list of references to Article 3, italics were omitted in a few Latin species names. This should be corrected before submitting the article to a journal. These very minor editorial shortcomings do not detract from the high value of the presented work. I mention them only as a part of my reviewer's duty.

Final conclusions

In conclusion, my assessment of the scientific research presented by M.Sc. Hedvig Kriszta Csapó in his doctoral thesis is highly positive. The statements of the co-authors confirm M.Sc. Csapó's significant contribution to the articles presented, including fieldwork, laboratory and statistical analyses, data processing, manuscripts preparations and revisions. M.Sc. Csapó demonstrated extensive theoretical knowledge in the field of science being studied, and the presented research provides an original solution to a scientific problem. The leading role of M.Sc. Csapó in the presented studies is proof of Her readiness to independently conduct scientific activity. The reviewed dissertation meets the statutory requirements specified in Act of 14 March 2003 on Academic Degrees and Academic Title and on Degrees and Title in the Field of Art (consolidated text: Journal of Laws of 2017, item 1789, as amended) in connection with the Act of 3 July 2018, Provisions introducing the Act – Law on Higher Education and Science (Journal of Laws of 2018, item 1669, as amended) and I request the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences in Sopot to admit M.Sc. Hedvig Kriszta Csapó to the further stages of the doctoral procedure. Noting the high scientific level of the presented research, some of which has already been published in highly rated journals, and the wide range of M.Sc. Csapó's skills, I propose that the work be considered for distinction.



Wnioski końcowe

Podsumowując, moja ocena badań naukowych przedstawionych przez mgr Hedvig Csapó w jej rozprawie doktorskiej jest bardzo pozytywna. Oświadczenia współautorów potwierdzają dominujący wkład mgr Hedvig Csapó w powstanie trzech prezentowanych artykułów, obejmujący m.in. pracę terenową, analizy laboratoryjne i statystyczne, przetwarzanie danych, przygotowanie oraz korektę manuskryptów. Doktorantka wykazała się szeroką wiedzą teoretyczną z dziedziny będącej jej obszarem zainteresowań, a prezentowane badania są oryginalnym rozwiązaniem problemu naukowego. Wiodąca rola Doktorantki w prezentowanych badaniach jest dowodem jej gotowości do samodzielnego prowadzenia działalności naukowej. Stwierdzam, że recenzowana przeze mnie rozprawa doktorska mgr Hedvig Csapó spełnia wszelkie warunki ustawowe stawiane pracom doktorskim na podstawie Ustawy z dnia 14 marca 2003 roku o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (t. jedn. Dz. U. z 2017 r. poz. 1789 ze zm.) w zw. z Ustawą z dnia 3 lipca 2018 r. Przepisy wprowadzające ustawę – Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1669 ze zm.) i wnioskuję do Rady Naukowej Instytutu Oceanologii Polskiej Akademii Nauk w Sopocie o dopuszczenie mgr Hedvig Kriszta Csapó do dalszych etapów przewodu doktorskiego. Zwracając uwagę na wysoki poziom naukowy przedstawionych badań, z których część została już opublikowana w wysoko punktowanych czasopismach, oraz szeroki wachlarz umiejętności Doktorantki, składam wniosek o wyróżnienie pracy.

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