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Instytut Systematyki i Ewolucji Zwierząt
Polskiej Akademii Nauk

ul. Sławkowska 17 • 31-016 Kraków

dr hab. Łukasz Kajtoch, prof. ISEZ PAN

Institute of Systematics and Evolution of Animals

Polish Academy of Sciences

Sławkowska 17 31-016 Kraków

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REVIEW

of the doctoral dissertation authored by BERNARDO FLORES ANTUNES

titled „Landscape genomics of newts: habitat, population connectivity and multi-scale gene flow”,

PhD student in the Doctoral School, Faculty of Biology, Jagiellonian University in Kraków,

the Genomics and Experimental Evolution Group

prepared in connection with applying for a doctoral degree

in the field of natural sciences and the discipline of biological sciences

Supervisor: Prof. dr hab. WIESŁAW BABIK

Auxiliary Supervisor: dr. PIOTR ZIELIŃSKI

I was appointed to perform the review by decision of the Council in the Discipline of Biological Sciences of the Jagiellonian University dated June 27, 2023.

INTRODUCTION

Understanding current population structure and connectivity becomes a crucial task in ecological studies, as well as in research intended to understand factors important for population conservation or management. Formerly, knowledge of populations was limited by the availability of data, which allowed for populations to be described and understood in space and time. Classical studies based on observational information or capturing and marking individuals were not sufficient to resolve many issues. Also, knowledge of the environment was limited to a general visualization of landscape characteristics. The development of two techniques, high-throughput sequencing and geographic information systems, allowed for the structure and connectivity of wild populations to be understood with respect to various landscape and environmental characteristics. In this way, landscape genetics came into being and this technique was used by the PhD candidate, Mr. Antunes, to understand which landscape factors share in the population structure and connectivity of two sibling newt species. These taxa were objects of extensive previous research conducted by his Supervisors in the last decades. This previous research on phylogeny, phylogeography and hybridization of *Lissotriton* newts offered a strong background for the dissertation that is the object of this doctoral procedure.

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FORMAL EVALUATION

The dissertation presented for review was based on three English-language chapters: one article already published, one manuscript accepted for publication at the time of submitting the dissertation (published on June 30, 2023) and one manuscript not yet submitted to any journal.

1) **Antunes, B**., Figueiredo-Vázquez, C., Dudek, K., Liana, M., Pabijan, M., Zieliński, P., & Babik, W. (2022). Landscape genetics reveals contrasting patterns of connectivity in two newt species (*Lissotriton montandoni* and *L. vulgaris*). Molecular Ecology, 00, 1–16.

2) **Antunes, B**., Dudek, K., Pabijan, M., Zieliński, P., & Babik, W. (2023). Past forest cover explains current genetic differentiation in the Carpathian newt (*Lissotriton montandoni*), but not in the smooth newt *(L. vulgaris*). Diversity and Distributions, 00, 1–12.

3) **Antunes, B**., Palomar G., Dudek, K., Pabijan, M., Zieliński, P., & Babik, W. Habitat connectivity inferred from landscape genetic models at multiple spatial and temporal scales in two newt species (*Lissotriton montandoni* and *L. vulgaris*)

The articles were published in reputable and high-ranked journals that are classified in the 1st quartile (Molecular Ecology, IF=4.90; Diversity and Distribution, IF=4.60).

In all of these chapters, the PhD candidate is listed as the first and a corresponding author, which clearly indicates his substantial role in conducting research and writing scientific publications. This is supported by the statements provided by all other authors. According to these statements, the contribution of Mr. Antunes was 52%, 60% and 60%, respectively. These statements informed that other authors participated in all steps of this research at various contribution levels.

The materials provided have been prepared with due diligence and, in my opinion, meet the formal requirements.

GENERAL EVALUATION

The dissertation submitted for review consists of 161 pages, including summaries, an introduction to the dissertation, three chapters (manuscript and publications), conclusions, statements from the co-authors of the publications, additional statements and acknowledgements. The dissertation was prepared in English (except the Polish version of the summary).

The title of the doctoral dissertation is adequate for the research problems undertaken and the content of the doctoral dissertation. The dissertation as such is an important contribution to understanding the landscape genetics of newts in the Carpathians. The cycle of three chapters, together with the

introductory and summary part of the dissertation, constitutes a coherent and logical whole. It is worth appreciating the rich graphic design, well made and clearly showing the information and results.

DETAILED EVALUATION

The summary is clear and concise. It presents the general ideas, results and conclusions of the undertaken research.

In general, the introductory part of the dissertation presents the necessary background of the study. It starts with an overall summary of what could be achieved in conservation biology when using the newest technological achievements in genotyping and geographic information systems. This chapter introduces concepts of landscape genetics, in order to examine species structure and connectivity and to link genetic characteristics to data about geography and habitats. The author also explains the importance of proper study design, which could be challenging considering "time lags" in population responses to environmental constraints. Next, the introduction chapter describes amphibians as the objects of landscape genetic studies. Finally, Mr. Antunes introduces the major objects of his studies, Carpathian newts and smooth newts, with references to previous studies by the research group led by his Supervisor, Prof. W. Babik, as well as other researchers. This elaboration on the state of knowledge is then used as justification for the aims of the dissertation. Previous research, starting with phylogenetic relations and revealing speciation processes, through to phylogeographic analyses, and on to the examination of recent and current hybridization, showed that the genetics among populations of both newts species is complex, with many interesting phenomena, but they still have unclear relations with the environment. That issue is selected for examination in the PhD dissertation and broken down into three parts, which constitute logical and chronological research sequences. The major idea of the whole study is that common and generalist species (e.g., the smooth newt) is less susceptible to unfavorable landscapes than the Carpathian newt, which inhabits only mountain forests. In the general introduction, no methodological information about sampling, GIS measurements, laboratory works, bioinformatics or statistics is presented but all of these are clearly presented in the following chapters, which are either articles or manuscripts.

The first chapter explains how landscape constraints (restricted to the presence of forests and agricultural areas) affect gene flow in populations of both newt species collected in several plots localized in the Eastern Carpathians. Specifically, this study compared models describing isolation by distance to models describing isolation by (environmental) resistance. The genetics of the Carpathian newt population were found to be restricted by the presence of forest cores, whereas

genetics of the smooth newt were found to be associated with forest edges. Surprisingly, the second species was found to be more affected by anthropogenic landscapes, which resulted in stronger isolation and lower effective sizes in relation to the first species of newt.

The second chapter comes from the previous article but extends the research aims into the temporal framework. In detail, the authors used previous data on the genetics of both newt species but extended their analyses to approximately half a century ago, considering changes in forest coverage and continuity since 1963. Resistance models based on these historical data revealed a time lag for the Carpathian newt, where the genetic structure of the population could be better explained by forest data from 40 years ago (c. 10 generations in the past). On the other hand, no time lag was detected for smooth newt, as the genetic structure of this species' population was best explained by current habitat destruction and fragmentation. Contrary to the previous chapter, this article considers two subspecies of smooth newt separately (*L.v. vulgaris* and *L. v. ampelensis*).

The third chapter also comes from previous research, but this time it focuses on intensively sampled populations in the Polish Eastern Carpathians and includes more variables describing the landscape (incl. topography, hydrography and roads) and the set of spatial scales, extending all the way back to 1860. Results support previous findings about the crucial role of forest cover and connectivity for the genetic structure of both newt species' populations, whereas the negative effect of anthropogenic areas was clear for the Carpathian newt but almost undetectable for the smooth newt. Moreover, models built using the land cover available within short distances predicted the populations' genetic differentiation better than models based on larger scales. Finally, this study showed that relatively recent changes in landscapes (c. 50 years) better explain the genetic structure of the newt species' populations, likely being a reflection of forest restoration.

The dissertation continues with the "General Discussion" chapter, in which Mr. Antunes describes all the findings of his studies, referring to previous research on newts as well as other genetic research conducted involving the landscape of other amphibians and animals. Findings and interpretations are provided in detail on the connectivity of newt populations, an emphasis on the need for replicating the sampled populations in order to make generalizations and the advantages of examining data on multiple spatial and temporal scales, which enable explanations to be unraveled that otherwise cannot be detected using other means. This chapter is very interesting and well prepared but as it was read after reading certain other chapters, it is also redundant, since many parts describe the same information as preceding chapters in nearly the same way.

Finally, the dissertation ends with two chapters. The first includes recommendations for the conservation of newts. The PhD candidate lists findings that could be useful to better manage the habitats of Carpathian and smooth newts, namely implications about the impact of forest discontinuity on effective gene flow among populations, considered on various spatial and temporal scales. The last chapter summarizes the findings of the study; it is again well written but highly redundant, given the information in the "Summary," the articles and the "General Discussion."

The task of the reviewer is to indicate both the strengths and ambiguities of the doctoral dissertation. I found several linguistic errors and words that were seemingly translated incorrectly in the Polish version of the summary. While reading this dissertation I found other issues that, in my opinion, were questionable and I noticed that some issues were omitted (like missing topography or hydrography as landscape factors in modeling or not considering possible differences between the two subspecies of the smooth newt). The majority of these questions were resolved when I moved through subsequent chapters, where I found that Mr. Antunes already noticed and incorporated these issues in consecutive studies. In this way, I found answers to nearly all my questions in the dissertation. I describe some of the remaining comments and questions about the dissertation below.

1) The sampling was organized in the Carpathians. It is a typical habitat of the Carpathian newt. However, the smooth newt is mostly a lowland species, with some populations in the lower mountains, where it is sympatric with the former species. I am aware that the sampling for this research was taken from previous research already published. Also, the sampling required the presence of both species in either the same or similar landscapes. However, I wonder if sampling smooth newts on the altitudinal verge of their range in mountains could have some consequences for the genetic patterns and conclusions observed in the landscape. I can imagine that some findings, like the lower impact of forest cover on the genetic structure of the smooth newt population and having detected higher isolation and low effective population sizes, could be affected by the fact that the sampled populations were from suboptimal habitats for this species. It would be interesting to see the results of landscape genetics on the lowland populations of this newt, as forest distribution and continuity could be very different in the lowlands than mountains. Moreover, the dispersal abilities of smooth newts could be more restricted in mountains than in lowland areas. The authors of the articles and manuscript justify their choice to study populations of both newts in foothills and in the lower mountains, but it seems that the decisions made for purposes of previous hybridization studies prevail over any potential problems caused by sampling the smooth newt in a suboptimal

environment. Or maybe I am wrong and the Carpathians are in fact an optimal habitat for this species? This question is supported by the sentences found on page 108, which support that elevation was an important factor contributing to the models used to explain the genetics of newt populations.

2) Although hydrography was used in the modeling in the third chapter, I still think that it could not be enough. The authors used data about the distribution of major rivers but the location of bodies of water (e.g., natural lakes or artificial ponds and pits) was never included among the environmental factors in any part of this research. I suppose that perhaps there are no appropriate GIS layers including such types of habitats in the Eastern Carpathians. Was it not possible to generate a layer showing the distribution of bodies of water based on the field inventory and an investigation of the maps? Mr. Antunes was also aware of this problem of omitting bodies of water from the variables used in modeling (p. 134).

3) On page 11 is the sentence: "The ranges of the two species meet across an ecotone at lower elevations in the Eastern Carpathians (…)" I wonder if such a zone is absent in the Western and Southern Carpathians or the Eastern Sudetes?

4) On page 134, among the implications for conservation is a sentence suggesting that reforestation "…does not seem to be dense enough to increase connectivity…" in order to restore proper habitats for newt populations. Certainly it is important for newt protection, however, the trend of reforestation in the Carpathians accelerating in recent decades (by natural succession or planting trees) is not beneficial for many other organisms in these mountains that are rare and threatened.

ASSESSMENT OF ACHIEVEMENTS

According to a search on Web of Science (08/08/2023), Mr. Antunes authored six publications, including two articles, as a part of his PhD thesis. He is the first author listed in two of the remaining articles. These articles were published in reputable journals in the fields of evolutionary biology (*Heredity*, *Evolutionary Applications*, *Evolutionary Ecology*) and conservation biology (*Conservation Genetics*). The topics of all these papers are related to the landscape genetics and conservation genetics of amphibians (newts and salamanders). Three of these articles come from research conducted outside of his doctoral research. His works have been cited approximately 60 times. He obtained funding from the National Science Center (Preludium grant) for the topic strictly related to his PhD thesis. Everything mentioned above shows that the current achievements of Mr. Antunes are extraordinary for a young scientist and they are good predictors of his future professional career.

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FINAL CONCLUSIONS

In my opinion, the doctoral dissertation of Mr. Antunes have very high scientific value. His doctoral research contributed significantly to the state of knowledge on the landscape genetics of amphibians in Europe, with particular significance placed on his research on understanding the impact of landscape characteristics on population and conservation genetics of protected species: Carpathian newts and smooth newts. In his research, the PhD candidate demonstrated his knowledge and laboratory skills, GIS skills and analytical work. The proficiency of Mr. Antunes is noteworthy in the use of molecular and bioinformatics methods, as well as spatial analyses and advanced statistics.

I, the undersigned, certify that the reviewed doctoral dissertation of Bernardo Flores Antunes, MSc, meets the conditions set out in Art. 187 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws of 2018, item 1668 with amendments) and I apply to the Council in the Discipline of Biological Sciences of the Jagiellonian University for advancement of the PhD student in further stages of the procedure for conferring a doctoral degree.

Łukasz Marcin Kajtoch

Elektronicznie podpisany przez Łukasz Marcin Kajtoch
DN: c=PL, serialNumber=PNOPL-80010206478, cn=Łukasz Marcin Kajtoch, givenName=Łukasz Marcin, sn=Kajtoch
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Dr. hab. Łukasz Kajtoch

The doctoral dissertation of Mr. Antunes is an original study on an important research topic that was successfully completed by the PhD candidate, as evidenced by the completion of scientific manuscripts, two of which have already been published in reputable journals. The PhD candidate undertook the exploration of research topics that constitute a major inventive, methodological and computational challenge. The research conducted by Mr. Antunes significantly broadens the collective knowledge about the landscape genetics of two vulnerable newt species. His works also contributed substantially to analytical approaches based on genetic and digital geographic data with respect to various spatial and temporal scales. Particularly, his analyses that allow for a historical review when explaining contemporary population genetics are innovative and worthy of appreciation. The implications presented for the conservation of protected newts and forest management, in order to ensure proper connectivity of amphibian populations, are also important. For the above reasons, I recommend the doctoral dissertation of Bernardo Flores Antunes, MSc, for distinction.

Łukasz Marcin Kajtoch

Elektronicznie podpisany przez Łukasz Marcin Kajtoch
DN: c=PL, serialNumber=PNOPL-80010206478, cn=Łukasz Marcin Kajtoch, givenName=Łukasz Marcin, sn=Kajtoch
Data: 2023.10.05 15:12:22 +02'00'

Dr. hab. Łukasz Kajtoch