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Review of the doctoral dissertation of MSc Bernardo Flores Antunes „*Landscape genomics of newts: habitat, population connectivity and multi-scale gene flow*”

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Formal comments

The doctoral dissertation of MSc Bernardo Flores Antunes consists of two published papers: 1) Antunes et al. 2022. *Landscape genetics reveals contrasting patterns of connectivity in two newt species (Lissotriton montandoni and L. vulgaris)*. *Molecular Ecology* 32: 4515-4530; 2) Antunes et al. 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 *Diversity and Distributions* 29: 1129-1140; and 3) an unpublished manuscript entitled “*Habitat connectivity inferred from landscape genetic models at multiple spatial and temporal scales in two newt species (Lissotriton montandoni and L. vulgaris)*” and enclosed Supplementary materials. The dissertation contains also the Summary in Polish and in English, the Introduction, the General Discussion, Statements of all of the co-authors of three works and Acknowledgments. The papers have been published in very high impact peer-reviewed scientific journals - the first in *Molecular Ecology* (IF = 4.9, Q1 in Ecology and Evolutionary Biology) and the second in *Diversity and Distribution* (IF = 4.6, Q1 in Biodiversity Conservation and Ecology). Mr Antunes' PhD study was supported by several sources of funding: the ATUT PhD Programme in Biology implemented at the Faculty of Biology at the Jagiellonian University funding (co-funded by the European Union), the Polish National Centre Preludium grant, and the DSC grant from the Faculty of Biology at the Jagiellonian University awarded to the author.

Evaluation of the candidate

Mr Antunes is the first and the corresponding author in two papers and the unpublished manuscript presented in the dissertation and the PI of the Preludium and DSC grants, that supported the PhD study. Based on the statements of the co-authors, I assume that the contribution of MSc Bernardo Antunes to the published papers and the manuscript varied between 52% (Antunes et al. 2022) and 65% (Antunes et al. 2023). According to the authors' contributions in the published papers, Mr Antunes participated in the design of the study (as described in the first and second papers), the field work, the laboratory work, the genomic

analyses (in the second paper) and the writing of the manuscript (in the first and second papers). On the basis of the information provided in the published papers, I assume that the author has been the main person in charge of the landscape genetic analyses in the whole project. Unfortunately, I did not find in the submitted PhD thesis any detailed information on the contribution of MSc Antunes to the manuscript of the third (unpublished) paper. It will be easier to assess the role of the candidate in the submitted study if the author provides a clear statement of his contribution to the PhD project in the submitted dissertation. As one of the requirements of the review is an evaluation of the PhD candidate, it would be helpful if the dissertation also contained a list of all other publications by MSc Bernardo Antunes. In any case, I have no doubt that Mr Antunes has played a significant role in the presented study and has fully complied with the formal requirements for PhD candidates in Poland.

Scientific significance of the study

Landscape genetics is a rapidly developing discipline, especially in recent years, that links population genetic (or genomic) data with landscape variables to answer the question of how landscape structure shapes genetic variation and gene flow between populations of the species under study. The aim of Mr Antunes' study was to investigate landscape connectivity in two closely related but ecologically distinct newt species, the widespread generalist *Lissotriton vulgaris* and the Carpathian endemic *L. montandoni*, using genomic and landscape data and advanced statistical methods. The material used in this study is very large (varying between 554 and 3544 samples collected in the years 2010-2021 from 73 to 179 localities in 6 regions of the Eastern Carpathians and between 6453 and 22846 SNPs, depending on the datasets used in different types of analyses). Some of the genomic data used in the study came from previously published studies.

The novelty of this project lies in the combination of genomic and landscape data at multiple spatial and temporal scales to gain a better understanding of movement and gene flow in two sister newt species. The study design is rather unique in landscape genetic research as it included replicated sampling (several regions), considered the effect of current and past forest cover on the current genetic variability of the studied organisms, and included genomic data of two closely related species with different ecological requirements. The landscape genetic analyses were carried out at a large spatial scale, covering the Eastern Carpathians, and at regional and local scales in the Podkarpackie region (south-eastern Poland), in areas where both species occur in parapatry. The data used in the study included contemporary landscape variables and historical forest cover data (since 1960 for the large scale and from 1860 to 1970 years for the regional and local scale analyses).

The results obtained showed that forested habitats at distances close to the species' maximum dispersal range per generation (1 to 2 km) had a positive effect on connectivity. *L. vulgaris* was associated with open forest habitats, whereas *L. montandoni* preferred closed forests. *L. vulgaris* populations were smaller and more isolated compared to *L. montandoni*. Although *L. vulgaris* is less sensitive to anthropogenic landscapes than *L. montandoni*, landscape change at local scales had a significant impact on the survival of the population. In my opinion, the most important, novel and interesting results of this study are those showing that genetic variation within populations reflects long-term population size and historical connectivity, and

that differentiation between populations corresponds to recent landscape connectivity, not older than 40 years (approximately 10 newt generations). Although *L. vulgaris* populations were smaller and vulnerable at local scales, genetic variation was still present and could be protected if suitable forested habitats for *L. vulgaris* were restored. The study also clearly showed that each of the species (even closely related and occurring in the same area) responded differently to landscape change. It should be stressed that the results of this study are important for planning effective conservation strategies for a globally declining group of vertebrates such as amphibians.

Detailed comments

I noticed that the Summary in Polish contains many grammatical and editorial errors, which sometimes make the text difficult to understand and follow. There are also grammatical errors in the co-authors' statements. I think they could easily be revised with a little help from the Polish-speaking collaborators.

Most of the figures in the text are small, the fonts are very small, the lines are very thin and therefore many of the figures are difficult to read. The dissertation is in manuscript form, so there is no space limitation and it would be possible to make them larger and easier to read.

There is not much detailed information about the study areas and study sites, and the maps showing the study areas are difficult to read. It would be worth adding more details about the study regions and sites, e.g. in the supplementary material.

Chapter I

Figure 1a - It would be easier to recognise the locations of your study areas if you at least added geographical coordinates to the map.

Study area - more details about the sampling areas would be helpful (e.g. in which countries they are located).

Status of the study area - it would be helpful if the author could also describe the management status of the study area, e.g. does the study area include nature reserves or is it mainly managed state forests, agricultural and urban areas? This is important information in the context of future landscape changes and implications for the long-term existence of the studied populations.

Sampling areas and regions: do they mean the same thing? This is not clear to me. Also, full names and their abbreviations should be used together for the first time in the text.

Chapter II

In the Abstract and in the Introduction you mentioned about two species studied. According to the Admixture analyses both species are divided into two genetic clusters but then you presented the results of landscape genetic analyses of three evolutionary units (*L. montandoni* and two subspecies of *L. vulgaris*). Could you explain this discrepancy?

Fig 1 Polygons representing different study areas are difficult to distinguish from the background.

Are the study sites and regions similar or partly the same as in the Chapter I?

Supplementary materials:

I could not find any information about laboratory where the sequencing on the Illumina MiSeq platform was performed.

I think that Tables S1-S5 should be included in the text of the dissertation. It would be helpful to have them in the manuscript as supplementary material rather than having to search for them on the internet.

Discussion

It will be worth discussing the quality of the reforested areas (e.g. whether the young forests are equally suitable habitats for both species). Are they rather dense or open forests? Is it a natural reforestation process or are they planted (managed) forests? These aspects may be worth mentioning in the context of the competition between these two species.

Chapter III

Lack of a statement on the contribution of each of the co-authors to the study presented.

Similar comments regarding the size of figures and their readability as above.

Tables S1-S3 should be included in the manuscript.

General discussion

I think it will be interesting to mention here if any of the regions studied could play a role as a refugia for the long-term existence of the studied species? Are in your study regions areas where there will be no significant changes in land-use in long-term perspective? And in opposite, in which regions the studied populations are most threatened by urbanisation and infrastructure development plans?

What will be your recommendations for the conservation of the studied species in the context of landscape change plans? It will be worth adding them in the final conclusions of the General discussion.


Final conclusions

The above comments are rather minor and do not diminish the scientific and conservation value of the study presented in the PhD thesis written by MSc Bernardo Antunes. The results of the analyses carried out by the Doctoral candidate significantly increase our knowledge of

connectivity and gene flow between populations of two sister but ecologically distinct species of newts at different spatial and temporal scales. The obtained results also have important implications for planning an effective and long-term conservation strategy for the studied species.

Finally, the PhD thesis submitted by Mr Bernardo Flores Antunes, entitled: "*Landscape genomics of newts: habitat, population connectivity and multiscale gene flow*" fully complies with the requirements for doctoral theses set forth in Article 187 of the Law on Higher Education and Science of July 20, 2018 (Journal of Laws of 2018, item 1668, as amended). I request the Council of the Discipline of Biological Sciences of the Jagiellonian University to admit Mr. Bernardo Flores Antunes, M.Sc. to the further stages of the doctoral procedure.

Podsumowując, rozprawa przedłożona przez Pana mgr. Bernardo Floresa Antunesa pt.: „*Landscape genomics of newts: habitat, population connectivity and multi-scale gene flow*” w pełni spełnia wymogi stawiane pracom doktorskim określone w artykule 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późn. zm.). Wnioskuje do Rady Dyscypliny Nauki biologiczne Uniwersytetu Jagiellońskiego o dopuszczenie Pana mgr. Bernardo Floresa Antunesa do dalszych etapów przewodu doktorskiego.



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