PhD Thesis Acceptance Report

Scientific Council of Institute of Dendrology Polish Academy of Sciences

Candidate's name and surname: Berika Beridze

PhD Thesis Title: Evolutionary history and conservation genetics of *Castanea sativa* Mill. in the South Caucasus

Thesis Supervisor: dr hab. Monika Dering, Poznań University of Life Sciences

Co-supervisor: dr Katarzyna Sękiewicz, Institute of Dendrology, Polish Academy of Sciences

Reviewer: dr hab. Elżbieta Cieślak, W. Szafer Institute of Botany, Polish Academy of Sciences

THESIS EVALUATION

1. Scientific merit of the thesis
   a. Originality of the research:

The reviewed doctoral dissertation addresses the fundamental issue of molecular biogeography/phylogeography, focusing on reconstructing the history and dynamics of processes leading to the formation of contemporary flora ranges in the Caucasus region. Due to its unique location and ecological conditions, this region is one of the most exceptional in Europe and globally. The research conducted by the doctoral candidate concentrates on analyzing the historical formation of the disjunctive distribution of the relict species *Castanea sativa* in the Caucasian forests and modeling future changes in its genetic structure, level of variability, and distribution in the context of ongoing and projected climate changes in the area.

Phylogeographic analyses populations of *C. sativa* from the Caucasus introduce new data compared to earlier research results on European populations. This provides comprehensive knowledge about the genetic variability of this species across both the Mediterranean basin and the Caucasus. Therefore, there is potential for understanding the evolutionary processes influencing the genetic diversity of *C. sativa* in the area of distribution. It is crucial to emphasize that comprehensive studies of the genetic structure of trees, based on good sampling, are essential for accurate inferences about the processes shaping the biodiversity of the Caucasian flora.

Given the already noted extinction processes of *C. sativa* populations in Europe, the dissertation also attempts to evaluate of survival of the species with observed and predicted climate changes in various Caucasus regions, using climate modeling methods. One of the research goals was determining the pace of the genetic pool depletion of Caucasian populations due to ongoing climatic changes in the area. An important aspect was evaluating the nature conservation management known as the "assisted gene flow" (AGF) strategy and determining its potential contribution to preserving genetic diversity in populations of *C. sativa* in the Caucasus.

Considering the limited information regarding the genetic variability of *C. sativa* in the part of the Caucasian range, the set objectives and research hypotheses are pivotal for understanding biodiversity in this strategic area. Given the biodiversity richness of the Caucasus and its significance as an endemic center, studying and interpreting genetic patterns at a regional level is fundamental for effective biodiversity conservation both locally and globally.

b. Scientific merit of the chapters / articles:

The dissertation encompasses three articles published in renowned, interdisciplinary scientific journals (JCR). All are based on comprehensive data and provide scientifically substantiated results. Below, I summarize their main achievements.

Using Bayesian inference and species distribution modeling, the research estimated the historical demographic scenario of the Caucasian *C. sativa* population. Findings confirmed that the fragmentation and divergence between the Caucasian and European *C. sativa* lineages originated during the Early Middle Pleistocene. Differences in the level of genetic diversity of population within the Caucasian population were attributed to survival in refugia in the region. One of the most important refugia is the Colchic Lowland, which served as a major climatic. Additionally, based on the level of allelic diversity of population, other refugia were identified in regions such as the Northern Caucasus and Northern Azerbaijan, which were previously overlooked in discussions about C. sativa evolution (Publication 1). This pattern suggests the possible existence of other refugia in the region beyond the major ones defined in Colchis and Hyrcania.

It was inferred that geographic isolation in the Caucasus was less intense than for common chestnut in Europe. Statistical analysis emphasized the significant role of long-distance gene flow in counteracting differentiation processes, suggesting that the complex topography of the Caucasus and neighboring regions did not hinder gene flow. Climate (isolation-by-ecology) was inferred as a significant factor shaping the genetic diversity of *C. sativa* in the Caucasus.

The subsequent research phase identified factors shaping the current genetic structure of Caucasian chestnut populations concerning historical climatic shifts and ecological conditions. Forecasts regarding the species' future distribution amid projected climate changes were then conducted (Publication 2). These studies hold significant potential for nature conservation, transitioning from traditional protective concepts to Assisted Gene Flow (AGF) strategy. Using landscape genetics and Species Distribution Models (SDMs), it was determined that Great Caucasus populations, given their significant variability and allelic diversity, exhibit great resilience against climate changes that may compromise their survival. Based on niche modeling and genetic diversity, it was possible to indicate a priority in the conservation management of *C. sativa* according to an in situ or ex-situ strategy.

Considering findings from Publication 2, indicating increased biotic stress for C. sativa due to anticipated climate changes in the Caucasus, advanced analyses were undertaken to evaluate the efficacy of the Assisted Gene Flow (AGF) strategy (Publication 3). The primary goal of AGF in endangered species conservation programs is to bolster adaptive

processes in vulnerable species by introducing individuals with beneficial alleles, enhancing population resilience against anticipated climate shifts. Based on detailed analyses of future species distribution models concerning projected climate scenarios from 2071-2100, the AGF strategy for *C. sativa* was deemed insufficient. This stemmed mainly from observed mismatches between species distribution changes and future climatic models. Notably, the dissertation's hypothesis verification offers a robust foundation for future research, especially concerning adaptive variability. Out of the presented hypotheses, five were confirmed, one received partial support, and one was rejected.

2. Layout and Register

The dissertation comprises three original publications in English, published in journals from the JCR database, consistently related to the title and main goal of the work. These are multi-authored works, with Mr. Berika Beridze serving as the first author. The predominant involvement in the research is deduced mainly from the authorship position rather than formal declarations. The research was supported by the National Science Center (a project no 2017/26/E/NZ8/01049).

The manuscript of the dissertation starts with a list of publications included in the doctoral thesis. It is followed by a summary in English, Polish, and Georgian, providing general information about the initiative, its outcomes, and conclusions. In the Introduction section, the doctoral candidate discusses issues related to the historical biogeography of the Caucasus forest ecosystems, offering a comprehensive summary of current knowledge in this field, supported by an extensive set of literary sources. The introduction also justifies the selection of the studied species, *Castanea sativa* Mill, highlighting its economic significance to human activities and the risk of extinction due to human pressure and climate change. The "Research Objectives" chapter outlines the primary research goal, expanded with detailed objectives in the form of questions and seven research hypotheses.

The research mainly relies on molecular data, emphasizing the analysis of genetic structure based on microsatellite analysis. Methodological aspects are presented using a graphical scheme called the "Methodological pipeline." A pivotal section is "Major Outcomes" discussing the outcomes of publications 1-3. They are organized into four subsections, relating to the detailed objectives of the work. The combination of three scientific publications in this dissertation is thoughtfully arranged and logical. Each article forms an integral part of the whole, and their individual readings provide only a fragmented view of the research topic. The concluding section includes general conclusions, literary references, a list of other publications and additional popular science articles, and supplementary files. In summary, the doctoral dissertation's structure is logical, fitting academic and scientific requirements and is presented professionally. The three main scientific articles were meticulously written and published in reputable journals (attached in Supplementary Files). All additional elements - tables, figures, and supplementary information - are presented following international standards.

3. Critical notes

My comments pertain to the methodological aspect. The conclusions in all three presented works rely on results from a single type of analysis. This raises questions about the basis for planning the research, as it seems to be exclusively centered on microsatellite analysis. Given the rapid mutation rates of microsatellite sequences, these mutations can become significant evolutionary factors or dominate migration processes, thereby reducing the genetic drift effect. Consequently, in biogeographical studies, a comprehensive approach involving a broad spectrum of analyses based on various molecular markers is preferred. This allows for a more thorough discussion of the actual processes shaping a particular genetic structure pattern.

In the case of domesticated or economically used species, material translocation might alter natural demographic patterns, potentially occurring with *Castanea sativa*. Hence, the definition of natural populations of *Castanea sativa* in the Caucasus, chosen for the study, becomes essential. I believe that when discussing species conservation strategies, including evaluating the effectiveness of the AGF strategy, understanding this natural variability, shaped by natural processes, is crucial. The AGF strategy assumes material translocation from other areas, leading to changes in natural genetic variability. Therefore, for a proper assessment of this strategy's effectiveness, real knowledge about adaptive processes specific to the species is vital. Evaluating the chosen strategy solely based on microsatellite markers may not provide adequate justification.

4. Final grade

The doctoral dissertation of Berika Beridze, MSc provides valuable and innovative scientific contributions to the biogeography of species in the Caucasus. The research conducted as part of the doctoral studies offers invaluable insights into the mechanisms and factors underlying the maintenance of high levels of genetic diversity in C. sativa in the Caucasus region and helps to understand how this diversity was shaped. Based on his studies (2 and 3), the doctoral candidate demonstrated that the results of the analysis of the genetic structure of contemporary species have significant practical applicability, providing useful measures of biological diversity in conservation and management efforts.

It shows that the candidate is able to efficiently prepare scientific publications and has developed excellent competences and skills across a wide array of methods and analyses. These include handling and analyzing genetic data, utilizing various population genetic methods, and conducting modeling analyzes of future range changes based on various climate scenarios. It is worth emphasizing that all the work was carried out within an interdisciplinary research team.

As such, I hereby declare that the reviewed PhD thesis Berika Beridze, MSc meets the criteria for doctoral dissertations specified in art. 187 of the Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended). I request that the Scientific Council of the Institute of Dendrology Polish Academy of

Sciences accepts Berika Beridze, MSc for further stages of doctoral proceedings in the field of natural sciences, in the discipline of biological sciences.

Taking into account the quality of articles and the prestige of the journals in which they were published, the significant contribution to advancing our understanding of the history of the development of flora in Europe and the Caucasus, the wide-ranging competences gained by the candidate during his PhD, and important research perspectives opened by the thesis outputs, I hereby request that the thesis is accepted with distinctions.

[Stwierdzam, że rozprawa doktorska mgr. Beriki Beridze spełnia warunki określone w art. 187 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (t.j. Dz. U. z 2023 r. poz. 742 z późn. zm.) wnioskuję do Rady Naukowej Instytutu Dendrologii Polskiej Akademii Nauk o dopuszczenie mgr. Beriki Beridze do dalszych etapów postępowania w sprawie nadania stopnia doktora.

Wniosek o wyróżnienie

Biorąc pod uwagę jakość artykułów i prestiż czasopism, w których zostały opublikowane, wartość naukową uzyskanych wyników i ich znaczący wkład w pogłębienie naszej wiedzy o historii kształtowania się współczesnej flory Europy i Kaukazu oraz szerokie kompetencje zdobyte przez kandydata podczas doktoratu oraz ze względu na ważne perspektywy badawcze, jakie otwierają dorobek rozprawy, wnioskuję o przyjęcie rozprawy z wyróżnieniem.]

Date: 09 January 2024

Elżbieta Ciś Loch

Reviewer's signature