



Review of the doctoral dissertation

by Daniel Sanchez Garcia, M.Sc. entitled

Evolutionary trajectories of reintroduced and source *Phengaris teleius* butterflies thirty years after reintroduction

Introduction

The doctoral thesis of Mr Daniel Sanchez Garcia, presented for review, was carried out under the supervision of Dr Magdalena Witek and Dr Luca Pietro Casacci at the Museum and Institute of Zoology of the Polish Academy of Sciences.

The dissertation addresses issues in evolutionary ecology, genetics, behaviour, morphology and conservation. The subject of the research is the endangered species of the day butterfly *Phengaris teleius* (Bergsträsser, 1779) belonging to the family Praying Mantis (Lycaenidae).

Dissertation characteristics

The assessed dissertation runs to 141 pages and is written in English. It begins with a list of the three manuscripts included in the dissertation, followed by an abstract in English and Polish. Two pages constitute the acknowledgements and the table of contents. The articles that form the basis of the dissertation, are accompanied by a detailed introduction, description of materials and methods with all types of analyses carried out, results, and conclusions in great detail. At the end, there is a literature list of 43 items. This way of organising the dissertation is clear and reader-friendly. This was followed by the three manuscripts, each manuscript has a layout characteristic of a scientific publication with a division into introduction, material and methods, results, discussion and also a considerable number of appendices. Two articles are in peer review in English-language journals indexed in Journal Citation Reports (Web of Science), one article is

prepared for submissions. The declarations of author contributions as well as the first position of the PhD student among the authors of all articles indicate his leading role in the creation of the publication. The dissertation has been carefully prepared and the thoughts - both in dissertation and in the articles - have been clearly formulated. Also noteworthy is the aesthetically produced, colourful cover, whose graphics relate to the research problem presented in the dissertation.

Scientific value of the dissertation

Daniel Sanchez Garcia's MSc dissertation is an important contribution to the understanding of the adaptation mechanism of the reintroduced butterfly species, and I rate its scientific value very highly. The level of the journals in which the work will be published indicates that the articles included in the dissertation present research of a high scientific level that significantly advances knowledge of butterfly reintroduction. It should be noted that studies carried out on such a wide range of reintroduced metapopulations of *Phengaris* butterfly are rare (this may be the only such comprehensive study, I don't think as many analyses have been done for *Phengaris arion* introduced from Sweden to the UK). Thirty years after the introduction of the species, the changes that have occurred in the Dutch metapopulation in adults and caterpillars were investigated by analysing cuticular hydrocarbon profiles, vibroacoustic and behavioural analysis, wing morphological analysis, and analysis of the genetic structure of this metapopulation. The study also considered interactions between the butterfly species studied and their host ants of the genus *Myrmica*. The broad scope of the research has made it possible to trace the process of coevolution and the emergence of adaptation to new conditions, and the conclusions drawn are reliable, based on the application of various types of research, experiments and measurements. This carefully planned and carried out research testifies to the scientific maturity of Mr Daniel Sanchez Garcia and his ability to take a synthetic view of the problem studied. I highly appreciate the ability to introduce the research topic and the clarity of the formulated research hypotheses, as well as the choice of research methods, statistical tools for data analysis, the way the results are presented and the ability to interpret them against the background of the literature on the subject.

The interpretation of the results and the conclusions obtained are correct, clear and formulated in accordance with the art of inference in Science. Studies have shown that adult butterflies show morphological differences between metapopulations, manifested in differences in

body weight, thorax width and wing size as well as in wing shape and dot pattern variation. An explanation for these changes may be the connectivity of the metapopulation as an important factor influencing changes in morphological traits of adult individuals (Manuscript 2 and 3). In the case of caterpillars, differences were observed in chemical and vibroacoustic signals and thus in the adaptation potential of the introduced metapopulations (Manuscript 1). Dutch caterpillars have a less similar CHC profile to the host ants before and after their adoption compared to the Polish ones. Also vibroacoustic signals produced by ants and caterpillars differs among metapopulations – the caterpillars from each metapopulation produce sounds more similar to the ones of their sympatric host ants..

Very interesting results were obtained in studies on the impact of reintroduction on the genetic structure of metapopulations - despite passing through the bottleneck effect and founder effect, the Dutch metapopulation shows a distinct genetic structure and resistance to loss of genetic variation (Manuscript 2). The effective population size of the reintroduced metapopulation was estimated to be significantly lower than that of the native Polish population. The second manuscript is valuable in terms of conservation management implications. The results of the study indicate that a proper long-term habitat management in reintroduced butterfly metapopulations and habitat restoration are key factors influencing the success of reintroduction. Maintaining source and reintroduced metapopulations in healthy conditions should be a priority in species conservation practice.

The very large number of analyses carried out and the interesting results obtained entitle the doctoral student to come to the correct conclusion that *Phengaris teleius* shows adaptability at all stages of its life cycle; any morphological changes that have occurred in the reintroduced butterfly are a response to environmental pressure. The Dutch metapopulation shows a distinct genetic structure, as well as resistance to loss of genetic variation. The ability of this species to adapt in new habitats indicates that its introduction into areas where it is extinct is most feasible. *Phengaris teleius* is good indicator of habitat changes and it is able to adapt to environmental alterations in a relatively short period of time. It only took 86 reintroduced individuals for the only population of this species in the Netherlands to survive, adapt, prosper and expand its range after 30 years.

Critical comments

The doctoral dissertation deserves a high evaluation both for its content value and for its editorial side. I do not see any errors or carelessness in it, but a sound scientific workshop, and the aforementioned. It may have been worth adding a separate chapter to the dissertation describing the hypotheses and research questions.

- As I have no experience in analysing the morphological characteristics of butterflies I wanted to know why only the underside of the hind wing was measured? Why wasn't the front wing, on which there are more prominent discoid spots, analysed?
- I was also interested to know why the 4th larval stage was used in the vibroacoustic study and why the recording was carried out in the morning?
- Why were *Myrmica scabrinoids* investigated for chemical and acoustic studies? It is the most common and most abundant species, but was this the only ant species found in Polish and introduced metapopulations? In Europe, parasitism of this butterfly on 9 ant species has been proven.
- Is there any information on the genetic condition of the *P. teleius* population in neighbouring countries, in Germany or in slightly more distant France. In both countries the sites of occurrence of this species are known. And whether gene flow between populations was possible at all?
- I am also interested in what the doctoral student thinks about the prospects for conservation of myrmecophilous *Phengaris* species.

Final evaluation

The doctoral thesis submitted for review brings an original solution to an important scientific problem of coevolutionary processes and adaptations to new conditions and genetic changes in introduced species. The leading role of the doctoral student in the creation of the dissertation is not in doubt. He has demonstrated extensive knowledge, the ability to conduct scientific work independently by applying appropriate research methods and analysing the results obtained as well as critically discussing them. It can be confidently predicted that the PhD student's publications will be cited by other researchers, thus making a lasting contribution to

Science. the Candidate's skills and general theoretical knowledge in the field of biological sciences presented in the doctoral dissertation are sufficient to independently conduct scientific work.

In view of the above, I conclude that the evaluated doctoral dissertation of Daniel Sanchez Garcia fully meets the conditions specified in art. 187 of Act of 20 July 2018, Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended). I therefore request the Scientific Council of the Museum and Institute of Zoology of the Polish Academy of Sciences to admit Mr Daniel Sanchez Garcia to the further stages of the doctoral dissertation.

Furthermore, given the high scientific level of the dissertation of M.Sc. Daniel Sanchez Garcia and his significant contribution to the understanding of evolutionary changes occurring in introduced species, I request that the dissertation be awarded.

Kraków, 30.07.2024

A handwritten signature in blue ink, appearing to read "Ladkowski - Ciertik".