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**Review of the doctoral dissertation by magister Daniel SANCHEZ GARCIA, entitled
"Evolutionary trajectories of reintroduced and source *Phengaris teleius* butterflies
thirty years after reintroduction"**

Introduction

The doctoral thesis presented for review, authored by Daniel Sanchez Garcia, M.Sc., was carried out at the Museum and Institute of Zoology of the Polish Academy of Sciences under the supervision of Magdalena Witek, Ph.D., and Luca Pietro Casacci, Ph.D.

The dissertation topics include evolutionary ecology, behaviour, morphology, genetics and conservation. The subject of the research is the endangered day-flying butterfly species *Phengaris teleius* (BERGSTRÄSSER, 1779), belonging to the family Lycaenidae.

Evaluation of the editorial side of the dissertation

The assessed dissertation runs to 141 pages and is written in English. Its layout is clear and in line with the rules used in this type of dissertation. The study begins with a list of the three manuscripts included in the dissertation. This is followed by an abstract in English and Polish. The next two pages are acknowledgements and the table of contents. The main part of the dissertation is 14 pages long and is divided

into an introduction, objectives of the study, material and methods with all types of analyses carried out in great detail, results, and conclusions. This part ends with a literature list of 43 items. This is followed by three comprehensive manuscripts of 59, 28 and 19 pages respectively. Each has a layout typical of a scientific publication with an introduction, material and methods, results, discussion and a considerable number of appendices. Noteworthy is the colour-compatibility of the sections of illustrations relating to the source and introduced populations consistently applied in all three manuscripts. The aesthetic value of the work is greatly enhanced by the elegant and thoughtful binding (both front and back) intriguingly informing the reader of the subject matter and object of study.

Evaluation of the merits of the thesis

a) Originality of the research

The dissertation presented for evaluation is a completely original study and the leading role of the Doctoral Student in planning and conducting the experiments and preparing the manuscript does not raise any doubts. In each of the three manuscripts that are parts of the dissertation, the PhD student is the first author which indicates his key role in the process of preparing the publication. This is clearly confirmed by the accompanying statements, which also show that the PhD student is the sole author of almost all 31 figures illustrating the results of the analyses carried out. The publications included in the dissertation contain a wide spectrum of hitherto unknown information on the characteristics of the reintroduced population of the studied butterfly species. The conclusions are based on the application of different types of research, experiments and measurements. Throughout the dissertation, the author's endeavour to elaborate as broadly as possible on the topic undertaken is evident. The source and reintroduced populations are analysed biochemically, morphologically, vibroacoustically, behaviourally and genetically, and most of these analyses were carried out on both imagoes and caterpillars. Interactions between the studied butterfly species and the ants were also included in the study. This demonstrates - in my opinion - the

maturity of the PhD student as a scientist and his ability to take a synthetic view of the issue under study.

b) Scientific value of the dissertation.

I assess the substantive value of the dissertation highly. Research hypotheses are clearly formulated and the methods used are adequate for their verification. Also the interpretation of the research results does not raise any objections and the final conclusions are justified. It turns out that the plasticity of the species (differences between the source population and the reintroduced population) is expressed both in changes in the characteristics of vibroacoustic signals produced by the caterpillars and in the analysed morphological parameters of the imago, as well as in reduced genetic variability. In this last aspect, the results indicate that, despite the loss of about half of the allelic richness and thus a very pronounced so-called 'bottleneck effect', the population is able to adapt to the different conditions at the reintroduction site (Manuscript 2). In the same manuscript, the conclusion on the methodology of conservation activities and studies is noteworthy. It points to the need for continuous monitoring of the quality not only of the reintroduced population and its 'new' habitat, but also of the source population, whose condition should allow future 'support' of the introduced population.

Host-parasite relationships are analysed in detail in terms of chemistry and vibroacoustics (Manuscript 1). In both cases, the similarities are more pronounced in the source population, although the results indicate that the vibroacoustic communication in the Dutch population has undergone a rapid and precise adjustment to the pattern that was displayed by the local host ant population.

Detailed morphometric differences in hind wing pattern between the source and reintroduced populations revealed a much higher variability within the former (Manuscript 3). The PhD candidate interprets the results obtained by a great variety of factors of both genetic and environmental nature but without more detailed analyses. It appears that the use of appropriately chosen methodologies (a significant plus of the dissertation) is much easier than justifying the results. This is not, however, a reproach, but rather a general remark concerning all research, especially in the biological sciences, where the exceptional complexity of the still superficially

known relationships makes it difficult or even impossible to explain clearly the facts discovered.

In light of the very interesting results of the varied analyses, the PhD student's assumption that *P. teleius*, despite its complex life cycle and interactions with its host ants, is such a plastic species that its reintroduction in areas where it has become extinct is entirely possible.

Strengths and weaknesses of the dissertation/Critical remarks

The doctoral dissertation deserves a high mark both on the merits and on the editorial side. Personally, I very much like the idea of the doctoral student to replace the typical hardcover with an interesting collage of photographs and diagrams. The critical remarks presented below should be regarded more as my personal suggestions for further research or missing details, regarding the broader context of the issues elaborated rather than shortcomings of the dissertation itself. First of all, the lack of information on the isolation of the butterfly's reintroduction area drew my attention. The fact that the species became extinct in the Netherlands (which, by the way, is a small country) does not inform whether there was a possibility of migration, even of single individuals from populations in neighbouring countries. Probably not, but such clear information would be welcome. My surprise regarding the morphometric analyses was aroused by the analysis by the doctoral student (together with his team) of only the details of the shape and pattern of the underside of the hind wing. Why was the front wing and perhaps even the upper side of the wing having their characteristic pattern not examined? The dissertation lacks any explanation of the approach taken. I am intrigued by the question of how the observed differences between the source population and the reintroduced population would look if a third small but highly isolated, natural population were analysed. This would have given an interesting context to the evolutionary changes observed in the study, whether the degree of differences detected between populations should be related more to the 'founder effect' or to long-term, more natural microevolution in isolation. A minor technical shortcoming is the failure to isolate the conclusions in

the first manuscript (p. 49), which should begin with the paragraph 'To summarize... "which presently is part of "Behavioural responses...'.

Last but not least, as a taxonomist but also as an experienced editor, I would like to point out that throughout the dissertation the scientific, Latin name of the butterfly is not once given in full, i.e. together with the author and the year in which the species was described. This omission does not affect the merits of the study at all. However, similarly as a detailed description of the methodology makes it possible to repeat analyses and experiments, so a precise identification of the organisms under study avoids any doubt as to what the researcher was dealing with. In the case of the butterfly species studied, this may not be so important, but proper habits become much more important when the subject of the research is a much less obvious taxon.

Final evaluation

Based on the detailed analysis of the dissertation, I conclude that it represents an original solution to an important scientific problem. At the same time, it indicates the Doctoral Student's broad knowledge and his ability to conduct scientific work independently by applying appropriate research methods, ability to comprehensively analyse the obtained results as well as their discussion based on available sources of information. His leading role in the creation of the dissertation does not raise any doubts.

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.

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