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Review of the PhD thesis of M.Sc. Alberto García-Rodríguez

„The role of the brown bear *Ursus arctos* as seed disperser:

a case study with the bilberry *Vaccinium myrtillus*”

supervised by

dr hab. Nuria Selva Fernández and dr Jörg Albrecht

Seed dispersal is one of most crucial stages of plant development. The fruit-frugivore seed dispersal mutualism fascinates scientists for decades. The PhD thesis of M.Sc. Alberto García-Rodríguez fits well in the excellent tradition of seed dispersal investigations conducted by Spanish scientists. He decided to study a relevant topic. His thesis considers the role of megafauna for seed dispersal and plant regeneration processes. The thesis refers to the role of brown bear *Ursus arctos* as seed disperser, addressing both the quantitative and qualitative components of seed dispersal. The study focused on dispersal of bilberry *Vaccinium myrtillus* seeds, however it took into account also a long list of other plant species dispersed by brown bear. The thesis demonstrates comprehensive knowledge of the ecology of brown bear and bilberry. Both study species are well known. Surprisingly, knowledge of the ecosystem services provided by brown bear as a seed disperser is rather poor. The PhD thesis of M.Sc. Alberto García-Rodríguez clearly fills this gap. On the other hand, generative reproduction of bilberry is an important and interesting issue in the context of reproductive ecology of clonal plants and the presented PhD thesis considerably enriches our knowledge in this area. In his PhD thesis the Author raised several important questions, e.g.: (1) What is the contribution of fleshy fruits in brown bears' diets across biomes and particularly in the Tatra mountains? (2) How ingestion by brown bears affect germination of seeds? (3) Is there an effect of human disturbance on seed dispersal provided by brown bear? (4) What is the role of brown bear as bilberry disperser in relation to other mammal and bird dispersers? (5) What is the germination and survival rate of bilberry seedlings dispersed by brown bear?

M.Sc. Alberto García-Rodríguez dealt excellently with his tasks. The thesis is well structured and easy to follow. The objectives and hypothesis are well defined and clearly presented. To answer raised questions, the Author applied a wide range of well suited methods and scientific tools. He prepared a wide review of bibliographical data, collected large number of samples in the field, performed experiments and applied molecular analysis. Data collection was very labour-consuming but it profited in a really large dataset. To analyse the data, he used up-to-date analytical and statistical analysis tools (e.g. metabarcoding, generalized linear mixed models), which are clearly presented in each chapter. The results are presented in detail and profoundly discussed using recent references. In each chapter the Author places his study in a wider ecological context. The complexity of the aspects of seed dispersal by brown bear taken under consideration in this study is impressive. The Author

placed his study in a range of spatial scales: from wide scale of biomes to fine scale of the Tatra Mts.

As the most important results of the presented PhD thesis I consider: (1) The brown bear is a legitimate seed disperser providing highly effective dispersal services and may have a substantial impact on plant regeneration processes in its distribution range. (2) Fleshy fruits, especially *V. myrtillus* and *Rubus idaeus*, play a very important role in brown bear's diet, especially in the hyperphagia period. (3) The brown bear has a potential to disperse large quantities of seeds comparing to other seed dispersers. Moreover, brown bear's digestion does not destroy seeds, but increases the probability of their germination comparing with seeds germinating from whole fruits. (4) Bilberry seedlings germinating from brown bear faeces may survive in vital numbers at least one year. (5) Dispersal of bilberry seeds provided by brown bear complement a more effective seed dispersal provided by birds.

The PhD thesis consists of four main chapters which corresponds to manuscripts prepared for submission, submitted or already published:

García-Rodríguez, A., Albrecht, J., Szczutkowska, S., Valido A., Farwig N., Selva N. 2021. The role of the brown bear *Ursus arctos* as a legitimate megafaunal seed disperser. *Scientific Reports* 11, 1282 <https://doi.org/10.1038/s41598-020-80440-9>

García-Rodríguez A., Selva N., Zwijacz-Kozica T., Albrecht J., Lionnet C., Rioux D., Taberlet P., De Barba M. The bear-berry connection: ecological and management implications of brown bears' food habits in a highly touristic protected area. Submitted to *Biological Conservation*

García-Rodríguez A., Albrecht J., Farwig N., Frydryszak D., Parres A., Schabo D.G., Selva N. Functional complementarity of seed dispersal services provided by birds and mammals in an alpine ecosystem. In preparation for resubmission to *Journal of Ecology*

García-Rodríguez A., Selva N. Constant gardeners: endozoochory promotes repeated seedling recruitment in the bilberry *Vaccinium myrtillus*. Submitted to *Biology Letters*

The presented PhD thesis consists of multi-author papers. In all papers M.Sc. Alberto García-Rodríguez is the first author. The authors' contribution chapter, present in each of the papers, showed that his contribution to study conception and design, data collection, data analysis and manuscript preparation was leading and considerable. The number of co-authors proves that M.Sc. Alberto García-Rodríguez successfully collaborated with scientists from various international institutions.

The thesis covers multiple aspects and consists of four papers but it focuses clearly on one topic. There is a strong and coherent connection among the topics and methodology of the different papers that comprise the thesis. The introduction and conclusions of the thesis provide a unifying picture of the whole research. Four main chapters present various aspects of the role of brown bear in seed dispersal of fleshy fruited species with special focus on *V. myrtillus*, and seed dispersal of *V. myrtillus* by mammals and birds, with a special focus on brown bear. The studies supplement each other, giving a very complete picture of the studied problem which is clearly presented in the Introduction chapter. In the Introduction, which is very well-written and easy to follow, M.Sc. Alberto García-Rodríguez presents a theory regarding seed dispersal effectiveness with its quantitative and qualitative components. The

literature reviewed in this chapter is current and complete and it presents the broad background of present-day knowledge of seed dispersal. In this chapter the Author presents also in detail two main studied species (*U. arctos* and *V. myrtillus*) and study area. At the end of this chapter, M.Sc. Alberto García-Rodríguez lists very clearly the main objectives of the thesis.

In the first paper, in the series of four main chapters of PhD thesis, M.Sc. Alberto García-Rodríguez presented meta-analysis concerning effectiveness of brown bear as a seed disperser worldwide. He based the results on a detailed review of published data. He searched literature database using criteria which were carefully defined and precisely described in the methods section. On the basis of the collected data, M.Sc. Alberto García-Rodríguez presented a very detailed and multi-aspect analysis showing the dispersal of fleshy-fruited seeds by brown bear. The analysis of brown bears' diet took into account not only the plant species identity but also the effect of biomes on frequency of occurrence of plant taxa consumed by brown bear. He found that brown bears consume more than one hundred fleshy fruited plant species worldwide, with fruits from Rosaceae and Ericaceae family most frequently consumed. This chapter shows that M.Sc. Alberto García-Rodríguez have gained excellent knowledge about brown bear diet as a seed disperser in the scale of its global range. The results of this analysis would have been enough for a sound and very informative study, however the Author did not limited this chapter of his PhD thesis to this part. He completed his meta-analysis with results from an ex-situ germination experiment checking if seeds of 11 fleshy fruited species, commonly eaten by brown bear, are damaged by gut passage. Although, M.Sc. Alberto García-Rodríguez was not conducting the experiment himself, he efficiently analysed the data collected previously by his collaborators using generalized mixed effects models. He found that seeds ingested by brown bear usually germinated better than those embedded within the pulp and in the case of some species - even better than seeds depulped manually. Thus, thanks to a profound literature review and a well-planned laborious experiment, the results of this chapter constitute a very complete analysis of quantitative and qualitative aspects of dispersal effectiveness of brown bear worldwide. In the discussion section, M.Sc. Alberto García-Rodríguez managed to clearly join all aspects of this study and discuss them really profoundly. Moreover, he placed the results of the study in the context of consequences of hazard for brown bears' population persistence for ecosystem services it provides.

In his second paper from the PhD thesis, M.Sc. Alberto García-Rodríguez moved from the worldwide perspective of his first paper to the narrower scale of the Tatra Mountains and focused on the diet of brown bears in a more restricted area. He studied in detail not only diet composition but also its within year temporal patterns. Additionally, the Author placed his study of ecosystem services provided by brown bear in the context of anthropogenic pressure which occurs in the Tatra National Park. The base for the analysis was a large amount of brown bear scats (~250) collected by M.Sc. Alberto García-Rodríguez and his collaborators within 2 years. At this point I would like to ask the Author my first question. Although I understand limitations of the investigations conducted within his PhD studies, I wonder whether a two-year period of data collection in a field could influence the results? In other words, how much among year variation of seed production could decide about the obtained results? This aspect of the study was not addressed in the discussion section. In this study, the diet of brown bear was determined using metabarcoding. The species were divided in four

main groups: plants, insects, birds and mammals. Moreover, within plants 7 different categories were distinguished: fleshy-fruited plants, plant species producing hard mast, tree species with neither fleshy fruit nor hard mast, plant species with edible roots, graminoids, forbs and cryptogams. Here is my second question. It is unclear for me why did the Author decide to distinguish such groups? There was no explanation for such approach in the text. Moreover, the discussion section only commented on fleshy-fruited plants in bear diet. One of the most important results from this study was the finding that fleshy fruits, and especially *V. myrtillus* and *Rubus idaeus*, play a very important role in brown bear diet in the Tatra Mountains, especially in the hyperphagia period. In the discussion section, the Author provided an extensive passage in which he put his results in the context of anthropogenic pressure on areas inhabited by brown bears and its consequences for their diet and quality of ecosystem services. The paper ends with recommendations for conservation management strategies to ensure dispersal of fleshy fruited plants provided by brown bear.

While reading the second paper, I wondered whether the dispersal of bilberry by brown bear is really effective comparing with for example dispersal provided by birds. Surprisingly, third paper of the presented thesis answered this question in detail, as well as several others. In the third paper, M.Sc. Alberto García-Rodríguez presented seed dispersal services provided by birds and mammals in coniferous forests and alpine meadows. More than 1800 bird droppings and mammal scats were collected. Elevation and type of microhabitat were taken into account (i.e. bare soil, dead wood, vegetation and stone). It was a laborious approach, and delivered a huge number of data for the analysis. Seed dispersers were identified with the help of metabarcoding methods. The results from this part of the study showed, that dispersers differed considerably in terms of quantity of dispersed seeds. The brown bears provided the majority of the seed rain (93%). Moreover, the field data were supplemented by a well-planned ex-situ germination experiment to determine the effects of the microhabitat and seed density on the germination rate of bilberry seeds. The Author simulated in a set of pots four microhabitat types and three densities of seeds. It was found that bilberry germination rates significantly decreased with increasing seed densities and that among microhabitats, the largest germination rates were found on dead wood, followed by bare soil, stones and vegetation. Thanks to the results from this part of the study, the Author could detect that the seed dispersers differed also in terms of quality components of dispersal. For example, the Author stated that small passerines, that dispersed only few seeds per dropping and defecated mainly on stones, provided the highest quality of bilberry seed dispersal in terms of germination rates. At this point I have some doubts. I wonder if indeed stones are such a good microhabitat for germination? In the pot experiment, the positive effect of germination on stones could be in my opinion the result of the fact that seeds were washed from the stones to the soil and germinated in fact in the soil. In natural mountain conditions, the stones are usually large or lying on the ground in various layers where the seeds would not have the appropriate conditions to germinate, as in the pot experiment. There is also another question related to this result. How much differences between dispersers in microhabitats where they deposit seeds could be affected by the differences in visibility of faecal samples on those types of microhabitats? For example, is it probable that bird droppings were detected more easily on stones than within vegetation? Is it possible, that as a consequence of such a bias, the Author might have concluded that passerines usually defecated on stones? I have also another question regarding the results from this paper. Why did the Author suggest that the quantity of the seed dispersal service is a better indicator of the total effect of the

dispersers on plant reproduction than the quality of the service they provide? It seems to me contrasting with the result that two species of thrushes were the most effective bilberry dispersers, while on the other hand brown bears generated most of seed rain. In spite of some doubts, I am impressed by the research plan and complexity of this study. Investigations concentrate most often on one of the components of seed dispersal effectiveness. M.Sc. Alberto García-Rodríguez found that quality and quantity components are negatively correlated. Thus, consideration of both quantitative and qualitative components of dispersal seems to be the most desirable approach. The presented results showed spatiotemporal complementarity of different dispersal vectors of bilberry seeds and make a sound and well-presented contribution to studies of seed dispersal effectiveness.

In the fourth paper, M.Sc. Alberto García-Rodríguez disputes that *V. myrtillus* follows “recruitment in windows of opportunity” strategy of regeneration. It is the shortest paper out of the four presented. For two years he followed the fate of seeds in ~60 faeces of brown bears, mesocarnivores and passerines. And he found that germination of seeds from faeces is frequent and seedlings appear in the first and second year after seed dispersal. In consequence, the Author stated that “repeated seedling recruitment” and not “recruitment in windows of opportunity” is the recruitment strategy of bilberry. A very interesting result is also the demonstration that resting sites of brown bears are especially important for bilberry seedling recruitment. Those findings improve our knowledge about bilberry biology. I have some questions about the methods and results. Why seedlings were counted at 1 m² plots around faeces and not only directly on the faeces? It was not explained in the methods section of the manuscript. Why did the Author decide to test whether distance between faeces and control plots (located 5, 10 and 30 m from marked faeces) affects seedling emergence?

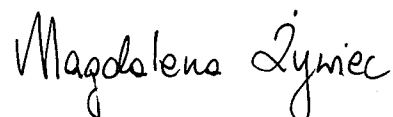
For a separate comment deserves the excellent illustrative and documental material of the presented PhD thesis. It consists of well-planned informative figures and tables included in the main body of the text and extremely rich supplementary materials added to each chapter. Those supplementary materials which could not be included in the main bodies of manuscripts are valuable illustrations of the results and detailed data description documenting the huge number of data collected by the Author. The thesis is also illustrated by beautiful photos taken by M.Sc. Alberto García-Rodríguez.

Concluding, M.Sc. Alberto García-Rodríguez has proved that he knows how to pose scientific questions, plan and conduct a series of field observations and experiments, and critically discuss the results of his research in the context of previous studies and place them in the wide context. His PhD thesis showed that he is a very careful observer of nature. The very complex approach to each of questions raised deserves to be highly valued. The investigations were conducted using various methods: profound literature review, laborious field observations and data collection, experiments planed in detail and molecular techniques. The statistical analysis using modern modelling methods is a very strong point of the presented PhD thesis. Moreover, he took on a challenge of preparing suggestions for conservation management which could protect ecosystem services provided by brown bear for the ecosystems. The results of his PhD thesis significantly broadens our knowledge of dispersal effectiveness of brown bear and generative reproduction of *V. myrtillus*. I am sure that M.Sc. Alberto García-Rodríguez proved with his PhD thesis that he deserves the PhD degree.

In conclusion, I believe that the doctoral dissertation presented for review, whose author is Alberto Garcia Rodriguez, M.Sc., meets the criteria for doctoral dissertations set out in Article 13 of the Act of 14 March 2003 on Scientific Degrees and Academic Title and Degrees and Titles in Art (Journal of Laws of 2017, item 1789) and in the Act of 20 July 2018, Law on Higher Education and Science (Dz. U. of 2018, item 1668, as amended) and the Act of 3 July 2018, Introductory provisions of the Act - Law on higher education and science (Journal of Laws of 2018, item 1669, as amended). In view of the above, I put forward a motion to the Scientific Council of the Institute of Nature Conservation of the Polish Academy of Sciences in Krakow to admit M.Sc. Alberto Garcia Rodriguez to further stages of the doctoral dissertation". I also believe that the work deserves an honourable mention.

Kraków, 26.05.2021

Dr hab. Magdalena Żywiec

A handwritten signature in black ink that reads "Magdalena Żywiec". The signature is written in a cursive style with a large initial 'M' and a distinct 'Ż'.