

Opponent's review - Comments on Ph.D. Thesis

to Ph.D. Thesis of RNDr. Alena Bartoňová „Factors influencing butterfly diversity of the species-rich Submediterranean region“

Supervisor: doc. RNDr. Martin Konvička, Ph.D.

Reviewer: Mgr. Vladimír Vrabec, Ph.D.

In total, the thesis ranges 165 pages, when introduction (including references) forms 30 pages. The rest of the thesis is composed of two peer reviewed scientific papers and one manuscript (both thesis works are first-authored by the Ph.D. candidate), brief summary and curriculum vitae.

After thorough review of the thesis, I do not have major comments on the presented articles, which were revised within their publication process in the prestigious scientific journals. Other minor flaws such as missing italics in latin names of the species were found in the text very rarely. Therefore, this review mainly aims to provide some bullets for further discussion about the topic during the Ph.D. defence.

Regarding the content of Ph.D. thesis, I would like to know the author's opinion on following topics:

1. Genus *Proterebia* is currently represented by only one species *P. afra* (= *P. phegea* in the past). Is this the final version of it's name from the taxonomic point of view? Could the author briefly describe some details about the genesis of current nomenclature of this species?
2. Could the author characterize the distribution of closely related taxons from *Callerebia* and *Loxerebia* genus? Does the author also know some details about their status of endemism and threat rates? How abundant are these species? And what can the author say about recently described taxons of such genera (e.g. *C. dibagensis*)?
3. Regarding distribution of organisms inhabiting non-forested habitats and steppe refugia in Czechia and Slovakia, well known (but underestimated) theory of loess steppe refugia does exist. According to this theory, loess steppes used to represent very important glacial bioms for many relict species until the neolite, when humans began to use the land. Could the author describe genesis of landscape in the Czech Republic and compare it with that of Balkan peninsula? The theory was based on certain group of animals, does the author know which one? And which dispersal tracks of xerothermic and steppe species is the theory based on? Are there some of such species currently presented in the Czech Republic (e.g. in Elbe lowland area = Polabí)? Which Czech localities of quaternary sediments are considered to be the most important? Why does the author think, that main movements of the species do not lead through Pannonian lowland.
4. The results obtained within this study indicates, that refugia of *Proterebia afra* at Balcan peninsula could be considered as very important habitats. Does the author know any other similar biodiversity hot-spots at Balcan?
5. Can the author clarify the taxonomy of *Pseudophilotes* genus, which is sometimes considered as synonym of *Scolitantides*? Does the author know some sources (molecular genetic probably) to confirm this name as undoubtedly (certainly) valid?

6. In case of *Pseudophilotes bavius*, only two related species *P. fatma* and *P. barbagiae* are mentioned in the thesis. However, the genus contains more taxons, which are classified on the species level. Could the author briefly inform about those species, mainly about their distribution in Meditarrean area and their relation to populations of *P. bavius*?
7. Certain populations of related species and subspecies are known to be critically endangered in the area. Some of them have been even reintroduced there. Does the author know about such cases? Could she provide an information about the conservation of *P. bavius* (active management support)?
8. Does author think that some knowledge of ecology of blue butterflies *Phengaris* can be applied on *Pseudophilotes bavius*? In particular, I mean prediction of fragmentation, relations with ants and life span.
9. The study (as far as I know, not cited in the thesis) of Todisco et al. (2018): The origin of the Sardinian Blue, *Pseudophilotes barbagiae* (Lepidoptera: Lycaenidae): Out-of-Europe or Out-of-Africa? Genome, 58(5): 288-289 refers about interesting question. What is the situation of *P. barbagiae* and *P. fatma* from the taxon validity point of view? Sardinia lies between the areas of their distribution?
10. Similarly, what is the relation of *Pseudophilotes sinaicus* to other *Pseudophilotes* populations? Does the author have a knowledge of the conservation of the species?

In conclusion, the thesis is well written and deep entomological as well as ecological knowledge of the author is evident. Moreover, the publication list of the author, containing 10 peer reviewed papers and 6 contributions at international conferences, is impressive. Therefore, I recommend the thesis to pass the defence and also gladly recommend to grant Ph.D. title for the student.

In Kolín, 9th June, 2019



Vladimír Vrabec

Salzburg, 6.6.2019

Department of Biosciences,
Evolutionary Zoology group

Review on the PhD thesis of Alena Bartonová “Factors influencing butterfly diversity of the species-rich Submediterranean region” – by Prof. Dr. Jan Christian Habel

Univ.-Prof. Dr. Jan C. Habel
Hellbrunner Str. 34
5020 Salzburg
Austria

Tel +43.662.8044.5620

Janchristian.habel@sbg.ac.at

The PhD thesis of Alena Bartonová consists of three main chapters, one introduction and a summary i.e. conclusion chapter. The thesis focuses on the history, current state and conservation of butterfly diversity in the Submediterranean grasslands and Palaearctic steppes. The PhD thesis was performed on butterfly species, and uses a broad variety of methods, such as molecular analyses (here different markers such as DNA sequencing, AFLPs), Species Distribution Models, and ecological studies, such as transect counts to assess butterfly communities. In the following I will review the chapters in chronological order:

The **Introduction** provides an excellent overview on the history of the Eurasian grasslands, including the Eocene to Miocene / Pliocene and Pleistocene, as well as the Holocene. Mrs. Bartonová explains the biogeography and uniqueness of the Balkan region in detail here. This section ends with a clear summary of aims and scope of the thesis. Here, the candidate explains in detail the study setup, study species selected, and the methods applied. Furthermore, this first paragraph provides an excellent literature review on the biogeographic history of this region.

The **first study** focuses on the habitat demands of *Proterebia afra*, and associated butterfly communities. Field surveys were conducted along transects in two study areas (differing in environmental conditions and biogeographic history). Here, the study species (abundance) but also all other species has been recorded. Species were classified into Mediterranean vs non-Mediterranean species. Here I would suggest to increase the number of ecological traits! GLMs showed that the habitat demands of *P. afra* (but also butterfly community composition) showed contrasting trends for the two areas (Askion Mountains and Dalmatia). The author close this first study with very important advices for conservation management.

Question 1: Which traits would be appropriate to study specie' responses to environmental conditions?

The **second study** is on the phylogeography of *Proterebia afra* across the Palaearctic steppes during glacial–interglacial cycles. This second chapter starts with a wrong abstract. In this study, Alena Bartonová

combines various molecular markers, mtDNA and nuclear DNA Sequence data and Amplified Fragment Length Polymorphisms, with Species Distribution Models. The sampling was appropriate to answer the raised research questions. The analytical methods and statistical analyses such as SAMOVA were well selected and appropriate to test the phylogeographic history of this species. The data show sub-refugia in the Balkan area and eastwards. Based on this joint data set (molecular data with SDMs) it was possible to reconstruct the impact of past climate changes and subsequent range dynamics across this area. In the conclusion the authors broadened the scope and explain how mammoth steppe fauna shifted during the past.

Question 2: Explain potential reasons of differences obtained from different marker systems, against the background of mtDNA vs nuclear evolution processes.

Question 3: Why is Cytochrome c oxidase subunit I COI an important molecular marker when reconstructing phylogeographic histories and taxonomies?

Question 4: Explain strengths and shortcomings of Species Distribution Models in phylogeographic studies?

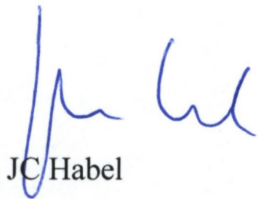
In the **third study**, the candidate studied recently lost connectivity among local populations of the lycaenid butterfly *Pseudophilotes bavius*. This study has been performed based on molecular data (DNA sequencing of five genes). The data show some spatial genetic differentiation, as well as high genetic diversity – despite the fact that today's populations occur highly fragmented and geographically isolated. Thus, the authors assume that the current population structure (highly fragmented) does not yet reflect the genetic structure (i.e. the high genetic diversity found). The authors close this last study with some conservation implications and state that historical disturbances (succession dynamics) provided by historical land use regimes are of relevance here.

Question 5: Steppe species or boreo-montane butterfly species often exist as small and isolated relict populations across the Western Palearctic; Are relict species / populations of relevance for nature conservations? (pros and cons)

The thesis ends with a short summary of the results and some concluding comments.

All data sets are sound, and well analysed, using appropriate statistical approaches. The interpretation of results were done in an appropriate way (without over-interpreting results or speculations).

I accept this very interesting PhD thesis – and wish the candidate good luck for her defence of this interesting work.

A handwritten signature in blue ink, appearing to be 'JC Habel', with a stylized, cursive script.

JC Habel