



# The origin of the Sardinian Blue, *Pseudophilotes barbagiae* (Lepidoptera, Lycaenidae):

## Out-of-Europe or Out-of-Africa?

Valentina Todisco<sup>1</sup>, Konrad Fiedler<sup>1</sup>, Elisabetta Puliga<sup>1</sup>, Vlad Dinca<sup>2,3</sup>, Raluca Voda<sup>3,4</sup> and Andrea Grill<sup>1</sup>

<sup>1</sup>. Department of Botany and Biodiversity Research, Division of Tropical Ecology and Animal Biodiversity, University of Vienna, Austria.

<sup>2</sup>. Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada.

<sup>3</sup>. Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain.

<sup>4</sup>. Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain.

### Introduction

Due to its geographic position and geological history, Sardinia is characterized by an extraordinary richness of endemic species. The butterfly *Pseudophilotes barbagiae* (de Prins & van der Poorten 1982) (Lycaenidae: Polyommatainae) is an emblematic endemic species of the Sardinian fauna (Fig. 1). The species is restricted to a few slopes in the central mountainous areas of the island, and has been described as distinct from the continental and Corsican *P. baton* by characteristics of the male genitalia and wing markings.

The aim of this study was to reconstruct the evolutionary history of the endemic Sardinian blue butterfly valuating an African versus European origin using the DNA barcoding region of the COI mitochondrial gene.

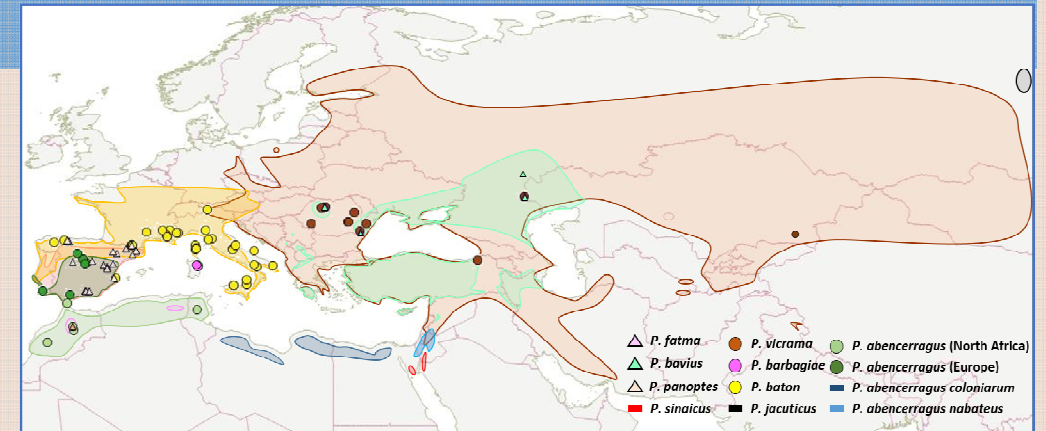
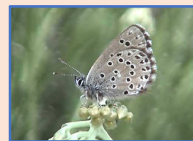
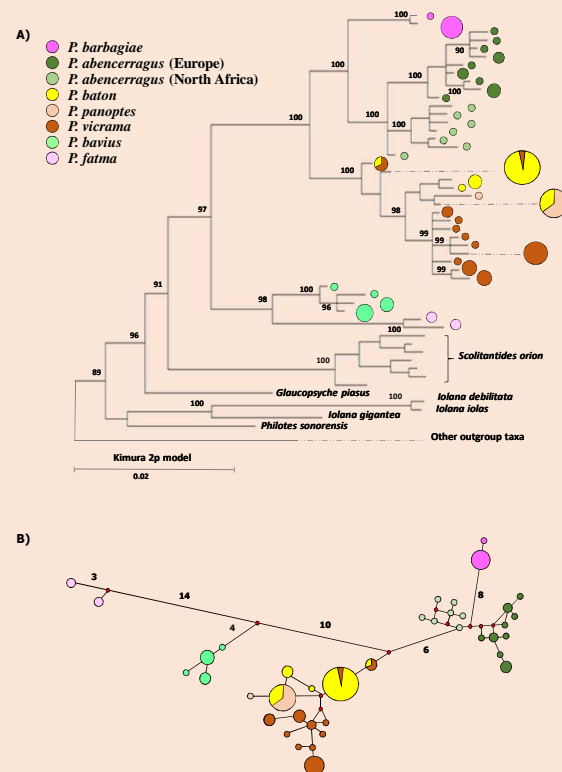


Fig. 1. Approximate *Pseudophilotes* taxa distributions<sup>1-6</sup> (shaded areas) and sampling localities (symbols) included in this study. The map was obtained using Quantum GIS 2.8.2 based on a map from Natural Earth ([www.naturalearthdata.com](http://www.naturalearthdata.com)).

### Material and Methods

A 658-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was sequenced for 115 individuals belonging to 7 species from 85 sampling locations (Fig. 1). We added to this dataset seven COI sequences from GenBank for *P. vicrama*, *P. bavius*, *P. baton* and *P. abencerragus*. We analyzed 11 individuals of *P. barbagiae* and compared them with all European and North African congeneric species: a group of 77 sequences representing *P. baton* (Corsica, Italy, France, Spain), *P. vicrama* (Romania, Turkey, Kazakhstan, Russia) and *P. panoptes* (Spain), as well as several BINs containing 20 sequences of *P. abencerragus* (Spain, Morocco, Tunisia, Portugal), 10 sequences of *P. bavius* (Romania, Russia) and 4 sequences of *P. fatma* (Morocco). A Bayesian analysis (K2p model) was performed to investigate the phylogenetic relationships of the mtDNA haplotypes. 110 sequences from various representatives of the *Glaucopteryx* section were retrieved from BOLD and used as outgroup. The program NETWORK 4.6.1.3 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)) was used to calculate a median joining (MJ) network representing the genealogical relationships among mtDNA haplotypes. Finally, average distances (K2p model) among species were calculated (MEGA 6<sup>7</sup>).

### Results

The Bayesian tree of 39 haplotypes showed that *Pseudophilotes* forms a monophyletic group with the *P. bavius* and *P. fatma* clade in the basal position (Fig. 1A). *P. abencerragus* and *P. barbagiae* formed another strongly supported group, as well as the group including all sequences from *P. baton*, *P. vicrama* and *P. panoptes*, although the relationships between the latter three taxa were not resolved. The MJ network analysis (Fig. 1B) was fully consistent with the Bayesian phylogenetic analysis and eight mutational steps separate *P. barbagiae* from *P. abencerragus*. The largest interspecific distance (4.8%) was found between *P. fatma* and the *baton* - *vicrama* - *panoptes* group, while the smallest (1.2%) was between the sister species *P. abencerragus* and *P. barbagiae*. The Sardinian endemic thus had closer affinity to *P. abencerragus* (1.2%) than to the *baton* - *vicrama* - *panoptes* group (1.8%).

### Discussion

The distribution pattern of *Pseudophilotes* species indicates that the genus probably had an Eurasiatic origin. Our results are consistent with the scenario that the speciation of *P. barbagiae* most likely took place in the Middle Pleistocene (about 0.6 Ma) as result of vicariance due to the isolation of Sardinia, when the sea re-flooded the Mediterranean basin after the Messinian crisis (about 5 Ma). The island had last contact to the mainland during the Messinian salinity crisis, as the result of desiccation of the Mediterranean Basin. In this period also the Strait of Gibraltar was closed and a land bridge connected Europe and North Africa. We hypothesize that the common ancestor of *P. barbagiae* and *P. abencerragus* was widely distributed from North Africa to South-West Europe and it colonized the island until the Pleistocene, when Sardinia became disconnected from the mainland after a rise of the sea level. It is however unknown if the colonization took place from Iberia or from a certain region of the Maghreb. However, an expansion from Africa has already been observed in other Sardinian endemic butterflies<sup>8,9</sup> and a similar scenario seems plausible for *P. barbagiae* as well, in view of the smaller geographical distance between Sardinia and the African continent. Several other groups besides butterflies<sup>10,11</sup> such as cave-beetles<sup>12</sup>, lizards<sup>13</sup> and snakes<sup>14</sup> speciated after the Messinian salinity crisis.

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Fig. 1. COI Bayesian Tree (A) and Median Joining Network (B) of 39 unique *Pseudophilotes* haplotypes. Circled areas are proportional to haplotype frequency. In (A) and (B) the numbers above and below branches represent Bayesian posterior probabilities and the number of nucleotide substitutions respectively.