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Factors influencing butterfly diversity of the species-rich Submediterranean region

Ph.D. Thesis

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Annotation

This thesis focuses on the history, current state, and conservation of butterfly diversity in the Submediterranean grasslands and Palaearctic steppes. The thesis is introduced with the summary of Cenozoic history of the steppe biome in Eurasia. Then it consists of three studies. The first study describes butterfly communities and microhabitat selection of a steppe specialist butterfly *Proterebia afra* in two relic grassland areas in the Balkans. Based on genetic markers and samples covering the whole distribution ranges, the second and third studies assess the phylogeography of two steppe butterflies, *P. afra* and *Pseudophilotes bavius*, distributed in the Irano-Anatolian region, the Balkans, as well as the northern Palaearctic steppes.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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České Budějovice, 29. 3. 2019

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Alena Bartoňová

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Cover image: Anna Vosolsobě

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List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. Bartoňová, A., Kolář, V., Marešová, J., Šašić, M., Šlancarová, J., Sucháček, P., Konvička, M., 2017. Isolated Asian steppe element in the Balkans: habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities. Journal of Insect Conservation 21, 559–571 (IF=1.562).
Alena Bartoňová designed the study together with MK, led the field experiments, analysed the data and wrote the manuscript with MK.

II. Bartoňová, A., Konvička, M., Korb, S., Kramp, K., Schmitt, T., Faltýnek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867–884 (IF=2.532).
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III. Bartoňová, A., Konvička, M., Marešová, J., Kolev, Z., Wahlberg, N., Faltýnek Fric, Z. Recently lost connectivity in the Western Palaearctic steppes: the case of a scarce specialist butterfly (manuscript).
Alena Bartoňová designed the study together with ZFF, obtained the samples with ZFF and ZK, carried out the laboratory work, analysed the data, and wrote the manuscript with MK.

Co-author agreement

Martin Konvička, the supervisor of this thesis and co-author of all presented manuscripts, fully acknowledges the contribution of Alena Bartoňová as the first author and her contributions as stated above.

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Contents

INTRODUCTION

1. Introduction

The Submediterranean grasslands represent the transition of the steppe to the Mediterranean biome, and are climatically similar to the Middle Asian steppes (Wesche et al., 2016). They are widely distributed in Asia Minor and occur as disjunct patches in the three Mediterranean Peninsulas, often as pastures in mountainous areas (Catorci et al., 2014). Such patches were formed under local conditions, such as altitude, water supply, specific bedrock or disturbance regime. They could represent, however, relic conditions of the past, which survived under specific circumstances. The Balkans is specifically rich in steppe elements (Polunin, 1980), regarding the proximity to the core Asian steppes and geomorphic diversity. While the lowland fertile Eurasian steppes are mostly threaten by transition into arable land (Török et al., 2016; Wesche et al., 2016), the Mediterranean mountain steppes face mostly the decrease in traditional land use, and successional overgrowing or direct afforestation (e.g., Debussche et al. 1999; Cremene et al. 2005; Mazzoleni et al. 2008).

The butterflies (Lepidoptera: Papilionoidea) became a popular model in biogeography due to several reasons: the knowledge of their current distribution is supreme to many insect groups (Boggs et al., 2003), and many of them specialize to a certain habitat type (due to their host plant, light, shelter, nectar, and many other, often very specific demands) with limited movement among habitat patches. Moreover, they could react swiftly as the habitat conditions change, including both rapid population crashes (Kadlec et al., 2010) and colonisations (Neve et al., 2009). Sadly, about a third of the populations of the European butterfly species is declining, and their main threat is the loss of the habitat or the habitat connectivity (Van Swaay et al., 2010). Many butterflies in Europe have fragmented distribution, with populations often remote to each other, but their area frequently covers large parts of the Palaearctic realm or the Northern Hemisphere (cf. Vila et al., 2010).

The biogeographic history of the steppe elements reaching Europe remains poorly studied (Stewart et al., 2010; Kajtoch et al., 2016), even though they probably represent a relic and endangered components of the European fauna. In this thesis, I focus on biogeography and ecology of butterfly species of Eurasian steppes with disjunct distribution in the Submediterranean parts of the Balkan Peninsula.

2. On the history of the vast Eurasian grasslands

Eurasian grasslands, as they are known, originated and formed during the Cenozoic period. Its elaborate history encompasses the cooling of the planet, accumulation of land masses, spread of grasses, the mighty mammalian megafauna and last but not least, us – the humans.

2.1 Eocene to Miocene

In early Tertiary, the whole Earth was warm and covered with evergreen forests up to polar regions (Wolfe, 1985; Sluijs et al., 2007). During the Eocene/Oligocene transition (34 Million years ago, Mya), the global temperatures decreased due to the establishment of the Antarctic Circumpolar Current, decrease in atmospheric CO_2, and a minimum in solar insolation (DeConto and Pollard, 2003; Liu et al., 2009). The Antarctic ice sheet started to form (Kennett, 1977) and the planet shifted from the “green house” stage into the “ice house”.

Major land and climate reorganisations of Eurasia started at the beginning of Oligocene. The Turgai Strait, connecting the warm Tethys sea in the South with the West Siberian Sea in the North, disappeared (Baraboshkin et al., 2003; Roberts et al., 2009), intensifying the effect of global cooling. A major faunal restructuring happened that time in Europe, when closed forest mammals were replaced with those of some extent more open and seasonal environments, referred as the *Grande Coupure* event (“the great break”) (e.g., Hooker et al., 2004).

The collision of the Indian and Eurasian tectonic plates uplifted the Tibetan plateau and the Himalaya Mountains, preventing the moisture inflow from the west to inner Asia (Ruddiman and Kutzbach, 1989; Ramstein et al., 1997). Open steppe was present since the beginning of Oligocene in Central Asia (Wang, 1997; Wang et al., 2006), dominated by herbs including some grass species, with localised forest patches (Song et al., 1981). The transition was reflected in the faunal composition change, termed as the Mongolian Remodeling (Meng and McKenna, 1998). The abundance of grass pollen in the palynological records did not rise until Early Miocene (Jacobs et al., 1999 and references therein). The establishment of the monsoon system around 8 – 9 Mya (Miocene) further enhanced the aridity in the area (Zhisheng et al., 2001).

The African tectonic plate approached, causing the closure of the Tethys Sea and the Alpine orogeny (Dewey et al., 1973; Dercourt et al., 1986). The epicontinental Paratethys Sea, covering the central Eurasia during most of the Oligocene and representing the most important source of moisture in the area, gradually shrunk during Miocene, significantly changing the Eurasian climate towards the more continental conditions (Ramstein et al., 1997). Between Oligocene and Miocene (Paleogene/Neogene boundary), the evergreen thermophilous trees were replaced by deciduous cold-adapted forest taxa (Jacobs et al., 1999 and references therein). In Central Europe, this exchange took place by the Middle Miocene, whereas in the south, the evergreen taxa were still abundant until the Late Miocene. The later Miocene era is characterized by enhanced aridification of the continent (Fortelius et al., 2006). The Mediterranean Sea had become isolated from the oceans and to some extent (or completely) desiccated during the event termed as the Messinian salinity crisis about 6 Mya, at the Miocene/Pliocene boundary (Hsü et al., 1973; Krijgsman et al., 1999). Low sea levels and increased aridity could allow steppe taxa from Asia to reach the Western Mediterranean, mirrored in recent disjunct distribution between Spain and Central Asia (Ribera and Blasco-Zumeta, 1998). The landscape opening was pronounced in the Eastern Europe when compared to Central Europe, which remained relatively closed (Fortelius et al., 2006).

The browsing ungulates (leaves-eating) dramatically declined in North America during middle to late Miocene, and were replaced by grazers (grass-eating) (Janis et al., 2000). These so-called *Hipparion* faunas (such as bovids, giraffids, and horses) expanded across Eurasia in later Miocene, traditionally interpreted as the response of spread of grass dominated ecosystems, but the Eurasian fauna consisted primarily of mixed-eating ungulates until the Pliocene (Janis, 1993; Jacobs et al., 1999). The phytolith data suggested that the grass-dominated ecosystems had been already present in Anatolia since the Early Miocene, and the *Hipparion* fauna radiation could have rather been triggered by increased drying and opening of habitats (Strömberg et al., 2007). Around 7 Mya, C_4-photosynthethising grasses spread over the Earth, bearing an innovation for coping with severe conditions, but their quantity remained rather low in Europe and over the northerly latitudes (Cerling et al., 1997; Osborne et al., 2014).

2.2 Pliocene and Pleistocene

The Plio-Pleistocene is characterized by further decrease of CO_2 in the atmosphere, climatic cooling, gradual formation of the Northern Hemisphere ice sheets, establishment of glacial cycles and their increasing amplitude.

Even since the late Miocene, the first ice covers were periodically arising in the Northern Hemisphere (Jansen and Sjøholm, 1991). Repeated and synchronous advances of the Greenland, Laurentide (North American) and Scandinavian ice sheets were documented from 3 Mya, and large scale glaciation in the regions encompassing Nordic Seas and North Atlantic ocean were present since 2.75 Mya onwards (Flesche Kleiven et al., 2002). At that time, permafrost was formed for the first time in areas adjacent to the ice sheets and a circumpolar tundra belt evolved (Kahlke, 2014 and references therein). The water retention in ice caused lowering of the sea levels, revealing more land for terrestrial ecosystems (Miller, 2005). The advances of the ice sheets further reduced the moisture inflow to the Palaearctic region (Guthrie, 2001).

The official boundary between Pliocene and Pleistocene (Tertiary/Quaternary) has been established at 2.59 Mya according to the International Commission on Stratigraphy (Cohen et al., 2013). During the Quaternary, the glacial cycles of 19 -23 thousand years (ky) long changed their periodicity to 41 ky in the early Pleistocene at 1.4 Mya, and increased to 100 ky cyclers at about 0.7 Mya (mid-Pleistocene transition) (Ruddiman et al., 1989; Lisiecki and Raymo, 2007). The mid-Pleistocene transition also triggered the onset of the first major Pleistocene glaciation in the Alps (Muttoni et al., 2003).

Central Asia was experiencing further aridification during the early Pleistocene, which was driving the evolution of the local mammalian fauna, gradually alternating the life strategy towards grazing. In the circumpolar tundra, another fauna of open landscapes evolved, first separated by taiga belt from the continental steppes. Between 480 and 400 thousand years ago (kya), during the long lasting cold conditions of MIS 12 (Marine Isotope Stage), the taiga belt retreated and the two faunas co-occurred for the first time, forming the famous cold mammoth steppe (Guthrie, 2001; Kahlke, 2014). The cold mammoth steppe supported a large biomass of the megaherbivores (e.g., Zimov et al., 2012) and was arid, but botanically diverse, composed of grasses, sedges and forbs such as *Artemisia* L. (Zazula et al., 2003). This new biome had Palaearctic-wide distribution during the cold and long glacials of the later Pleistocene. A small-extent present-day analogue could be found in the South-Eastern Russian Altai Mountains and is characterized as a landscape mosaic of different habitat types, both dry and wet (Ricankova et al., 2014; Chytrý et al., 2019).

The taiga belt separating the two faunas should cyclically re-appear during the shorter interglacials (Kahlke, 2014). Zimov (Zimov, 2005; Zimov et al., 2012) argues that the numerous large herbivores of the mammoth steppe could have maintained the highly productive grasslands over a wide range of climates, and preventing tundra to establish in the arctic during interglacials. Hence, the mammoth ecosystem was probably insensitive to climatic variations.

In Europe, the Pleistocene large mammalian faunas were alternating between continental glacial and oceanic interglacial stages, and the landscape basically oscillated between steppe and tree savannah/different types of woodland, but consisting of rich mosaic of different habitats (Kahlke et al., 2011). Vera (2000) argued that the large herbivores were the major force once maintaining a shifting mosaic of habitats in the temperate Europe. This is in agreement with the dung beetle assemblages from the previous interglacial (Sandom et al., 2014) suggesting that the herbivores were able to maintain the landscape relatively open and heterogeneous during the warmer stage. The interglacial small mammal and malacofaunal records indicate the warm and humid woodland environment (Horáček and

Ložek, 1988; Ložek, 2001). On the other hand, the central European full glacial loess molluscan assemblages are characterized by the widely distributed steppe, arctic, and even southern xeric steppe elements (Horáček and Ložek, 2004).

The fauna of the last glacial maximum (LGM) in Southern Europe comprised both temperate-adapted and cold-adapted elements (Sommer and Nadachowski, 2006). A similar peculiar mixture was harboured in the Carpathians and Sudety Mountains (Lozek, 2006; Sommer and Nadachowski, 2006; Juřičková et al., 2014).

In the late Quaternary, the modern humans spread across the Eurasia. Starting about 50 kya, many of the key mammoth steppe species reduced its populations in Eurasia or went extinct (e.g., Koch and Barnosky, 2006) (including the woolly mammoth, which latest populations survived until early Holocene, Stuart et al., 2002). The almost worldwide event, named the Late Quaternary Extinctions, have been attributed to climate change, human hunting (the *overkill* hypothesis), or both (e.g., Koch and Barnosky, 2006; Lorenzen et al., 2011; Araujo et al., 2017). Nevertheless, much evidence supports the major human role in Palaearctic mammoth fauna extirpation (Zimov et al., 2012; Araujo et al., 2017).

2.3 Holocene

The Holocene era started approximately 11,700 before present (BP), with rapid melting of the remaining continental ice sheets (Walker et al., 2009; Cohen et al., 2013), and it is essentially linked to the age of men. We were capable of transforming the whole landscape since the first spread.

In the early Holocene (Preboreal period), the grasslands were still widespread in Central Europe (Feurdean et al., 2018). The herbivore pressure was probably already less effective in the Northern and Western Europe (Svenning, 2002; Sandom et al., 2014). The extent of forest cover in Central Europe increased between 9000 and 4000 BP, before farming and the domestic herds overtook the role in landscape opening (Feurdean et al., 2018). The first farmers spread from the eastern Mediterranean into the Balkans and then to the rest of Europe during the early to mid Holocene (Bogucki, 1996) and the livestock numbers first increased in Atlantic period in Eastern Europe (Schumacher et al., 2016). Given the biodiversity richness of grasslands, herbivore pressure, frequent fires, and the presence of specific soils, the usually termed secondary grasslands in Central Europe (cf. Wesche et al., 2016), attributed to be the results of the long-term human activities, could have persisted in some extent during the whole Holocene (Feurdean et al., 2018). This is supported by uninterrupted presence of > 9% of Poaceae in a pollen-based Holocene vegetation reconstruction (Abraham et al., 2016). Pokorný et al. (2015) provided evidence for continuous local occurrence of steppe grasslands in Central Europe, immediately followed by Neolithic farming.

In Anatolia and NW Iran, the early Holocene vegetation of semi-arid savannah and *Artemisia* steppe was gradually encroached by open forest of deciduous oaks and *Pistacia* L., and such habitat was highly deforested by men in later Holocene (Djamali et al., 2008; Asouti and Kabukcu, 2014; Biltekin et al., 2018). The deciduous oak forests, with some but minor evergreen elements, broadly established in the Mediterranean, and suffered the same fate as in Anatolia (Reille et al., 1996; Hajar et al., 2010).

From the Carpathians to the Ural Mountains, the forest steppe ecotone had been present during the Holocene before the recent conversion into agricultural landscape (Shumilovskikh et al., 2018). The steppe belt (Pontic and Middle Asian steppes) transformed from cold and arid mammoth steppe (with some cold-adapted conifers) to present-day steppe with scattered deciduous woodland, varying ratio of *Artemisia* spp. and Chenopodiaceae, but with continuous presence of grasses (Tarasov et al., 1997; Kremenetski et al., 1999; Chlachula, 2010). In Ukraine and southern Russia, the steppe belt has been affected by nomadic populations since the mid-Holocene (Gumilev, 2008; Kremenetski et al., 1999).

Nomadic pastoralism was the first human land use of steppes, with seasonal migrations across the plains or uphill/downhill. The pastoralism remained in regions where the arid or mountainous conditions hamper the pasture transition into arable land (e.g. transhumance between Caucasus and the steppes in eastern Georgia). The steppes of Europe and southern Russia developed on the nutrient-rich dark soils (chernozem) and were extensively converted into cropland, and only fragments survived. The transformation, however, occurred relatively lately, as this region was, for most of historical epoch, a crossroad and battleground between nomadic/predatory cultures from the East and South, and settled agriculturalists from the North (Sunderland, 2004). A huge area of steppes of Kazakhstan and adjacent parts of Russia were turned into arable land as late as in the 1950s. In the 1990s, the state farm system collapsed in the Eastern Bloc. Up to 60 million hectares of cropland were abandoned in the former USSR, and species richness partly recovered (Kamp et al., 2015, 2018). However, the recovered biotopes could suffer from absence of management in future (Mathar et al., 2016). For instance, grazing and time since abandonment positively affected the reestablishment of steppe species in Western Siberia, but they were negatively affected by the cover of competitive grasses (Kämpf et al., 2016). Recently, a large portion of abandoned cropland has been reclaimed (Kamp et al., 2015).

The proportion of converted steppes decrease from the West to the East: almost all steppes were ploughed in Ukraine and hardly any in Mongolia (Török et al., 2016). In Anatolia, the steppes are threatened by the conversion to cropland, overgrazing but also rural abandonment and succession (Ambarlı et al., 2016).

In present, the grasslands (from which the Palaearctic steppes form a great proportion) are the world´s most endangered ecosystems, but only a small fraction is under any legal protection (Hoekstra et al., 2005). Regarding their extent, a long history, unique biodiversity and importance in human civilizations, the steppes are worthy of our attention.

3. Diversity of the Balkans: on a crossroad

The geographic, biotic and cultural diversity of the Balkans is undoubtedly spectacular. The biotopes are changing and intermixing on a small spatial scale. The Balkan biota is composed of several elements (Polunin, 1980): (1) taxa of temperate deciduous biome, which persisted in the area during glacial stages, present in mountains of inner Balkans; (2) species of evergreen Mediterranean formations, situated mostly along the coastline and on the islands; (3) Balkan endemics; and (4) continental taxa with present Pontic and/or Irano-Turanian distribution. The latter forms and inhabits steppe-like grasslands and shrublands in middle altitudes, maintained by both climate and pastoralism as a traditional land use.

The Balkans served as an important large mammalian fauna migratory crossroad between Africa and Eurasia during its formation in Neogene (Koufos et al., 2005). In early Quaternary, the migration waves were related to the southwest expansion of steppes. Some of the new arrivals represented northern elements. Both cold and temperate taxa are known from the late Pleistocene Greece. In the late Pleistocene long glacial periods, sea levels declined, opening extensive land masses in the Aegean region (Perissoratis and Conispoliatis, 2003), facilitating migration. The Adriatic Sea was also diminished, connecting the North-Eastern Adriatic with Italian Peninsula, with Croatian islands as mountain ridges at the edge of the plain, and river Po forming its delta at the ridge of the Mid-Adriatic (Maselli et al., 2011). From the mid Pleistocene onwards, the fauna of continental Croatia indicated an open steppe (Mauch Lenardić et al., 2018).

In the last glacial period, the landscape of continental Greece was principally dominated by herbaceous steppes with spatially restricted patches of trees, both in lowlands and mountains (Willis, 1992a; Magyari et al., 2008). The topographic complexity could have buffered the extreme effects of climate and residual tree populations survived (Tzedakis et al., 2002). The Irano-Turanian floral elements probably reached the Balkans during the glacial periods via the Thracian plain (Magyari et al., 2008).

Moreover, glaciers covered the mountainous parts of the Balkan Peninsula during the glacial times. The most prominent ice sheet formed over Montenegro during the coldest phase of the Pleistocene (MIS 12) (Hughes et al., 2011). There is some evidence of lowland glaciations in Eastern Adriatic (e.g. the Pag Island) from the same time (Marjanac and Marjanac, 2004). In the LGM, the largest glaciers were present in the mountain chain alongside the west of the peninsula (Hughes and Woodward, 2008).

Both the inner lowlands and coastline are characterized by the presence of the steppe vegetation during the end of the last glacial period and onset of deciduous oak forests in the climatic transition (Willis, 1992b; Šoštarić, 2005; Kotthoff et al., 2008). On the Thracian Plain, the deforestation and the transition to present day open vegetation with e.g. the evergreen oak *Quercus coccifera* L. and *Paliurus spina-christi* Mill. happened during the drier period (~ 4000 BP) and is being attributed to human influence, based on the increased presence of charred particles, and indicators of grazing and erosion (Connor et al., 2013). In coastal Eastern Adriatic, first (~ 8000 BP), the oak forests changed to evergreen vegetation (*Phillyrea* L. and *Juniperus* L.) and this change cannot be attributed to humans. Later, the vegetation switched to communities dominated by *Quercus ilex* L. (Šoštarić, 2005). Further inland, there is an unbroken dominance of sub-Mediterranean deciduous oak forests since ~ 7000 BP and indications of anthropogenic impact are recorded only from the Roman times.

Although the history of the Balkan biota is complex and dynamic, the mosaic of different habitats has been sustained over ages. Heterogeneous Balkan landscapes could allow coexistence of open forest and grassland butterflies (Bartoňová et al., 2017). Species often coexist at a very fine spatial scale thank to their diverging exploitation of sources (Slancarova et al., 2015). Species richness and many endemics are associated with open habitats rather than dense forests in the Balkans (Grill and Cleary, 2003; Slancarova et al., 2016). However, this could rapidly change when introducing factors non-analogous through history. Both land-use abandonment and intensification of agriculture could result in landscape homogenization, and could develop in the loss of species diversity (Cremene et al., 2005).

4. Brief biogeography of Palaearctic species

De Lattin (1967) suggested existence of a three most important biogeographic elements in the Western Palaearctic biota: (a) the Mediterranean (temperate- or warm-adapted), (b) the arctic-alpine (cold-adapted), and (c) Siberian (continental) elements.

The first two represented a latitudinal temperature-driven gradient. The temperate-adapted elements should have withstood the unfavourable conditions of the cold stages in southern refugia (i.e., a maximally contracted range of a particular species). Since the mid-Pleistocene transition, the glacial cycles lasted about 100 ky, with predominant cold climate and the long-term isolation of populations in refugia resulted in genetic differentiation and even speciation (Hewitt, 1996, 1999). These lineages expanded northwards as the conditions change, which could be traced with genetic markers. The temperate-adapted elements often comprised broad-leaf trees or taxa adapted to woodland environment, which widely re-established during the Holocene (Stewart and Lister, 2001). Additional data revealed that the situation was more complex: many of the temperate-adapted species survived also in extra-Mediterranean (cryptic) refugia, which could had been areas with sheltered topography providing a favourable microclimate (Stewart and Lister, 2001). For instance, *Polyommatus coridon* (Poda, 1761) survived in the southern margins of the Alps and Pyrenees (Kühne et al., 2017). The European beech, *Fagus silvatica* L., survived in multiple refugia including Central Europe (Magri et al., 2006). Moreover, the fossil record suggest the simultaneous presence of woodland species with steppe and tundra species in some places in LGM (Stewart and Lister, 2001; Lozek, 2006; Sommer and Nadachowski, 2006).

The cold-adapted elements inhabited vast areas of the cold periglacial steppe and shifted its area to the deglaciated European north and/or up along the mountain slopes. The areas inhabited in the glacial periods could be traced by genetic similarity of often very remote populations. For instance, *Zygaena exulans* (Reiner & Hohemwarth, 1792) had a continuous distribution between the Alps and the Pyrenees (Schmitt and Hewitt, 2004). Descendant populations, which have not been isolated for a long time, could still shelter a high genetic diversity. Populations of *Coenonympha rhodopensis* (Elwes, 1900) from the Balkan Mountains are genetically diverse and not differentiated among individual massifs, and were probably distributed continually in the lowlands during the last glacial (Louy et al., 2013). Higher proportion of alpine elements, however, seem to have survived the glaciations in close proximity of the mountains (Schmitt, 2017). This could have resulted in a number of different lineages observed within single mountain ranges (Schönswetter et al., 2005). For instance, *Gentiana nivalis* L. formed three lineages restricted to the Alps and a fourth lineage present apart from the Alps in other European mountains and in

Scandinavia. There is also evidence of nunatak survival of some plants (Stehlik et al., 2002). Deglaciated Scandinavia was often colonized from Siberia rather than from Europe (Dalén et al., 2007; Marešová et al., 2019).

The Siberian elements were primarily considered as reaching Europe (including the Balkans, but not the majority of the Mediterranean coast, and excluding the Atlantic coast) in the current interglacial. Schmitt and Varga (2012) reviewed that such elements were present in Europe for several glacial cycles and were probably surviving during the glacials as many fragmented populations scattered over Europe, as it was observed, e. g., in *Erebia medusa* (Denis & Schiffermüller, 1775) (Hammouti et al., 2010). The continental taxa do not seem to change their distribution areas extensively between the glacial-interglacial conditions (Schmitt and Varga, 2012).

Stewart et al. (2010) argue that continental taxa represent a part of another, longitudinal humidity-driven gradient. The continental-adapted species should extend their range during glacials, and oceanic species in interglacials. In the case of continental-adapted species, the authors aim to the “true” steppe species, such as ground squirrels *Spermophilus* F. Cuvier, 1825 spp. or saiga antelope *Saiga tatarica* (Linnaeus, 1766), known to be components of the mammoth steppe and distributed further westerly during the last glacial period (e.g., Sokolov and Zhirnov, 1998). In theory, these species should be present nowadays in more continuous manner in Asian steppes, and have isolated populations in Europe (e.g., in Thrace or Pannonia), which serve as interglacial cryptic refugia. The European ground squirrel, *Spermophilus citellus* (Linnaeus, 1766), which had, according to the fossil record, a wider distribution in the north-western direction than today, appears to be currently surviving in refugia (Říčanová et al., 2013).

Kajtoch et al. (2016) reviewed that many steppe elements in Central Europe, often with fragmented populations, formed distinct genetic units, which pointed to a long-term *in situ* survival precluding a recent, Holocene expansion or long-distance dispersal. A Pontic-Pannonian steppe plant, *Linum flavum* L., probably persisted for a long time in its Central European localities, and had arrived prior to Holocene (Cieslak, 2014; Plenk et al., 2017).

The areas situated further east, like the Southern Ural Mountains and Kazakhstan, provided a stable environment without strong range shifts of its inhabitants during the later Pleistocene (Danukalova et al., 2009; Horsák et al., 2010). A few existing studies of steppe taxa on a large geographic scale revealed existence of widely longitudinally distributed lineages (Zinenko et al., 2015; Feoktistova et al., 2017; Bartonova et al., 2018).

To conclude, the available studies agree that the continental elements did not arrive to the west in the Holocene, but were probably present for a longer time, also during glacial maxima. The problematic part is the timing of their range expansions and contractions. Humidity would play a significant role. Megafauna could contribute both to the landscape openness and productivity, and cause that the water is not widely kept in the landscape (Zimov et al., 2012). Some steppe elements could have extended their ranges during the milder interstadial events during glacials (Lebedev et al., 2018; Plenk et al., 2017). The highly studied European biogeography should be complemented by more examples of steppe taxa and taxa distributed further easterly and south-easterly to provide a comprehensive picture.

5. Aims and scope of the thesis

In this thesis, I focused on butterfly taxa of Asian steppes with fragmented, presumably relic distribution in the Balkans. As the study models, I chose a Satyrine butterfly *Proterebia afra* (Fabricius, 1787) (*Proterebia phegea* (Borkhausen, 1788) according to the newly published checklist of the European butterflies, Wiemers et al., 2018, but I keep the name *P. afra* for the purposes of this thesis) and a specialized Lycaenid butterfly *Pseudophilotes bavius* (Eversmann, 1832). Apart from the Balkans, these two species are distributed both in the southern mountain steppes of the Irano-Anatolian biogeographic region (Asia Minor, Caspian Sea southern bank) and in Eastern European and Middle Asian steppes.

These two species meet in one special place in the Balkans – Askion Mountains (West Macedonia region, Greece), a limestone steppe area, which differs from its agricultural and wooded surrounding and resembles Anatolian mountain steppes.

In the first chapter, I focus on two isolated Balkan distribution areas of *P. afra* – Askion Mountains and Croatian Dalmatia. In a field study, I assessed the microhabitat selection of the focal species, its distribution in the areas, the co-occurring butterfly communities, and threats posed on the species in the Balkans.

The second and third chapters focus on biogeography of the two species. The second chapter assesses the phylogeography of *P. afra* based on specimens from its whole distribution range, mitochondrial and nuclear markers, amplified fragment length polymorphism and subsequent biogeographic analyses. The genetic data are compared with species distribution modelling to elucidate the climatically suitable areas of the species in recent and in the last glacial maximum.

The third chapter reveals the phylogeographic history of *P. bavius* based on one mitochondrial and four nuclear markers. This species has several small isolated populations within the Balkans and Eastern Europe with possible conservation concern.

This thesis provide three case studies on steppe species, and could support their conservation and enhance our knowledge on Quaternary biogeography of the Palaearctic region.

6. References

Abraham, V., Kunes, P., Petr, L., Svobodova, H.S., Kozakova, R., Jamrichova, E., Svarcova, M.G., Pokorny, P., 2016. A pollen-based quantitative reconstruction of the Holocene vegetation updates a perspective on the natural vegetation in the Czech Republic and Slovakia. Preslia 88, 409–434.

Ambarlı, D., Zeydanlı, U.S., Balkız, Ö., Aslan, S., Karaçetin, E., Sözen, M., Ilgaz, Ç., Gürsoy Ergen, A., Lise, Y., Demirbaş Çağlayan, S., Welch, H.J., Welch, G., Turak, A.S., Bilgin, C.C., Özkil, A., Vural, M., 2016. An overview of biodiversity and conservation status of steppes of the Anatolian Biogeographical Region. Biodiversity and Conservation 25, 2491–2519.

Araujo, B.B.A., Oliveira-Santos, L.G.R., Lima-Ribeiro, M.S., Diniz-Filho, J.A.F., Fernandez, F.A.S., 2017. Bigger kill than chill: The uneven roles of humans and climate on late Quaternary megafaunal extinctions. Quaternary International 431, 216–222.

Asouti, E., Kabukcu, C., 2014. Holocene semi-arid oak woodlands in the Irano-Anatolian region of Southwest Asia: natural or anthropogenic? Quaternary Science Reviews 90, 158–182.

Baraboshkin, E.Y., Alekseev, A.S., Kopaevich, L.F., 2003. Cretaceous palaeogeography of the North-Eastern Peri-Tethys. Palaeogeography, Palaeoclimatology, Palaeoecology 196, 177–208.

Bartoňová, A., Kolář, V., Marešová, J., Šašić, M., Šlancarová, J., Sucháček, P., Konvička, M., 2017. Isolated Asian steppe element in the Balkans: habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities. Journal of Insect Conservation 1–13.

Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867–884.

Biltekin, D., Eriş, K.K., Çağatay, M.N., Ön, S.A., Akkoca, Di.B., 2018. Late Pleistocene–Holocene environmental change in eastern Turkey: multi-proxy palaeoecological data of vegetation and lake-catchment changes. Journal of Quaternary Science 33, 575–585.

Boggs, C.L., Watt, W.B., Ehrlich, P.R., 2003. Butterflies: Ecology and Evolution Taking Flight. University of Chicago Press, Chicago.

Bogucki, P., 1996. The Spread of Early Farming in Europe. American Scientist 84, 242–253.

Catorci, A., Cesaretti, S., Malatesta, L., Tardella, F.M., 2014. Effects of grazing vs mowing on the functional diversity of sub-Mediterranean productive grasslands. Applied Vegetation Science 17, 658–669.

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene/Pliocene boundary. Nature 389, 153–158.

Chlachula, J., 2010. Pleistocene climate change, natural environments and Palaeolithic occupation of East Kazakhstan. Quaternary International 220, 64–87.

Chytrý, M., Horsák, M., Danihelka, J., Ermakov, N., German, D.A., Hájek, M., Hájková, P., Kočí, M., Kubešová, S., Lustyk, P., Nekola, J.C., Pavelková Řičánková, V., Preislerová, Z., Resl, P., Valachovič, M., 2019. A modern analogue of the Pleistocene steppe-tundra ecosystem in southern Siberia. Boreas 48, 36–56.

Cieslak, E., 2014. Phylogeography of Pontic-Pannonian Species in Central Europe. Polish Botanical Studies 30, 1–53.

Cohen, K.M., Finney, S.C., Gibbard, P.L., Fan, J.-X., 2013. The ICS International Chronostratigraphic Chart. Episodes 36, 199–204.

Connor, S.E., Ross, S.A., Sobotkova, A., Herries, A.I.R., Mooney, S.D., Longford, C., Iliev, I., 2013. Environmental conditions in the SE Balkans since the Last Glacial Maximum and their influence on the spread of agriculture into Europe. Quaternary Science Reviews 68, 200–215.

Cremene, C., Groza, G., Rakosy, L., Schileyko, A.A., Baur, A., Erhardt, A., Baur, B., 2005. Alterations of Steppe-Like Grasslands in Eastern Europe: a Threat to Regional Biodiversity Hotspots. Conservation Biology 19, 1606–1618.

Dalén, L., Nyström, V., Valdiosera, C., Germonpré, M., Sablin, M., Turner, E., Angerbjörn, A., Arsuaga, J.L., Götherström, A., 2007. Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. Proceedings of the National Academy of Sciences of the United States of America 104, 6726–6729.

Danukalova, G., Yakovlev, A., Kosintcev, P., Agadjanian, A., Alimbekova, L., Eremeev, A., Morozova, E., 2009. Quaternary fauna and flora of the Southern Urals region (Bashkortostan Republic). Quaternary International, Quaternary data from the Southern Urals region 201, 13–24.

de Lattin, G., 1967. Grundriss der Zoogeographie. VEB Gustav Fischer Verlag, Jena.

Debussche, M., Lepart, J., Dervieux, A., 1999. Mediterranean landscape changes: evidence from old postcards. Global Ecology and Biogeography 8, 3–15.

DeConto, R.M., Pollard, D., 2003. Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO2. Nature 421, 245–249.

Dercourt, J., Zonenshain, L.P., Ricou, L.-E., Kazmin, V.G., Le Pichon, X., Knipper, A.L., Grandjacquet, C., Sbortshikov, I.M., Geyssant, J., Lepvrier, C., Pechersky, D.H., Boulin, J., Sibuet, J.-C., Savostin, L.A., Sorokhtin, O., Westphal, M., Bazhenov, M.L., Lauer, J.P., Biju-Duval, B., 1986. Geological evolution of the tethys belt from the atlantic to the pamirs since the LIAS. Tectonophysics 123, 241–315.

Dewey, J.F., Pitman, W.C., Ryan, W.B.F., Bonnin, J., 1973. Plate Tectonics and the Evolution of the Alpine System. Geological Society of America Bulletin 84, 3137–3180.

Djamali, M., de Beaulieu, J.-L., Shah-hosseini, M., Andrieu-Ponel, V., Ponel, P., Amini, A., Akhani, H., Leroy, S.A.G., Stevens, L., Lahijani, H., Brewer, S., 2008. A late Pleistocene long pollen record from Lake Urmia, NW Iran. Quaternary Research 69, 413–420.

Feoktistova, N.Y., Meschersky, I.G., Bogomolov, P.L., Sayan, A.S., Poplavskaya, N.S., Surov, A.V., 2017. Phylogeographic structure of the Common hamster (*Cricetus cricetus* L.): Late Pleistocene connections between Caucasus and Western European populations. PLoS ONE 12, e0187527.

Feurdean, A., Ruprecht, E., Molnár, Z., Hutchinson, S.M., Hickler, T., 2018. Biodiversity-rich European grasslands: Ancient, forgotten ecosystems. Biological Conservation 228, 224–232.

Flesche Kleiven, H., Jansen, E., Fronval, T., Smith, T.M., 2002. Intensification of Northern Hemisphere glaciations in the circum Atlantic region (3.5–2.4

Ma) – ice-rafted detritus evidence. Palaeogeography, Palaeoclimatology, Palaeoecology 184, 213–223.

Fortelius, M., Eronen, J., Liu, L., Pushkina, D., Tesakov, A., Vislobokova, I., Zhang, Z., 2006. Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. Palaeogeography, Palaeoclimatology, Palaeoecology 238, 219–227.

Grill, A., Cleary, D.F.R., 2003. Diversity patterns in butterfly communities of the Greek nature reserve Dadia. Biological Conservation 114, 427–436.

Gumilev, L.N., 2008. Древняя Русь и Великая степь [Ancient Rus and Great Steppe, in Russian]. Ayris-press, Moscow.

Guthrie, R.D., 2001. Origin and causes of the mammoth steppe: a story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. Quaternary Science Reviews 20, 549–574.

Hajar, L., Haïdar-Boustani, M., Khater, C., Cheddadi, R., 2010. Environmental changes in Lebanon during the Holocene: Man vs. climate impacts. Journal of Arid Environments 74, 746–755.

Hammouti, N., Schmitt, T., Seitz, A., Kosuch, J., Veith, M., 2010. Combining mitochondrial and nuclear evidences: a refined evolutionary history *of Erebia medusa* (Lepidoptera: Nymphalidae: Satyrinae) in Central Europe based on the COI gene. Journal of Zoological Systematics and Evolutionary Research 48, 115–125.

Hewitt, G.M., 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68, 87–112.

Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58, 247–276.

Hoekstra, J.M., Boucher, T.M., Ricketts, T.H., Roberts, C., 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters 8, 23–29.

Hooker, J.J., Collinson, M.E., Sille, N.P., 2004. Eocene–Oligocene mammalian faunal turnover in the Hampshire Basin, UK: calibration to the global time scale and the major cooling event. Journal of the Geological Society 161, 161–172.

Horáček, I., Ložek, V., 2004. Ledová doba z pohledu zoologa. I. Glaciální fauna a historie její výpovedi [The Ice Age through the Eyes of the Zoologist I. Glacial Fauna and the History Involved in its Testimony, in Czech]. Živa 1/2004: 5–8.

Horáček, I., Ložek, V., 1988. Palaeozoology and the Mid-European Quaternary Past: Scope of the Approach and Selected Results. Academia, Prague.

Horsák, M., Chytrý, M., Danihelka, J., Kočí, M., Kubešová, S., Lososová, Z., Otýpková, Z., Tichý, L., 2010. Snail faunas in the Southern Ural forests and their relations to vegetation: an analogue of the Early Holocene assemblages of Central Europe? Journal of Molluscan Studies 76, 1–10.

Hsü, K.J., Ryan, W.B.F., Cita, M.B., 1973. Late Miocene Desiccation of the Mediterranean. Nature 242, 240–244.

Hughes, P.D., Woodward, J.C., 2008. Timing of glaciation in the Mediterranean mountains during the last cold stage. Journal of Quaternary Science 23, 575–588.

Hughes, P.D., Woodward, J.C., van Calsteren, P.C., Thomas, L.E., 2011. The glacial history of the Dinaric Alps, Montenegro. Quaternary Science Reviews 30, 3393–3412.

Jacobs, B.F., Kingston, J.D., Jacobs, L.L., 1999. The Origin of Grass-Dominated Ecosystems. Annals of the Missouri Botanical Garden 86, 590–643.

Janis, C.M., 1993. Tertiary Mammal Evolution in the Context of Changing Climates, Vegetation, and Tectonic Events. Annual Review of Ecology and Systematics 24, 467–500.

Janis, C.M., Damuth, J., Theodor, J.M., 2000. Miocene ungulates and terrestrial primary productivity: Where have all the browsers gone? Proceedings of the National Academy of Sciences of the United States of America 97, 7899–7904.

Jansen, E., Sjøholm, J., 1991. Reconstruction of glaciation over the past 6 Myr from ice-borne deposits in the Norwegian Sea. Nature 349, 600–603.

Juřičková, L., Ložek, V., Horáčková, J., Tlachač, P., Horáček, I., 2014. Holocene succession and biogeographical importance of mollusc fauna in the Western Sudetes (Czech Republic). Quaternary International 353, 210–224.

Kadlec, T., Vrba, P., Kepka, P., Schmitt, T., Konvicka, M., 2010. Tracking the decline of the once-common butterfly: delayed oviposition, demography and population genetics in the hermit *Chazara briseis*. Animal Conservation 13,

Kahlke, R.-D., 2014. The origin of Eurasian Mammoth Faunas (Mammuthus–Coelodonta Faunal Complex). Quaternary Science Reviews 96, 32–49.

Kahlke, R.-D., García, N., Kostopoulos, D.S., Lacombat, F., Lister, A.M., Mazza, P.P.A., Spassov, N., Titov, V.V., 2011. Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe. Quaternary Science Reviews 30, 1368–1395.

Kajtoch, Ł., Cieślak, E., Varga, Z., Paul, W., Mazur, M.A., Sramkó, G., Kubisz, D., 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. Biodiversity and Conservation 25, 2309–2339.

Kamp, J., Reinhard, A., Frenzel, M., Kämpfer, S., Trappe, J., Hölzel, N., 2018. Farmland bird responses to land abandonment in Western Siberia. Agriculture, Ecosystems & Environment 268, 61–69.

Kamp, J., Urazaliev, R., Balmford, A., Donald, P.F., Green, R.E., Lamb, A.J., Phalan, B., 2015. Agricultural development and the conservation of avian biodiversity on the Eurasian steppes: a comparison of land-sparing and land-sharing approaches. Journal of Applied Ecology 52, 1578–1587.

Kämpf, I., Mathar, W., Kuzmin, I., Hölzel, N., Kiehl, K., 2016. Post-Soviet recovery of grassland vegetation on abandoned fields in the forest steppe zone of Western Siberia. Biodiversity and Conservation 25, 2563–2580.

Kennett, J.P., 1977. Cenozoic evolution of Antarctic glaciation, the circum-Antarctic Ocean, and their impact on global paleoceanography. Journal of Geophysical Research 82, 3843–3860.

Koch, P.L., Barnosky, A.D., 2006. Late Quaternary Extinctions: State of the Debate. Annual Review of Ecology, Evolution, and Systematics 37, 215–250.

Kotthoff, U., Mueller, U.C., Pross, J., Schmiedl, G., Lawson, I.T., van de Schootbrugge, B., Schulz, H., 2008. Lateglacial and Holocene vegetation dynamics in the Aegean region: an integrated view based on pollen data from marine and terrestrial archives. Holocene 18, 1019–1032.

Koufos, G.D., Kostopoulos, D.S., Vlachou, T.D., 2005. Neogene/Quaternary mammalian migrations in Eastern Mediterranean. Belgian Journal of Zoology 135, 181–190.

Kremenetski, C.V., Chichagova, O.A., Shishlina, N.I., 1999. Palaeoecological evidence for Holocene vegetation, climate and land-use change in the low Don basin and Kalmuk area, southern Russia. Vegetation History and Archaeobotany 8, 233–246.

Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. Nature 400, 652–655.

Kühne, G., Kosuch, J., Hochkirch, A., Schmitt, T., 2017. Extra-Mediterranean glacial refugia in a Mediterranean faunal element: the phylogeography of the chalk-hill blue *Polyommatus coridon* (Lepidoptera, Lycaenidae). Scientific Reports 7, srep43533.

Lebedev, V., Poplavskaya, N., Bannikova, A., Ryurikov, G., Surov, A., 2018. Genetic differentiation in Cricetulus migratorius Pallas, 1773 (Rodentia, Cricetidae). Mammalian Biology 92, 115–119.

Lisiecki, L.E., Raymo, M.E., 2007. Plio–Pleistocene climate evolution: trends and transitions in glacial cycle dynamics. Quaternary Science Reviews 26, 56–69.

Liu, Z., Pagani, M., Zinniker, D., DeConto, R., Huber, M., Brinkhuis, H., Shah, S.R., Leckie, R.M., Pearson, A., 2009. Global Cooling During the Eocene-Oligocene Climate Transition. Science 323, 1187–1190.

Lorenzen, E.D., Nogués-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K.A., Ugan, A., Borregaard, M.K., Gilbert, M.T.P., Nielsen, R., Ho, S.Y.W., Goebel, T., Graf, K.E., Byers, D., Stenderup, J.T., Rasmussen, M., Campos, P.F., Leonard, J.A., Koepfli, K.-P., Froese, D., Zazula, G., Stafford, T.W., Aaris-Sørensen, K., Batra, P., Haywood, A.M., Singarayer, J.S., Valdes, P.J., Boeskorov, G., Burns, J.A., Davydov, S.P., Haile, J., Jenkins, D.L., Kosintsev, P., Kuznetsova, T., Lai, X., Martin, L.D., McDonald, H.G., Mol, D., Meldgaard, M., Munch, K., Stephan, E., Sablin, M., Sommer, R.S., Sipko, T., Scott, E., Suchard, M.A., Tikhonov, A., Willerslev, R., Wayne, R.K., Cooper, A., Hofreiter, M., Sher, A., Shapiro, B., Rahbek, C., Willerslev, E., 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. Nature 479, 359–364.

Louy, D., Habel, J.C., Abadjiev, S., Schmitt, T., 2013. Genetic legacy from past panmixia: high genetic variability and low differentiation in disjunct populations of the Eastern Large Heath butterfly. Biological Journal of the Linnean Society 110, 281–290.

Lozek, V., 2006. Last glacial paleoenvironments of the west Carpathians in the light of fossil malacofauna. Sbornik Geologickych Ved Antropozoikum 26, 73–84.

Ložek, V., 2001. Molluscan fauna from the loess series of Bohemia and Moravia. Quaternary International 76–77, 141–156.

Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gömöry, D., Latałowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., van der Knaap, W.O., Petit, R.J., de Beaulieu, J.-L., 2006. A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. New Phytologist 171, 199–221.

Magyari, E.K., Chapman, J.C., Gaydarska, B., Marinova, E., Deli, T., Huntley, J.P., Allen, J.R.M., Huntley, B., 2008. The ‘oriental’ component of the Balkan flora: evidence of presence on the Thracian Plain during the Weichselian late-glacial. Journal of Biogeography 35, 865–883.

Maresova, J., Habel, J.C., Neve, G., Sielezniew, M., Bartonova, A., Kostro-Ambroziak, A., Faltynek Fric, Z., 2019. Cross-continental phylogeography of two boreo-montane Nymphalid butterflies. PLoS ONE 14, e0214483.

Marjanac, L., Marjanac, T., 2004. Glacial history of the Croatian Adriatic and Coastal Dinarides, in: Ehlers, J., Gibbard, P.L. (Eds.), Quaternary Glaciations Extent and Chronology. Part I: Europe. Developments in Quaternary Sciences. Elsevier, Amsterdam, pp. 19–26.

Maselli, V., Hutton, E.W., Kettner, A.J., Syvitski, J.P.M., Trincardi, F., 2011. High-frequency sea level and sediment supply fluctuations during Termination I: An integrated sequence-stratigraphy and modeling approach from the Adriatic Sea (Central Mediterranean). Marine Geology 287, 54–70.

Mathar, W.P., Kämpf, I., Kleinebecker, T., Kuzmin, I., Tolstikov, A., Tupitsin, S., Hölzel, N., 2016. Floristic diversity of meadow steppes in the Western Siberian Plain: effects of abiotic site conditions, management and landscape structure. Biodiversity and Conservation 25, 2361–2379.

Mauch Lenardić, J., Oros Sršen, A., Radović, S., 2018. Quaternary fauna of the Eastern Adriatic (Croatia) with the special review on the Late Pleistocene sites. Quaternary International 494, 130–151.

Mazzoleni, S., Pasquale, G. di, Mulligan, M., Martino, P. di, Rego, F., 2008. Recent Dynamics of the Mediterranean Vegetation and Landscape. Wiley, Hoboken.

Meng, J., McKenna, M.C., 1998. Faunal turnovers of Palaeogene mammals from the Mongolian Plateau. Nature 394, 364–367.

Miller, K.G., 2005. The Phanerozoic Record of Global Sea-Level Change. Science 310, 1293–1298.

Muttoni, G., Carcano, C., Garzanti, E., Ghielmi, M., Piccin, A., Pini, R., Rogledi, S., Sciunnach, D., 2003. Onset of major Pleistocene glaciations in the Alps. Geology 31, 989–992.

Neve, G., Pavlicko, A., Konvicka, M., 2009. Loss of genetic diversity through spontaneous colonization in the bog fritillary butterfly, *Proclossiana eunomia* (Lepidoptera: Nymphalidae) in the Czech Republic. European Journal of Entomology 106, 11–19.

Osborne, C.P., Salomaa, A., Kluyver, T.A., Visser, V., Kellogg, E.A., Morrone, O., Vorontsova, M.S., Clayton, W.D., Simpson, D.A., 2014. A global database of C_4 photosynthesis in grasses. New Phytologist 204, 441–446.

Perissoratis, C., Conispoliatis, N., 2003. The impacts of sea-level changes during latest Pleistocene and Holocene times on the morphology of the Ionian and Aegean seas (SE Alpine Europe). Marine Geology 196, 145–156.

Plenk, K., Bardy, K., Höhn, M., Thiv, M., Kropf, M., 2017. No obvious genetic erosion, but evident relict status at the westernmost range edge of the Pontic-Pannonian steppe plant *Linum flavum* L. (Linaceae) in Central Europe. Ecology and Evolution 7, 6527–6539.

Pokorný, P., Chytrý, M., Juřičková, L., Sádlo, J., Novák, J., Ložek, V., 2015. Mid-Holocene bottleneck for central European dry grasslands: Did steppe survive the forest optimum in northern Bohemia, Czech Republic? The Holocene 25, 716–726.

Polunin, O., 1980. Flowers of Greece and the Balkans: a field guide. Oxford University Press, Oxford.

Ramstein, G., Fluteau, F., Besse, J., Joussaume, S., 1997. Effect of orogeny, plate motion and land–sea distribution on Eurasian climate change over the past 30 million years. Nature 386, 788–795.

Reille, M., Andrieu, V., De Beaulieu, J.-L., 1996. The main features of the vegetation history in western Mediterranean mountains. Ecologie (Brunoy) 27, 153–169.

Ribera, I., Blasco-Zumeta, J., 1998. Biogeographical links between steppe insects in the Monegros region (Aragón, NE Spain), the eastern Mediterranean, and central Asia. Journal of Biogeography 25, 969–986.

Ricankova, V.P., Robovsky, J., Riegert, J., 2014. Ecological Structure of Recent and Last Glacial Mammalian Faunas in Northern Eurasia: The Case of Altai-Sayan Refugium. PLoS One 9, e85056.

Říčanová, Š., Koshev, Y., Říčan, O., Ćosić, N., Ćirović, D., Sedláček, F., Bryja, J., 2013. Multilocus phylogeography of the European ground squirrel: cryptic interglacial refugia of continental climate in Europe. Molecular Ecology 22, 4256–4269.

Roberts, C.D., LeGrande, A.N., Tripati, A.K., 2009. Climate sensitivity to Arctic seaway restriction during the early Paleogene. Earth and Planetary Science Letters 286, 576–585.

Ruddiman, W.F., Kutzbach, J.E., 1989. Forcing of late Cenozoic northern hemisphere climate by plateau uplift in southern Asia and the American west. Journal of Geophysical Research 94, 18409–18427.

Ruddiman, W.F., Raymo, M.E., Martinson, D.G., Clement, B.M., Backman, J., 1989. Pleistocene evolution: Northern hemisphere ice sheets and North Atlantic Ocean. Paleoceanography 4, 353–412.

Sandom, C.J., Ejrnæs, R., Hansen, M.D.D., Svenning, J.-C., 2014. High herbivore density associated with vegetation diversity in interglacial ecosystems. Proceedings of the National Academy of Sciences of the United States of America 111, 4162–4167.

Schmitt, T., 2017. Molecular Biogeography of the High Mountain Systems of Europe: An Overview, in: Catalan, J., Ninot, J.M., Aniz, M.M. (Eds.), High Mountain Conservation in a Changing World. Springer International Publishing, Cham, pp. 63–74.

Schmitt, T., Hewitt, G.M., 2004. Molecular biogeography of the arctic-alpine disjunct burnet moth species Zygaena exulans (Zygaenidae, Lepidoptera) in the Pyrenees and Alps. Journal of Biogeography 31, 885–893.

Schmitt, T., Varga, Z., 2012. Extra-Mediterranean refugia: The rule and not the exception? Front Zool 9, 1–12. https://doi.org/10.1186/1742-9994-9-22

Schönswetter, P., Stehlik, I., Holderegger, R., Tribsch, A., 2005. Molecular evidence for glacial refugia of mountain plants in the European Alps. Molecular Ecology 14, 3547–3555.

Schumacher, M., Schier, W., Schütt, B., 2016. Mid-Holocene vegetation development and herding-related interferences in the Carpathian region. Quaternary International, Climate change in the Balkan-Carpathian region during Late Pleistocene and Holocene 415, 253–267.

Shumilovskikh, L.S., Novenko, E., Giesecke, T., 2018. Long-term dynamics of the East European forest-steppe ecotone. Journal of Vegetation Science 29,

Slancarova, J., Bartonova, A., Zapletal, M., Kotilinek, M., Fric, Z.F., Micevski, N., Kati, V., Konvicka, M., 2016. Life History Traits Reflect Changes in Mediterranean Butterfly Communities Due to Forest Encroachment. PLoS ONE 11, e0152026.

Slancarova, J., Vrba, P., Platek, M., Zapletal, M., Spitzer, L., Konvicka, M., 2015. Co-occurrence of three Aristolochia-feeding Papilionids (Archon apollinus, Zerynthia polyxena and Zerynthia cerisy) in Greek Thrace. Journal of Natural History 49, 1825–1848.

Sluijs, A., Bowen, G.J., Brinkhuis, H., Lourens, L.J., Thomas, E., 2007. The Palaeocene–Eocene Thermal Maximum super greenhouse: biotic and geochemical signatures, age models and mechanisms of global change, in: Williams, M., Haywood, A.M., Gregory, F.J., Schmidt, D.N. (Eds.), Deep-Time Perspectives on Climate Change: Marrying the Signal from Computer Models and Biological Proxies. The Geological Society of London on behalf of The Micropalaeontological Society, pp. 323–349.

Sokolov, V.E., Zhirnov, L.V., 1998. Saigak: filogeniya, sistematika, ekologiya, okhrana i ispol’zovanie [The saiga antelope: phylogeny, systematics, ecology, conservation, and use, in Russian]. Russian Academy of Sciences, Moscow.

Sommer, R.S., Nadachowski, A., 2006. Glacial refugia of mammals in Europe: evidence from fossil records. Mammal Review 36, 251–265.

Song, Z.-C., Li, H., Zheng, Y., Liu, G., 1981. Miocene floristic regions of China, in: Geological Society of America Special Papers. Geological Society of America, Boulder, pp. 249–254.

Šoštarić, R., 2005. The development of postglacial vegetation in coastal Croatia. Acta Botanica Croatica 64, 383–390.

Stehlik, I., Blattner, F.R., Holderegger, R., Bachmann, K., 2002. Nunatak survival of the high Alpine plant *Eritrichium nanum* (L.) Gaudin in the central Alps during the ice ages. Molecular Ecology 11, 2027–2036.

Stewart, J.R., Lister, A.M., 2001. Cryptic northern refugia and the origins of the modern biota. Trends in Ecology & Evolution 16, 608–613.

Stewart, J.R., Lister, A.M., Barnes, I., Dalén, L., 2010. Refugia revisited: individualistic responses of species in space and time. Proceedings of the Royal Society of London B: Biological Sciences 277, 661–671.

Strömberg, C.A.E., Werdelin, L., Friis, E.M., Saraç, G., 2007. The spread of grass-dominated habitats in Turkey and surrounding areas during the Cenozoic: Phytolith evidence. Palaeogeography, Palaeoclimatology, Palaeoecology 250, 18–49.

Stuart, A.J., Sulerzhitsky, L.D., Orlova, L.A., Kuzmin, Y.V., Lister, A.M., 2002. The latest woolly mammoths (Mammuthus primigenius Blumenbach) in Europe and Asia: a review of the current evidence. Quaternary Science Reviews 21, 1559–1569.

Sunderland, W., 2004. Taming the Wild Field: Colonization and Empire on the Russian Steppe. Cornell University Press, New York.

Svenning, J.-C., 2002. A review of natural vegetation openness in north-western Europe. Biological Conservation 104, 133–148.

Van Swaay, C., Cuttelod, A., Collins, S., Maes, D., López Munguira, M., Šašić, M., Settele, J., Verovnik, R., Verstrael, T., Warren, M., Wiemers, M. and Wynhof, I., 2010. European Red List of Butterfies. Luxembourg: Publications Office of the European Union.

Tarasov, P.E., Jolly, D., Kaplan, J.O., 1997. A continuous Late Glacial and Holocene record of vegetation changes in Kazakhstan. Palaeogeography, Palaeoclimatology, Palaeoecology 136, 281–292.

Török, P., Ambarlı, D., Kamp, J., Wesche, K., Dengler, J., 2016. Step(pe) up! Raising the profile of the Palaearctic natural grasslands. Biodiversity and Conservation 25, 2187–2195.

Tzedakis, P.C., Lawson, I.T., Frogley, M.R., Hewitt, G.M., Preece, R.C., 2002. Buffered Tree Population Changes in a Quaternary Refugium: Evolutionary Implications. Science 297, 2044–2047.

Vera, F.W.M., 2000. Grazing Ecology and Forest History. CABI, Wallingford.

Vila, R., Lukhtanov, V.A., Talavera, G., Gil-T., F., Pierce, N.E., 2010. How common are dot-like distributions? Taxonomical oversplitting in western European *Agrodiaetus* (Lepidoptera: Lycaenidae) revealed by chromosomal and molecular markers. Biological Journal of the Linnean Society 101, 130–154.

Walker, M., Johnsen, S., Rasmussen, S.O., Popp, T., Steffensen, J.-P., Gibbard, P., Hoek, W., Lowe, J., Andrews, J., Björck, S., Cwynar, L.C., Hughen, K., Kershaw, P., Kromer, B., Litt, T., Lowe, D.J., Nakagawa, T., Newnham, R., Schwander, J., 2009. Formal definition and dating of the GSSP (Global Stratotype Section and Point) for the base of the Holocene using the Greenland NGRIP ice core, and selected auxiliary records. Journal of Quaternary Science 24, 3–17.

Wang, B., 1997. The mid-Tertiary Ctenodactylidae (Rodentia, Mammalia) of eastern and central Asia. Bulletin of the the American Museum of Natural History 234. American Museum of Natural History, New York.

Wang, L., Lü, H.Y., Wu, N.Q., Li, J., Pei, Y.P., Tong, G.B., Peng, S.Z., 2006. Palynological evidence for Late Miocene–Pliocene vegetation evolution recorded in the red clay sequence of the central Chinese Loess Plateau and implication for palaeoenvironmental change. Palaeogeography, Palaeoclimatology, Palaeoecology 241, 118–128.

Wesche, K., Ambarlı, D., Kamp, J., Török, P., Treiber, J., Dengler, J., 2016. The Palaearctic steppe biome: a new synthesis. Biodiversity and Conservation 25, 2197–2231.

Wiemers, M., Balletto, E., Dincă, V., Fric, Z.F., Lamas, G., Lukhtanov, V., Munguira, M.L., van Swaay, C.A.M., Vila, R., Vliegenthart, A., Wahlberg, N., Verovnik, R., 2018. An updated checklist of the European Butterflies (Lepidoptera, Papilionoidea). ZooKeys 811, 9–45.

Willis, K.J., 1992a. The Late Quaternary Vegetational History of Northwest Greece. III. A Comparative Study of Two Contrasting Sites. The New Phytologist 121, 139–155.

Willis, K.J., 1992b. The Late Quaternary Vegetational History of Northwest Greece. I. Lake Gramousti. The New Phytologist 121, 101–117.

Wolfe, J.A., 1985. Distribution of Major Vegetational Types During the Tertiary, in: Sundquist, E.T., Broecker, W.S. (Eds.), Geophysical Monograph Series. American Geophysical Union, Washington, D. C., pp. 357–375.

Zazula, G.D., Froese, D.G., Schweger, C.E., Mathewes, R.W., Beaudoin, A.B., Telka, A.M., Harington, C.R., Westgate, J.A., 2003. Ice-age steppe vegetation in east Beringia - Tiny plant fossils indicate how this frozen region once sustained huge herds of mammals. Nature 423, 603–603.

Zhisheng, A., Kutzbach, J.E., Prell, W.L., Porter, S.C., 2001. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. Nature 411, 62–66.

Zimov, S.A., 2005. Pleistocene Park: Return of the Mammoth's Ecosystem. Science 308, 796–798.

Zimov, S.A., Zimov, N.S., Tikhonov, A.N., Chapin III, F.S., 2012. Mammoth steppe: a high-productivity phenomenon. Quaternary Science Reviews 57, 26–45.

Zinenko, O., Stümpel, N., Mazanaeva, L., Bakiev, A., Shiryaev, K., Pavlov, A., Kotenko, T., Kukushkin, O., Chikin, Y., Duisebayeva, T., Nilson, G., Orlov, N.L., Tuniyev, S., Ananjeva, N.B., Murphy, R.W., Joger, U., 2015. Mitochondrial phylogeny shows multiple independent ecological transitions and northern dispersion despite of Pleistocene glaciations in meadow and steppe vipers (*Vipera ursinii* and *Vipera renardi*). Molecular Phylogenetics and Evolution 84, 85–100.

CHAPTER I.

Isolated Asian steppe element in the Balkans: Habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities

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Chapter I.

Isolated Asian steppe element in the Balkans: Habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities

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Abstract

A characteristic butterfly of Asian steppes, *Proterebia afra* (Fabricius 1787), was studied in its two relic areas of occurrence within the Balkans—the Askion Mts (a single mountain massif in NW Greece) and Dalmatia (karstic inland S Croatia)—together with co-occurring butterfly communities during its early spring adult flight period. *P. afra* adults tolerate harsh continental conditions prevailing at its localities during its flight. Local densities were higher in the Askion Mts (up to 100 individuals per 50 m walk) than in Dalmatia (up to 30 individuals per 50 m). Within both areas of occurrence, it inhabits dry grasslands subject to intermediate grazing pressure that retains enough dry grass litter but still suppresses shrubs and trees. Hotter slopes are preferred in more northerly Dalmatia than in the more southerly Askion Mts. More co-occurring butterfly species and more Mediterranean species were recorded in the Askion Mts (69 vs 63; 23 vs 12), but more butterfly individuals and higher species' richness per transect were observed in Dalmatia. In ordination analyses, the main gradients organising the grassland butterfly communities distinguished between grassy plains and rocky slopes containing more woody plants. Given the distribution extents, local densities and present land use conditions, the Balkan *P. afra* is not currently endangered, but this may swiftly change with grazing abandonment, especially in Dalmatia, and some conservation actions should be considered in the near future.

Keywords

Proterebia afra, *Proterebia phegea*, Butterfly communities, Mediterranean grasslands, The Balkans, Steppe

Introduction

Proterebia afra (Fabricius 1787) (Nymphalidae: Satyrinae) is a butterfly with presumably relic distribution within Europe, the only representative of its genus worldwide, and deeply phylogenetically distinguished from other European Satyrines (Deodati et al. 2009; Peña et al. 2015). With its dark colour and early spring flight, it resembles the mountain genus Erebia in continental conditions.

Its rather continuous range covers the Pontic steppes and northern parts of the Irano-Turanian biogeographic region: Turkey, Transcaucasia, Northern Iran, Crimea, Northern Caucasus, Volga region, Southern Urals, up to Eastern Kazakhstan. A few additional isolated populations inhabit the Balkan peninsula; they are classified as *P. a. dalmata* (Godart 1824), occurring in Dalmatia, South Croatia (from Pag island to the Biokovo Mts.) and reaching Bosnia and Herzegovina (Koren et al. 2010; Koren and Trkov 2011); and *P. a. pyramus* de Louker and Dils 1987 in the Askion Mts, NW Greece, Kozani district (Pamperis 2009). A small population of the latter inhabits Greek Thrace (Xanthi district) (Pamperis 2011). Whereas the Askion population is limited to a single mountain massif and its surroundings (ca 250 square kilometres), the Croatian distribution covers approximately 5,000 square kilometres (Koren et al. 2010), encompassing altitudes from sea level up to ca 1,400 m a. s. l.

The Balkan steppe-like dry grasslands, exclaves of the Asian steppe biome, exist only locally within the biotically diverse Balkan Peninsula (Polunin 1980; Radovic et al. 2009), part of the Mediterranean global biodiversity hotspot (Myers et al. 2000; Mittermeier et al. 2005). They represent extra-zonal biome patches, presumable remnants of a more arid past. The Mediterranean basin was formed during the Tertiary as a descendant of the Thetys Sea (Dewey et al. 1973; Dercourt et al. 1986). From the Eocene onwards, repeated accumulation of water in glaciers caused reductions of sea (Fairbanks 1989; Ryan et al. 1997), extending the continental conditions in Eurasia (Allen et al. 1999). Under such circumstances, the Balkans acted as both a glacial refugium and land bridge allowing East–West migrations of steppe elements (Van Andel and Tzedakis 1996; Magyari et al. 2008). More humid periods, such as the recent, witnessed area shrinkage and disjunct distribution of the Balkan steppes (cf. Aufgebauer et al. 2012).

As with many South European biotopes, the Balkan steppe-like grasslands may be at risk of successional overgrowth associated with human land use change (Debussche et al. 1999; Mazzoleni et al. 2008; Slancarova et al. 2016). A crucial role of grazing herbivores, either wild or domesticated, for maintenance of steppe-like grasslands near the biome distribution limits is beyond doubt (e.g., Cremene et al. 2005) and grazing reduction could turn the steppe exclaves into a scrubland formation.

The species is not endangered either globally or within Europe, and its listing as Near Threatened for Croatia (Sasic et al. 2013) reflects its biogeographic uniqueness rather than decline. However, it may be viewed as an insect model for other Asian steppes' relics in Southern Europe, some of them probably more endangered.

The presented paper is a result of two 6-week surveys on the areas of *P. afra* European occurrence, aiming to collect more detailed data on this charismatic species. Specifically, we (a) describe adult behaviour observed in the two areas; (b) map distribution extent in Greece and Dalmatia; (c) describe and compare habitat conditions in the two areas; and (d) use ordination analyses to study co-occurrence patterns with other butterfly species in the two areas. We used this information to discuss future prospects of *P. afra* in Europe.

Materials and methods

The species

The single annual *P. afra* generation occurs from late March to early June (Koren et al. 2010; Pamperis 2009; Tolman and Lewington 2008); the March 3 records in Koren et al. (2010) are erroneous (2016, Verovnik pers. comm.). Festuca ovina L. agg. is the host plant in Europe, but oviposition was also observed on Bromus condensatus Hack. (Mihoci and Šašić 2007). After 2 weeks as an egg, the larva hatches and immediately starts feeding, probably diapauses in summer, overwinters as the 5th instar and the adults appear in spring after a short (20 days) pupal stage (Roos et al. 1984; Jutzeler and Lafranchis 2011). Reported habitats in the Balkans include dry limestone slopes or plains dominated by grasses (*Stipa* L., *Festuca* L., *Aegilops* L.), or *Astragalus* L. (Hesselbarth et al. 1995; Tolman and Lewington 2008).

The species' nomenclature has been recently discussed. G. Lamas (2012, by D. Bolt to Lepiforum.de, unreferenced) argued that the name *Proterebia afra* (Fabricius 1787), derived as feminine adjective of the original name *Papilio afer* Esper, 1783, was a junior primary homonym of *Papilio afer* Drury, 1782. This led Kudrna et al. (2015) and Numa et al. (2016) to revive the synonym *Proterebia phegea* (Borkhausen 1788). Here, we retain the name *Proterebia afra* (Fabricius 1787), as the one in common use (e.g., de Jong et al. 2014; Jutzeler and Lafranchis 2011; Tshikolovets et al. 2016).

Study areas

The Askion Mts (highest summit: Mt. Siniatsiko 2,111 m a. s. l., prominence 970 m, length × width ca 35 × 25 km), located in Western Macedonia, Greece (Fig. 1a, b) have a notably xeric character owing to the rain shadow produced by surrounding higher ranges—Pindos (2,637 m a. s. l.) in the West, Olympos (2,917 m a. s. l.) in the Southeast, Voras (2,524 m a. s. l.) in the Northeast, and Baba (2,601 m a. s. l.) in the North. The southern parts of the Askion Mts are built of limestone, the northern part of gneisses, amphibolites and slates (Androulakakis 2012). The northern parts are wooded; the southern ones are covered by steppe-like grasslands. Grazing (cattle, sheep, and goats) is practiced at accessible slopes, small crop fields are found in *polje* depressions.

Continental Dalmatia, southern Croatia, stretches from the Velebit Mts (the highest summit 1,757 m a. s. l.) in the North to the Prevlaka peninsula in the South and to the Bosnia and Herzegovina borders in the East (Fig. 1a, c). The evergreen Mediterranean scrubland typical for the Adriatic Sea coast recedes rapidly within a few kilometres inland, where it is replaced by the arid grasslands on karstic bedrock, which gradually rise towards the Dinaric Alps (1,913 m a. s. l.). The landscape is either abandoned or grazed, the pastures are often managed by fires; crop fields are restricted to villages' surroundings. The distribution of *P. afra* (Mihoci and Šašić 2005;

Koren et al. 2010; Koren and Trkov 2011; Tvrtković et al. 2015) includes the island of Pag, the southern part of the Velebit Mts, the inland except for the narrow evergreen coastal strip, the W slopes of the Dinarid Mts on the Bosnian border, and the northern slopes of the Biokovo Mts (1,762 m a. s. l.). The distribution area is ca 170 × 30 km. Landscape exploitation in the region is still hindered by the presence of land mines, placed there during the 1990s wars.

Field surveys

We visited the Askion Mts. between mid-April and early June, 2014, and Dalmatia in the same period of, 2015. In each area, we established 23 transects, designed to cover the entire range of natural grassland and scrubland habitats and elevations. The Askion Mts transects were situated within ca 200 square kilometres (Fig. 1b, Online Resource 1). The Dalmatian transects, reflecting the much larger *P. afra* distribution extent, were situated within ca 2,500 square kilometres, (Fig. 1c).

Each transect was 200 m long, divided into four 50 m segments. Each segment was defined by geographic coordinates and altitude (*lat*, *long* and *alt*) and the following habitat characteristics, recorded from a 50 × 50 m square dissected by the transect path, during the first visit (Table 1): first, *cover* as separated proportions of *rock*, *stone*, *scree*, *bare soil*, *tree* (>1.5 m), *shrub*, *green plants* (forbs and grass) and *grass litter*; furthermore, *inclination* (scale 0–4, from flat to steep); *aspect* (ordinal variable expressing thermal conditions of the slope: 1: North, Northeast, 2: Northwest, East, 3: flat, 4: Southeast, West, 5: Southwest, South); and *gully* (the presence/absence of a sheltered or wetter place, a track). Supply of nectar (1: <10 flowers, 2: <100 flowers, 3: flower rich site), was recorded separately for each visit and used as the mean value in analyses.

We visited each transect four times (mid-April, late April, early to mid-May and late May/early June) to record *P. afra* and associated butterflies. The butterflies were recorded by adjusted transect walking (Pollard 1977), in a ≈7 m cube in front of the recorder, with 30 min spent on the whole transect. The walks were restricted to conditions suitable for butterfly activity, between 9:00 and 16:00, local summer time. The timing of walks of individual transects was randomised among the visits. We recorded the abundance of *P. afra* and all co-occurring butterflies. We also noted down all observations of *P. afra* adult activity. The co-occurring butterflies were identified in flight, in the net, or, in case of difficult species, in the laboratory. The associated butterflies were classified into Mediterranean or non-Mediterranean species: the former distributed in the Mediterranean region, not crossing the Alps, sometimes reaching the Pannonian and Pontic lowlands. Butterfly nomenclature (Online Resource 2) follows Fauna Europaea (de Jong et al. 2014).

Apart from the transects, all other records of *P. afra* presence were noted and the coordinates used to produce a map of the overall distribution of the species in the two areas (Fig. 1).

Statistical analyses

All analyses were performed separately for Askion Mts and Dalmatia, and for pooled data from both study areas.

Habitats of P. afra

Presuming that adult abundance provides a good signal of habitat suitability, we modelled effects of environmental predictors recorded along the transects, using the information theory approach, selecting from multiple non-nested a priori hypotheses (van Strien et al. 2011) and relying on the Akaike information criterion (AIC) for model selection. For the whole dataset and for each study area separately, we constructed sets of generalised linear models (GLM) in R 3.2 (R Core Team 2016) with Poisson distribution of errors, for the following hypotheses: (i) As a xerophilous butterfly, *P. afra* should react positively to sward openness (predictors: *rock*, *soil*, *scree*, *stone*). (ii) Likewise, it should be negatively associated with *tree* and *shrub*. (iii) Inclination, *aspect* and *gully* are directly associated with microclimate. (iv) *Nectar* is an important adult resource for majority of butterflies. (v) Due to its association with grasses, *P. afra* abundance should respond to *litter* and *green plants* cover.

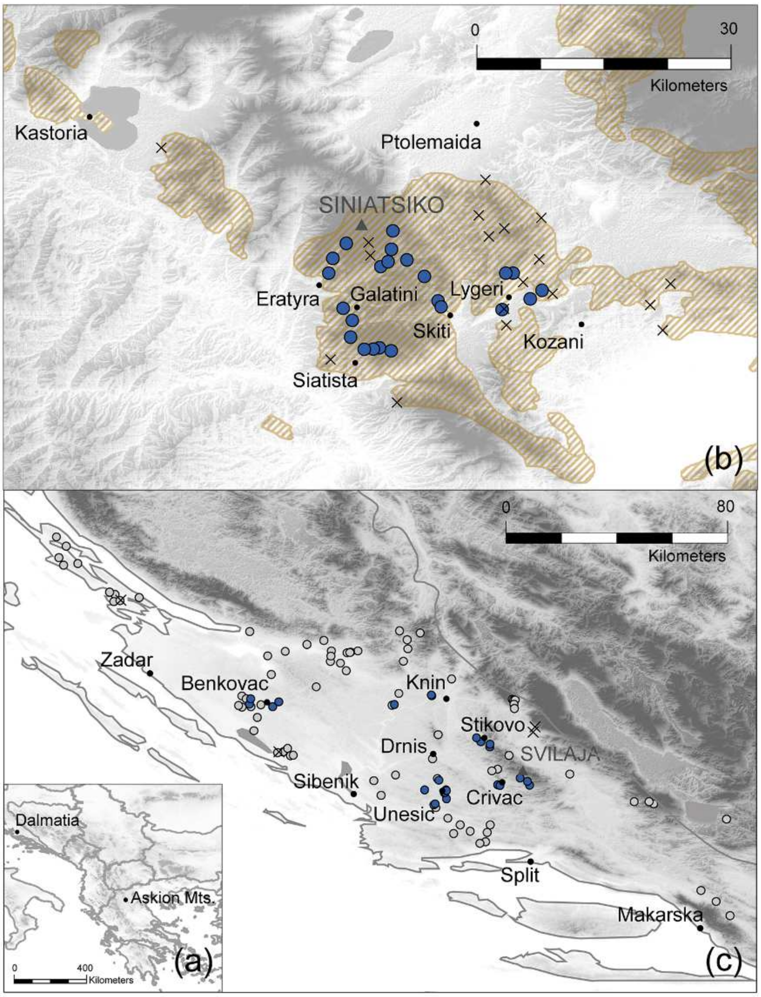


Fig. 1 a The map of the Balkans showing positions of areas of *P. afra* distribution. **b** Detailed map of the Askion Mts, showing positions of 23 transects used for butterfly recording (dark circles), our observations of *P. afra* outside of the transects (crosses) and major human settlements (black dots). The distribution of the species in Askion Mts mostly follows the calcareous bedrock (hatched). **c** Detailed map of Dalmatia, again showing the 23 transects (dark circles), our observations outside of the transects (crosses), records from literature (light circles; Mihoci and Šašić 2005; Koren et al. 2010; Koren and Trkov 2011; Tvrtković et al. 2015) and major human settlements (black dots). The map was produced using ArcGIS (ESRI 2011) and GTOPO30 terrain data (courtesy of the U. S. Geological Survey) as a background map

Table 1 Descriptive statistics of habitat characteristics recorded on 50 × 50 m study sites of *P. afra* occurrence in Askion Mts and Dalmatia

Area	Askion Mts.			Dalmatia		
Habitat characteristic	Mean (+/- STDEVA)	Median	Range	Mean (+/-STDEVA)	Median	Range
tree	2.0 (4.78)	0.5	0 – 30	8.2 (10.69)	5	0 – 50
shrub	5.7 (6.87)	3	0 – 30	11.7 (7.49)	10	0 – 30
rock	11.7 (13.05)	6	0 – 70	20.8 (18.01)	20	0 – 80
stone	19.4 (13.07)	20	0 – 50	13.3 (8.29)	10	0 – 35
scree	13.9 (17.0)	7	0 – 70	8.5 (9.15)	5	0 – 40
soil	10.4 (8.61)	10	0 – 40	5.4 (6.04)	3	0 – 25
green plants	51.3 (21.88)	50	10 – 90	32.0 (13.66)	30	8 – 65
litter	19.3 (16.69)	15	0.5 – 70	23.6 (13.8)	20	0 – 60
inclination	2.2 (1.06)	2	0 – 4	0.6 (0.81)	0	0 – 3
aspect	3.3 (1.38)	3	1 – 5	3.2 (0.97)	3	1 – 5
gully		0	23 yes/ 69 no		0	12 yes/ 80 no
nectar	1.7 (0.50)	1.75	1 – 2.75	2.0 (0.43)	2	1.25 – 3

Values for *cover* (tree to litter) are in percentages (they can overlap); *inclination*, *aspect* and *nectar* are in ranked scales (see text), gully as present or absent

Prior to assessing the above hypotheses, we constructed *P. afra* abundance models based solely on latitude, longitude and altitude of transect sections, including interactions. These covariate models were constructed iteratively, using backward and forward procedures, until models with the lowest possible AICs were achieved. Onto this model, i.e., onto the residuals of variation due to geographic position and proximity of transect sections, we one-by-one added predictor of the above hypotheses (i)–(v), searching for predictors combinations best explaining the data.

Co-occurring butterfly communities and their habitats

To investigate *P. afra* co-occurrence patterns with other butterflies, we used ordination analyses computed in CANOCO v. 5.0 (Ter Braak and Šmilauer 2012). In all analyses, we used per-segment abundance records for all butterfly species, *P. afra* included. Four species recorded as single specimens in the whole study were excluded: *Zerynthia cerisyi*, *Erynnis marloyi* and *Argynnis pandora* (all from Askion) and *Ochlodes sylvanus* (Dalmatia). The species abundances were log-transformed and the function downweighting rare species was used.

Unconstrained ordinations of species indicated gradient lengths =3.0 (pooled data), 2.6 (Askion Mts) and 2.5 (Dalmatia). Since the longest gradient was ≈3.0 and the data were compositional, we used unimodal ordinations (Ter Braak and Šmilauer 2012). First, we used the canonical correspondence analysis, CCA, a constrained ordination relating the composition of samples to external predictors, to construct a model based on latitude, longitude, and altitude of transect sections, including interactions. The CANOCO forward selection procedure and Monte-Carlo permutation tests (999 runs, $p < 0.01$) were used to define a significantly fitting combination of these predictors. This combination was used as a covariate model in a partial detrended correspondence analysis (DCA). This ordination depicted the co-occurrence patterns among the butterflies after removing the patterns attributable to geographic position and proximity of transect sections. Third, to describe the co-occurrence patterns, another CCA using the forward selection procedure was adopted to pick up the combination of habitat characteristics, with covariate model as in the previous step.

Results

Adult behaviour

Patrolling of males over grasslands was the most frequent activity. Females were also frequently seen in flight, being more sedentary than males but still not hiding as was observed in other species, probably seeking ovipositing possibilities. We repeatedly observed oviposition on *Festuca ovina* in the Askion Mts; this activity consisted of short settling on the grass tussock, dropping a single pinkish egg and flying towards another tussock. Nectaring was extremely rare, always in early afternoons (1 p.m.) on Alyssum L. sp. (n = 2) and an unidentified yellow-blooming *Ranunculus* L. sp. (n = 1) in the Askion Mts, and on *Thymus* L. sp. (n = 2), *Globularia cordifolia* L. (n = 1), and *Crepis rubra* L. (n = 5) in Dalmatia. Under less favourable weather, *P. afra* was often the only butterfly detectable, taking off from the grass when closely approached. In the Askion Mts, we observed a few individuals patrolling over rapidly melting snow after a snowstorm in mid-April.

Distribution in the Askion Mountains

The Askion Mts distribution follows the calcareous bedrock (Fig. 1b; for GPS see Online Resource 1). Besides the Askion massif, we recorded the butterfly also in an exclave SW of Lake Kastoria and on northern calcareous foothills of the Vourinos Mts. The species inhabited all types of grasslands existing on the mountains and surrounding: rocky southern slopes above Siatista, half-shaded oak savannas on eastern slopes, grassy plains at the foothills and on the karstic plateaux, mountains summits heavily grazed by cattle, and *Stipa* grasslands with *Juniperus* bushes in the Kozani surroundings (Fig. 2).

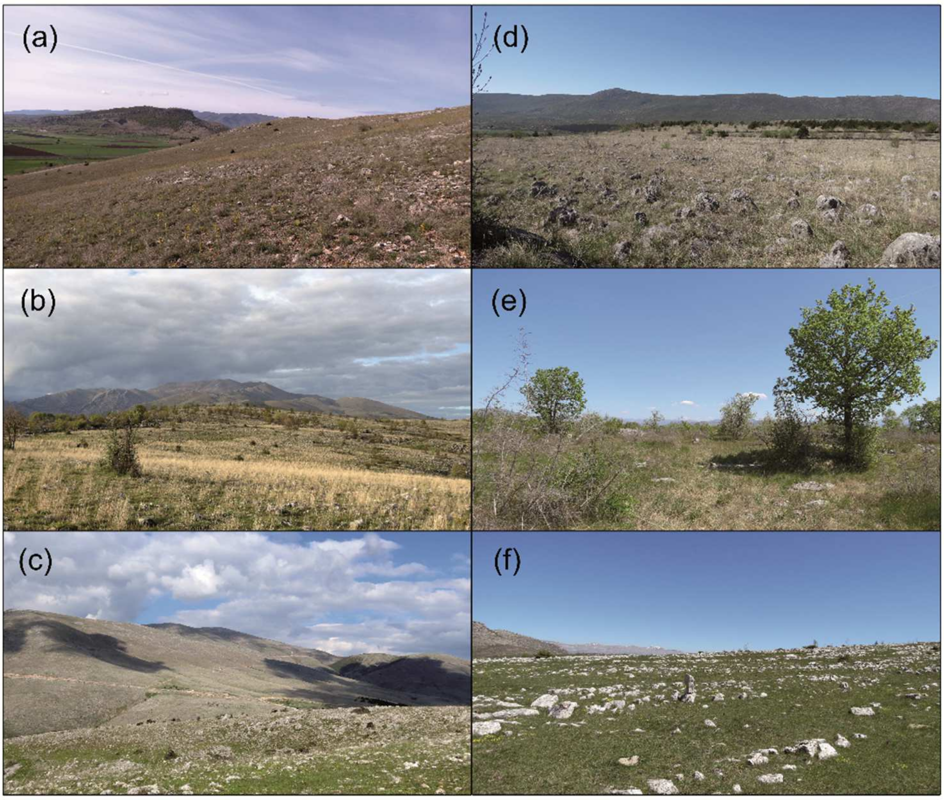


Fig. 2 Types of biotopes hosting *P. afra*. Askion Mts: **a** foothills near Lygeri village, altitude 700 m, a site without shrubs or trees and with high dry grass litter accumulation; **b** central karstic plateau with some shrubs above Galatini village, altitude 1100 m, Mt. Siniatsiko summit in the background; **c** rocky slopes above Siatista village, altitude 1300 m; besides *P. afra* inhabited by *Euchloe penia* and *Pseudophilotes bavius*. Dalmatia: **d** foothills of Svilaja Mt, altitude 500 m, lightly grazed grasslands with shrubby patches; **e** mainly abandoned landscape in the Knin surroundings, altitude 300 m; **f** heavily grazed cattle pasture on northern Svilaja Mt slopes, altitude 800 m. At sites **a** and **d**, we recorded the highest abundances in Greece and Croatia, respectively

On the foothills (<800 m a. s. l.), the flight period started prior to our arrival, peaked during the first visit (13 April), and heavily worn individuals still occurred on 9 May. On summits (ca 1,400 m a. s. l.), the first individuals appeared on 17 April and heavily worn individuals were still present in early June. The peak abundances were up to 100 individuals per 50 m transect segment. Mean abundances per segment were 9 (median 5, SD 13.9, range 0–100) for the first visit, 15 (median 11, SD 13.6, range 0–70) for the second visit, and 13 (median 6, SD 16.0, range 1–100) for the third visit; the species was present only on the two highest altitude transects during the fourth visit. The altitudinal range of records was 691–1,556 m.

Distribution in Dalmatia

Our findings corroborated those of Koren et al. (2010) (Fig. 1c). The habitats on Pag Island were heavily grazed stony plains with scarce vegetation (Fig. 2). Near Benkovac, the butterfly occurred in abandoned rural landscapes with tall grasslands, small crop fields, sheep and goat pastures, motorway embankments. Near Knin and near the Vransko Lake, where the abandoned landscapes were mostly covered by scrub, *P. afra* was limited to the remnant open patches. The localities near the village of Unesic were sheep and goat pastures, sometimes dominated by sedges. Svilaja Mt was mostly grazed by cattle; newly established pine plantations were present in some areas. On Svilaja Mt northern foothills (Štikovo village), open woodlands developing at sites of abandoned gardens and crop fields are used as sheep and goat pastures. The shrubs overgrowing the steppe-like pastures in Dalmatia included *Juniperus* spp. and *Pauliurus spina-christi* Mill.

The first individuals were observed on 12 April. With the progressing season, the butterfly occurred on almost all grasslands in the landscape, but only as a few individuals per segment. The abundance peaked in early May. Mean abundances per segment were 0.6 (median 0, SD 1.1, range 0–5) for the first visit, 4 (median 2, SD 5.8, range 0–30) for the second visit, and 3 (median 2, SD 2.8, range 1–15) for the third visit; no butterfly was recorded during the fourth visit. The altitudinal range recorded was from sea level to 1,056 m.

Table 2 The set of a priori GLM models of habitat selection of adults of *P. afra* within its Balkan localities

	Pooled data	df	RD	AIC	Askion Mts.	df	RD	AIC	Dalmatia	df	RD	AIC
Null model		183	4813	5597		91	2155	2627		91	551.3	864.7
Covariate model		11,172	2135	2940		8, 83	1670	2158		11, 80	155.9	491.3
(i)	*-rock -soil -scree -stone*	15, 168	1852	2665	*-rock -soil -scree -stone*	12, 79	1512	2008	*+rock*	12, 79	153.2	490.6
(ii)	*-tree -shrub*	13, 170	1954	2764	*-shrub*	9, 82	1629	2119	n.s.			
(iii)	*-/-inclination -/-aspect -gully*	16, 167	1989	2712	*-inclination -aspect -gully*	11, 80	1479	1973	*+inclination +aspect -gully*	14,77	139.3	480.6
(iv)	*+nectar*	12, 171	2125	2933	n.s				n.s			
(v)	*+green plants +/-litter*	14, 169	1782	2593	*+green plants +/-litter*	11, 80	1432	1927	n.s			

Covariate models: Pooled data: ~ + long + poly(lat, 2) + poly(alt, 2) + long * poly(alt, 2) + poly(alt, 2) * poly(lat, 2). Askion Mts: ~ + poly(alt,2) + poly(lat,2) + poly(alt,2)*poly(lat,2). Dalmatia: ~ + poly(alt, 2) + long + poly(lat, 2) + poly(alt,2) * poly(lat, 2) + long * poly(lat, 2)

Symbols + and − indicate the direction of a linear relationship, +/− or −/− the form and direction of a polynomial response. Only significant variables are shown

Habitat requirements

For pooled data from both areas as well as for the two areas analysed separately, *covariate model* (geography) fitted over 30% of variation in the data (Table 2). On residuals of these the *covariate models*, all groups of predictors decreased the deviance of pooled data, supporting our hypotheses regarding *P. afra* abundance. The best supported hypotheses were (v), concerning herbaceous cover and indicating the highest *P. afra* abundance at transect sections with intermediate grass *litter*; (i), indicating that *P. afra* avoided high cover of bare ground; and (iii), indicating that *P. afra* reached higher abundance at flat sites with low values of *aspect* (i.e., north- or east facing), but also avoided humid gullies. Taking this altogether, the best *P. afra* sites would be situated on relatively flat and not too hot surfaces, which would contain an intermediate amount of grass litter.

The picture was practically identical for the Askion data separately. Interestingly, *nectar* supply had no effect on *P. afra* abundance. For Dalmatia, only two hypotheses, (i) and (iii), gained statistical support, but the patterns were reverse of those in Greece: *P. afra* abundance increased, rather than decreased, with rock presence, and increased on steeper and sun-exposed surfaces.

Co-occurring butterfly communities

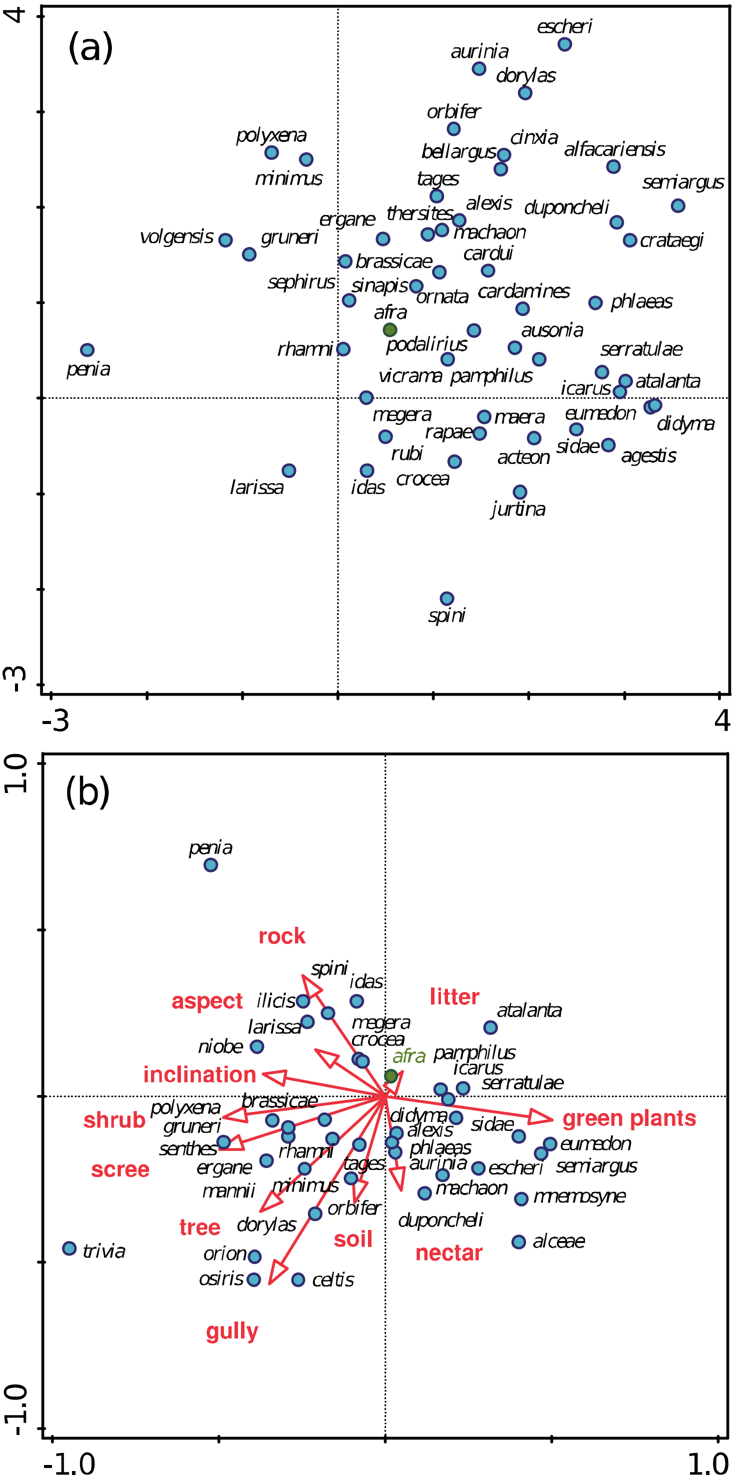
We recorded 83 butterfly species and 8,260 individuals: 69 from the Askion Mts and 63 from Dalmatia (Online Resource 2). *P. afra* was the dominant butterfly in both areas, with stronger dominance in Askion (≈70% of 4,987 butterfly individuals recorded) than in Dalmatia (≈20% of 3,273 individuals recorded).

There were fewer species per segment in Askion (mean 9, SD 4.4, range 2–20) than in Dalmatia (mean 12, SD 3.8, range 5–23) (t (DF 182) = −4.63, $p < 0.001$). The difference became less prominent per whole transects (Askion 20 (SD 6.7, range 10–36), Dalmatia 23 (SD 3.9, range 16–31), t (DF 35.5) = −1.86, $p = 0.07$). Both areas shared 49 species, 20 species were exclusive to the Askion Mts. and 14 to Dalmatia. A total of 25 Mediterranean species were detected (30% of the total), 23 in Askion

(33%) and 12 in Dalmatia (19%). Ten of them occurred in both areas (besides *P. afra*, these were *Euchloe ausonia*, *Melanargia larissa*, *Melitaea ornata*, *Libythea celtis*, *Pieris ergane*, *P. mannii*, *Polyommatus escheri*, *Pyrgus sidae*, *Zerynthia polyxena*); 13 in Askion Mts only (*Anthocharis gruneri*, *A. pandora*, *Cupido osiris*, *Euchloe penia*, *Erynnis marloyi*, *Hipparchia senthes*, *H. volgensis*, *Melitaea trivia*, *Leptidea duponcheli*, *Plebejus sephirus*, *Polyommatus anteros*, *Pseudophilotes bavius*, *Z. cerisyi*); two in Dalmatia only (*Iolana iolas*, *Limenitis reducta*; the latter, absent on our transects, occurred elsewhere in the Askion Mts).

For pooled data, the selected *covariate model* was: *species composition ~ long2 + alt*lat + alt + long + alt*long* (eigenvalues: 0.32, 0.10, 0.08, 0.04; explained variation 17.5%; first axis: F = 20.3, p = 0.001; all axes: F = 9.5, p = 0.001). The partial DCA ordination (eigenvalues: 0.15, 0.13, 0.11, 0.09; explained 18.9% of the variation; Fig. 3a) containing the above covariates was difficult to interpret, as species usually associated with identical habitat types ended up in both positive and negative extremes of the main gradient (horizontal axis: negative values, e.g., *Euchloe penia* – scree, *Melanargia larissa* – grasslands, *Zerynthia polyxena* – scrub; positive values, e.g. *Polyommatus bellargus* – rocks, *Cyaniris semiargus* – grasslands, *Aporia crataegi* – scrub). The same applied for positive values of the second (vertical) gradient.

The following habitat characteristics were selected in a partial CCA model (Fig. 3b): *species composition ~ covariate model + green plants + aspect + litter + gully + rock + scree + inclination + tree + nectar + shrub + soil* (eigenvalues: 0.07, 0.05, 0.05, 0.04; explained 8.36% of the variation; first axis: F = 4.8, p = 0.001, all axes: F = 2.4, p = 0.001). Except for stone, all the environmental predictors thus influenced the composition of butterfly assemblages after discounting the variation due to geography. The main canonical gradient distinguished between steep, scree-covered, sometimes shrubby or tree-covered slopes (negative values), and flat surfaces with high cover of herbaceous vegetation (positive values). The second gradient went from sun—exposed rocky sites (positive values) to cooler sites with exposed bare soil and abundant nectar (negative values).


(a)
4
-3
-3
4
escheri
aurinia
dorylas
orbifer
polyxena
minimus
cinxia
bellargus
alfacariensis
tages
semiargus
alexis
duponcheli
ergane
thersites
machaon
crataegi
volgensis
gruneri
brassicae
cardui
sephirus
sinapis
ornata
cardamines
phlaeas
afra
ausonia
rhamni
podalirius
serratulae
penia
vicrama
pamphilus
atalanta
icarus
megera
maera
eumedon
didyma
rapae
sidae
rubi
acteon
agestis
larissa
idas
crocea
jurtina
spini
(b)
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-1.0
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penia
rock
spini
idas
aspect
ilicis
litter
atalanta
larissa
megera
niobe
crocea
afra
pamphilus
icarus
inclination
serratulae
brassicae
polyxena
shrub
green plants
gruneri
didyma
alexis
sidae
senthes
rhamni
phlaeas
eumedon
tages
aurinia
escheri
semiargus
scree
ergane
mannii
minimus
machaon
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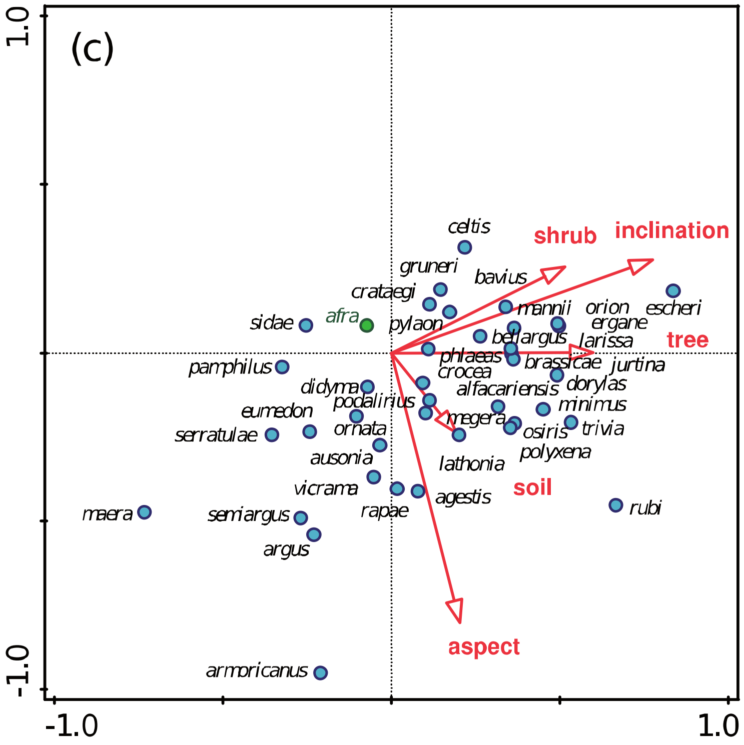
alceae
osiris
celtis
gully


(c)
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celtis
shrub
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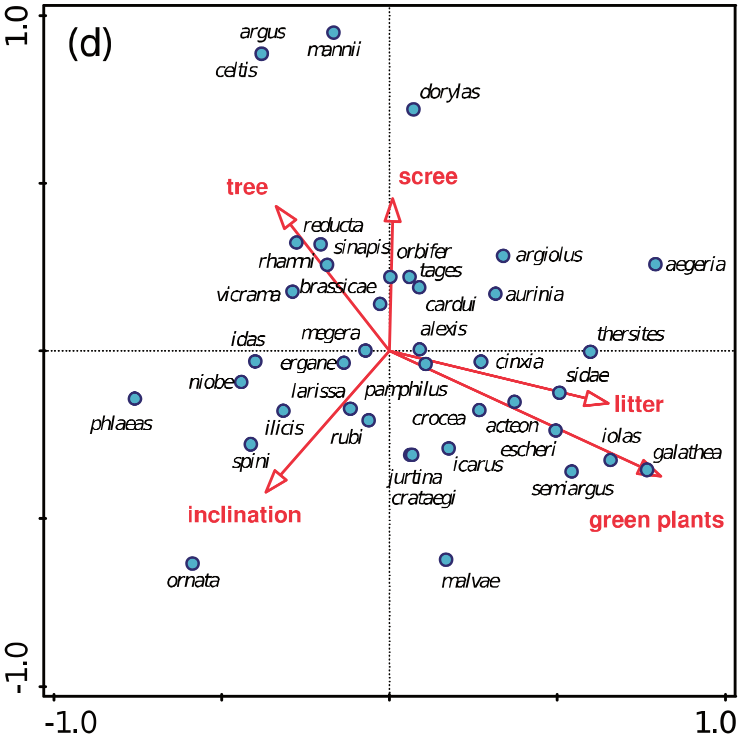
gruneri
bavius
crataegi
escheri
afra
mannii
orion
sidae
ergane
pylaon
bellargus
larissa
tree
phlaeas
brassicae
jurtina
pamphilus
crocea
dorylas
didyma
alfacariensis
podalirius
minimus
eumedon
megera
ornata
osiris
trivia
serratulae
ausonia
polyxena
lathonia
vicrama
soil
agestis
rubi
maera
rapae
semiargus
argus
aspect
armoricanus


(d)
1.0
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argus
mannii
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tree
scree
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sinapis
orbifer
argiolus
aegeria
rhamni
tages
vicrama
brassicae
aurinia
cardui
alexis
thersites
idas
megera
ergane
cinxia
niobe
sidae
larissa
pamphilus
litter
phlaeas
ilicis
crocea
acteon
rubi
iolas
escheri
galathea
spini
icarus
jurtina
semiargus
crataegi
inclination
green plants
ornata
malvae

Fig. 3 Partial ordinations (controlled for geographic covariates) relating *Proterebia afra* co-occurrence with other butterflies to habitat conditions of study sites. Scientific names are simplified to species names.

a Partial DCA, pooled data, showing mutual positions of 50 best-fitting species in our records.

b Partial CCA, pooled data, showing relations of 40 best-fitting butterfly species to habitat conditions.

c Partial CCA, Askion Mts, 40 species. *Covariate model: species composition ~ long2 + lat + alt*lat* (eigenvalues: 0.16, 0.14, 0.06, 0.18; 21.0% variation explained; first axis: F = 6.0, p = 0.001; all axes: F = 4.7, p = 0.001). Partial DCA: eigenvalues: 0.18, 0.13, 0.11, 0.08; explained variation 22.8%. Predictors (partial CCA): *species composition ~ covariate model + inclination + aspect + tree + soil + shrub* (eigenvalues: 0.11, 0.06, 0.06, 0.04; explained variation 12.6%; first axis: F = 4.5, p = 0.001; all axes: F = 2.7, p = 0.001).

d Partial CCA, Dalmatia, 40 species. *Covariate model: species composition ~ alt*lat + alt2 + alt + lat2* (eigenvalues: 0.16, 0.07, 0.05, 0.16; 20.5% explained; first axis: F = 6.9, p = 0.001; all axes: F = 4.4, p = 0.001). Partial DCA: eigenvalues: 0.16, 0.14, 0.09, 0.07; 25.2% explained. Predictors (CCA): *species composition ~ covariate model + green plants + inclination + scree + tree + litter* (eigenvalues: 0.08, 0.06, 0.05, 0.04; 12.5% explained; first axis: F = 4.0, p = 0.001; all axes: F = 2.6, p = 0.001)

The third gradient distinguished shrubby or tree covered sites (positive values) from sites with a high accumulation of dry litter (negative values).

The separate CCA models for the two areas differed slightly in significant variables (Fig. 3c, d) but the overall picture remained consistent. For the Askion Mts, the main gradient distinguished treeless plains (negative values) from slopes containing trees (positive values), and the second gradient was affected by aspect, from the northern-cold ones in positive values to southern—warm ones in negative values. For Dalmatia, the first gradient differentiated steep slopes (negative values) from grassy plains with accumulated dry litter (positive values) and the second gradient differentiated grassy and dry litter covered slopes from sites containing some trees and scree.

Discussion

Proterebia afra, an Asian steppe element with relic distribution in the Balkans, was studied in two areas hosting its largest populations, characterised by karstic bedrock and climates with cold winters and springs and arid summers—the Askion Mts in Greece and Dalmatia in Croatia. Its early spring flying adults experience episodes of harsh, swiftly changing weather. Within both study areas, the species inhabits a wide range of natural grassland types.

In both areas pooled, *P. afra* prefers more sun-exposed sites with a high accumulation of dry litter, few shrubs and trees, and low cover of rocks, stones, scree or bare soil, suggesting a preference for neither advanced succession nor sparse vegetation and exposed bedrock. A quite similar picture emerged in separate analyses for the Askion Mts, whereas only a preference for hotter slopes and rocks was apparent for Dalmatia. The lack of a negative shrubs or trees effect for Dalmatia could be due a more advanced successional state of Dalmatian vegetation, with some trees and bushes present at all transect sections (Table 1). The contrasting effects of inclination and aspect revealed a preference for warmer places in Dalmatia than in the Askion Mts, and it is well established that insects often require hotter microclimates near their cooler (i.e., northern) range limits (Thomas et al. 1999).

Grass litter cover is a proxy for grazing pressure (Vermeire and Gillen 2001). Heavy grazing prevents litter accumulation, causing first prevalence of forbs and ultimately, exposing barren bedrock. Under low grazing, a site ultimately succumbs to shrubs and trees. *P. afra* may prefer sites with grass litter accumulation not only because its larvae develop on grasses, but also because grass litter insulates overwintering larvae (cf. Örvössy et al. 2013; Stuhldreher and Fartmann 2014), buffers the climatic conditions, shelters against some predators (Möllenbeck et al. 2009), and may protect resting adults in early spring, when fresh green vegetation has not yet developed. The preference indicates the need for intermediate levels of grazing, not suppressing the grasses but keeping in check shrubs and trees. In both areas, the speed of woody regrowth is slowed down by the relative continentality of the climate, but shrubby or woody formations would eventually prevail even there, except perhaps on some stony slopes, in the absence of grazing. Slopes inaccessible to animals are covered by sparse oak savanna in the Askion Mts, while sites removed from grazing due to land mines from the 1990s wars are turning into scrub in Dalmatia (e.g., the Knin surroundings). Grazers, either wild roaming or domesticated, have arguably acted as ecosystem engineers throughout Eurasian steppe biome (Johnson 2009; Sandom et al. 2014) and their role may be even more critical in extrazonal insular steppe patches (e.g., Cremene et al. 2005; Pokluda et al. 2012).

Notably, a requirement for intermediate grazing (or mowing) is frequently reported for grassland butterflies from more northerly areas of Europe (e.g., Kruess and Tscharntke 2002; Eichel and Fartmann 2008; Loeffler et al. 2013; Stuhldreher and Fartmann 2014), whereas species associated with other biotopes, such as woodlands, typically prosper in mosaics of various successional stages (e.g., Slamova et al. 2011). On the relic steppe-like grasslands of the Balkans, the patchy presence of accumulated grass litter seems to indicate a grazing pressure not yet suppressing species sensitive to overgrazing.

In both areas, *P. afra* was the dominant butterfly of the spring phenological aspect. This was more prominent in the Askion Mts, where *P. afra* reached higher per-segment abundance, combined with lower per-segment butterfly species richness. The higher local abundance in the Greek Askion Mts. might be a case of a negative density-area relationship (Hambäck et al. 2007), a little understood ecological phenomenon, as the Greek area of occurrence is considerably smaller than the Dalmatian one.

Alternatively, the lower density in Dalmatia could be due to its existence near (northern) range margins (e.g. Lawton 1993), but such reasoning is hardly applicable for the disjunct range in the Balkans (Sagarin and Gaines 2002). An ecological explanation might be that most of the Dalmatian area of occurrence is an abandoned rural landscape, formed by a mosaic of grasslands, shrubs and trees, whereas the Askion area of occurrence is mainly continuous grassland. Finally, the lower density in Dalmatia might be a result of a more advanced succession due to faster woody plants growth in the more northerly region, perhaps hastened by grazing declines during and after the 1990s wars. We cannot decide between these explanations, as our results originated from two areas only. Data from more easterly parts of *P. afra* range could further elucidate the species' requirements.

Regarding associated butterflies, our study covered the spring aspect only (April to June), so parts of the regional species pools remained unrecorded. Still, it is evident that the Askion Mts host a higher proportion of Mediterranean species, as it is situated more southerly, within one of the most biotically diverse regions in Europe–the Greek–Macedonian borderland (cf. Kudrna et al. 2011 for butterflies; Kryštufek and Griffiths 2002 for mammals). Judging from their total distribution ranges, several species co-occurring with *P. afra* probably represent relics of more easterly distribution: *Anthocharis grunneri* (ranging from Southern Balkans to Iran); *E. penia* (reaching Turkey, Syria and Northern Iraq); *E. marloyi* (from the S Balkans through the Middle East to Transcaucasia); or *Pseudophilotes bavius* (steppes of NE Balkans, Crimea, Southern Urals) (cf. Pamperis 2009). In Dalmatia, a northerly projection of the Mediterranean biome where the Mediterranean and Euro-Siberian faunas intersect, there were fewer Mediterranean species, but higher total species count and more species per transect. The occurrence of such distinctly Euro-Siberian elements (cf. Kudrna et al. 2011, 2015) as *Argynnis niobe*, *Euphydryas aurinia*, or *Pararge aegeria* directly on *P. afra* sites was likely facilitated by higher representation of shrubs or trees. These "northern" species occur in mainland Greece as well, but prefer other habitats than the arid Askion Mts grasslands (cf. Pamperis 2009).

The observed situation was supported by ordination analyses (Fig. 3). The main gradients structuring butterfly communities in both pooled and separate data sets distinguished steep slopes with trees from grassland-covered plains. Arguably, the heterogeneous landscapes of the Balkans allow coexistence of open forest and grassland species on very small scales (e.g., the woodland species Satyrium spini and Z. polyxena, together with the grassland species M. larissa and Plebejus idas). The commonly used classifications of habitat specificity of European butterflies (Shreeve et al. 2001; Nylin and Bergström 2009) tend to reflect the situations in more northerly regions, where successional processes are faster and the biota homogenised by intensive land use (Ekroos et al. 2010). In the traditionally used areas of southern Europe, even species with contrasting requirements coexist at very minute scales (Loos et al. 2014; Slancarova et al. 2015).

Conservation message

The areas inhabited by *P. afra* in both the Askion Mts and Dalmatia are fairly large, the species dominates spring faunal aspects and inhabits a wide range of grassland types in both areas. The population densities seem to be lower in Dalmatia, but this is compensated by considerably larger distribution extent. It therefore seems that the species is not facing imminent threat in any of the regions. There are, however, warning signals, such as the total cessation of grazing and entire land-use abandonment in extensive parts of Dalmatia, the pine planting in Dalmatian karstic areas, or too heavy cattle grazing at mountain tops (both areas). There was a higher representation of trees and shrubs on Dalmatian transects, although our data do not allow deciding whether this is due to recently accelerating succession, or just due to a cooler and wetter climate in the more northerly region. In more northerly Europe, rapid crashes of seemingly common butterflies across large parts of ranges have occurred due to land use change (e.g., Kadlec et al. 2010), and such crashes should be avoided in the biotically richer European South. Furthermore, both areas are hosting other butterfly species with either restricted relic distribution (mainly the Askion Mts), or occurring near their northern range limits in the Balkans (Dalmatia). Some of these species exist there in much lower densities than *P. afra*, and may display more exacting biotope requirements and might be more susceptible to changing conditions.

Therefore, we propose to monitor the situation of *P. afra* in both areas closely. It is vital that current grazing levels at the Askion Mts are maintained, and conservation efforts in Dalmatia should lead to grazing re-establishment for the localities where shrub and tree succession has proceeded too far. Financial support for herdsmen would enhance attractiveness of traditional land use, which could, however, lead to overgrazing if not applied carefully. Direct shrub removal could be practised. Pasture management by fire could be used as a single-term method of grassland re-establishment, but this should be applied cautiously as repeated fires affect the biotic communities (Milberg et al. 2014). Deliberate afforestation should be avoided within *P. afra*'s range. Rewilding (i.e., reintroduction of wild roaming grazers) as a cost-efficient alternative to domesticated animals (e.g., Svenning et al. 2015), is already taking place in some parts of the Balkans (Jepson and Schepers 2016) and could be, in the future implemented in vast areas of *P. afra*'s Dalmatian distribution.

Acknowledgements

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Electronic supplementary material

The online version of this article (doi: 10.1007/s10841-017-9995-x) contains supplementary material, which is available to authorized users.

Online Resource 1 Geographic coordinates of the 23 transects set for butterfly recording in the Askion Mts and the 23 transects set for butterfly recording in inland Dalmatia, plus geographic coordinates of *Proterebia afra* records outside of the transects.

Online Resource 2 Overview of butterfly records along transects walked in the Askion Mts (Greece) and inland Dalmatia (Croatia) to study habitat associations of *Proterebia afra* and co-occurring butterfly species. ME = Mediterranean species (Y = yes).

References

Allen JRM, Brandt U, Brauer A, Hubberten H-W, Huntley B, Keller J, Kraml M, Mackensen A, Mingram J, Negendank JFW, Nowaczyk NR, Oberhansli H, Watts WA, Wulf S, Zolitschka B (1999) Rapid environmental changes in southern Europe during the last glacial period. Nature 400:740–743. doi:10.1038/23432

Androulakakis N (2012) Geological Map of Greece [Internet]. c2012–2016. Using: ArcGIS [GIS software]. Version 10.0. Redlands (CA): Environmental Systems Research Institute. https://www.arcgis.com/home/item.html?id=a26af2b211cb44a6af928739bfd29e9f. Accessed 10 Nov 2016

Aufgebauer A, Panagiotopoulos K, Wagner B, Schaebitz F, Viehberg FA, Vogel H, Zanchetta G, Sulpizio R, Leng MJ, Damaschke M (2012) Climate and environmental change in the Balkans over the last 17 ka recorded in sediments from Lake Prespa (Albania/F.Y.R. of Macedonia/Greece). Quatern Int 274:122–135. doi:10.1016/j.quaint.2012.02.015

Cremene C, Groza G, Rakosy L, Schileyko AA, Baur A, Erhardt A, Baur B (2005) Alterations of steppe-like grasslands in Eastern Europe: a threat to regional biodiversity hotspots. Conserv Biol 19:1606–1618. doi:10.1111/j.1523-1739.2005.00084.x

de Jong Y, Verbeek M, Michelsen V, Bjørn P de P, Los W, Steeman F, Bailly N, Basire C, Chylarecki P, Stloukal E et al (2014) Fauna Europaea—all European animal species on the web. Biodivers Data J 2:e4034. doi:10.3897/BDJ.2.e4034

Debussche M, Lepart J, Dervieux A (1999) Mediterranean landscape changes: evidence from old postcards. Glob Ecol Biogeogr 8:3–15. doi:10.1046/j.1365-2699.1999.00316.x

Deodati T, Cesaroni D, Sbordoni V (2009) Molecular phylogeny, classification, and biogeographic origin of *Callerebia* and other related Sino-Himalayan genera (Insecta: Lepidoptera: Nymphalidae: Satyrinae). In: Hartmann M, Weipert J (eds) Biodiversität und Naturausstattung im Himalaya III (Biodiversity and natural heritage of the Himalaya III). Verein der Freunde und Förderer des Naturkundemuseums, Erfurt, pp 107–114

Dercourt J, Zonenshain LP, Ricou L-E, Kazmin VG, Le Pichon X, Knipper AL, Grandjacquet C, Sbortshikov IM, Geyssant J, Lepvrier C, Pechersky DH, Boulin J, Sibuet JC, Savostin LA, Sorokhtin O, Westphal M, Bazhenov ML, Lauer JP, Biju-Duval B (1986) Geological evolution of the tethys belt from the atlantic to the pamirs since the LIAS. Tectonophysics 123:241–315. doi:10.1016/0040-1951(86)90199-X

Dewey JF, Pitman WC, Ryan WBF, Bonnin J (1973) Plate tectonics and the evolution of the alpine system. Geol Soc Am Bull 84:3137–3180. doi:10.1130/0016-7606(1973)84<3137:PTATEO>2.0.CO;2

Eichel S, Fartmann T (2008) Management of calcareous grasslands for Nickerl's fritillary (Melitaea aurelia) has to consider habitat requirements of the immature stages, isolation, and patch area. J Insect Conserv 12:677–688. doi:10.1007/s10841-007-9110-9

Ekroos J, Heliölä J, Kuussaari M (2010) Homogenization of lepidopteran communities in intensively cultivated agricultural landscapes. J Appl Ecol 47:459–467. doi:10.1111/j.1365-2664.2009.01767.x

ESRI (2011) ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands

Fairbanks RG (1989) A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. Nature 342:637–642. doi:10.1038/342637a0

Hambäck PA, Summerville KS, Steffan-Dewenter I, Krauss J, Englund G, Crist TO (2007) Habitat specialization, body size, and family identity explain lepidopteran density–area relationships in a cross-continental comparison. Proc Natl Acad Sci USA 104:8368–8373. doi:10.1073/pnas.0611462104

Hesselbarth G, Oorschot H van, Wagener S (1995) Die Tagfalter der Türkei unter Berücksichtigung der angrenzenden Länder. Goecke & Evers, Keltner

Jepson P, Schepers F (2016) Making space for rewilding: Creating and enabling policy environment. University of Oxford, Oxford. doi:10.13140/RG.2.1.1783.1287

Johnson CN (2009) Ecological consequences of Late Quaternary extinctions of megafauna. Proc R Soc B 276:2509–2519. doi:10.1098/rspb.2008.1921

Jutzeler D, Lafranchis T (2011) Die Larvalstadien von *Proterebia afra pyramus* (de Louker) aus dem Norden Griechenlands. Larvalentwicklung der dalmatinischen *P. afra dalmata* (Godart, 1824) im Vergleich und zur

Geschichte des Namens unserer Art (Lepidoptera Nymphalidae, Satyrinae). Entomol Romanica 16:5–18

Kadlec T, Vrba P, Kepka P, Schmitt T, Konvicka M (2010) Tracking the decline of the once-common butterfly: delayed oviposition, demography and population genetics in the hermit *Chazara briseis*. Anim Conserv 13:172–183. doi:10.1111/j.1469-1795.2009.00318.x

Koren T, Trkov D (2011) *Proterebia afra damlata* (Godart, 1824) (Lepidoptera, Satyrinae) recorded for the first time in Bosnia and Hercegovina. Nat Slov 13:57–58

Koren T, Burić I, Štih A, Zakšek V, Verovnik (2010) New data about the distribution and altitudinal span of the Dalmatian Ringlet, *Proterebia afra dalmata* (Godart, [1824]) (Lepidoptera: Satyrinae) in Croatia = Novi podatki o razširjenosti in razponu nadmorskih višin pojavljanja dalmatinskega rjavčka, *Proterebia afra dalmata* (Godart, [1824]) (Lepidoptera: Satyrinae) na Hrvaškem. Acta Entomol Slov 18:143–150

Kruess A, Tscharntke T (2002) Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. Conserv Biol 16:1570–1580. doi:10.1046/j.1523-1739.2002.01334.x

Kryštufek B, Griffiths HI (2002) Species richness and rarity in european rodents. Ecography 25:120–128. doi:10.1034/j.1600-0587.2002.250114.x

Kudrna O, Harpke A, Lux K, Pennerstorfer J, Schweiger O, Settele J, Wiemers M (2011) Distribution Atlas of Butterflies in Europe. Gesellschaft für Schmetterlingsschutz, Halle

Kudrna O, Pennerstorfer J, Lux K (2015) Distribution atlas of European butterflies and skippers. Wiss. Verl. Peks., Schwanfeld

Lawton JH (1993) Range, population abundance and conservation. Trends Ecol Evol 8:409–413. doi:10.1016/0169-5347(93)90043-O

Loeffler F, Stuhldreher G, Fartmann T (2013) How much care does a shrub-feeding hairstreak butterfly, *Satyrium spini* (Lepidoptera: Lycaenidae), need in calcareous grasslands? Eur J Entomol 110:145–152. doi:10.14411/eje.2013.020

Loos J, Dorresteijn I, Hanspach J, Fust P, Rakosy L, Fischer J (2014) Low-intensity agricultural landscapes in transylvania support high butterfly diversity: implications for conservation. PLoS ONE 9:e103256. doi:10.1371/journal.pone.0103256

Magyari EK, Chapman JC, Gaydarska B, Marinova E, Deli T, Huntley JP, Allen JRM, Huntley B (2008) The “oriental” component of the Balkan flora: evidence of presence on the Thracian Plain during the Weichselian late-glacial. J Biogeogr 35:865–883. doi:10.1111/j.1365-2699.2007.01849.x

Mazzoleni S, Pasquale G di, Mulligan M, Martino P di, Rego F (2008) Recent dynamics of the mediterranean vegetation and landscape. Wiley, Chichester

Mihoci I, Šašić M (2005) New findings of the butterfly Dalmatian Ringlet, *Proterebia afra dalmata* (Godart, [1824]) (Lepidoptera, Satyrinae) in Croatia. Nat Croat 14:121–129

Mihoci I, Šašić M. (2007) New distribution data on the endemic butterfly *Proterebia afra dalmata* (Godart, [1824]) (Nymphalidae, Satyrinae) in Croatia. Nat Croat 16:205–210

Milberg P, Akoto B, Bergman K-O, Fogelfors H, Paltto H, Talle M (2014) Is spring burning a viable management tool for species rich grasslands? Appl Veg Sci 17:429–441. doi:10.1111/avsc.12091

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Fonseca GAB da, Seligmann PA, Ford H (2005) Hotspots revisited: Earth’s biologically richest and most endangered terrestrial ecoregions, 1st edn. Conservation International, Mexico City

Möllenbeck V, Hermann G, Fartmann T (2009) Does prescribed burning mean a threat to the rare satyrine butterfly *Hipparchia fagi*? Larval-habitat preferences give the answer. J Insect Conserv 13:77–87. doi:10.1007/s10841-007-9128-z

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858. doi:10.1038/35002501

Numa C, van Swaay C, Wynhoff I, Wiemers M, Barrios V, Allen D, Sayer C, Lopéz Munguira M, Balletto E, Benyamini D, Beshkov S, Bonelli S, Caruana R, Dapporto L, Franeta F, Garcia-Pereira P, Karacetin E, Katbeh-Bader A, Maes D, Micevski N, Miller R, Monteiro E, Moulai R, Nieto A, Pamperis L, Peer G, Power A, Sasic M, Thompson K, Tzirkalli E, Verovnik R, Warren M, Welch H (2016) The status and distribution of Mediterranean butterflies. IUCN, Malaga

Nylin S, Bergström A (2009) Threat status in butterflies and its ecological correlates: how far can we generalize? Biodivers Conserv 18:3243. doi:10.1007/s10531-009-9640-4

Örvössy N, Kőrösi Á, Batáry P, Vozár Á, Peregovits L (2013) Potential metapopulation structure and the effects of habitat quality on population size of the endangered False Ringlet butterfly. J Insect Conserv 17:537–547. doi:10.1007/s10841-012-9538-4

Pamperis LN (2009) The butterflies of Greece. Editions Pamperis, Athens

Pamperis LN (2011) The presence of *Proterebia afra* (Fabricius, 1787) (Lepidoptera: Satyridae) in the Rhodope Mts, NE. Greece. Entomol Gaz 62.

Peña C, Witthauer H, Klečková I, Fric Z, Wahlberg N (2015) Adaptive radiations in butterflies: evolutionary history of the genus *Erebia* (Nymphalidae: Satyrinae). Biol J Linn Soc 116:449–467. doi:10.1111/bij.12597

Pokluda P, Hauck D, Cizek L (2012) Importance of marginal habitats for grassland diversity: fallows and overgrown tall-grass steppe as key habitats of endangered ground-beetle *Carabus hungaricus*. Insect Conserv Diver 5: 27–36. doi:10.1111/j.1752-4598.2011.00146.x

Pollard E (1977) A method for assessing changes in the abundance of butterflies. Biol Conserv 12:115–134. doi:10.1016/0006-3207(77)90065-9

Polunin O (1980) Flowers of Greece and the Balkans: a field guide. Oxford University Press, Oxford

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Radovic J, Civic K, Topic R, Posavec Vukelic V (eds) (2009) Biodiversity of Croatia. State institute for nature protection. Ministry of Culture—Republic of Croatia, Zagreb

Roos P, Arnscheid W, Stangelmaier G, Beil B (1984) Praimaginale Merkmale in der Gattung *Proterebia* Roos & Arnscheid: Beweise fur die phylogenetische Distanz zur Gattung *Erebia Dalman* (Satyridae). Nota Lepidopterol 7:361–374

Ryan WBF, Pitman WC, Major CO, Shimkus K, Moskalenko V, Jones GA, Dimitrov P, Gorür N, Sakinç M, Yüce H (1997) An abrupt drowning of the Black Sea shelf. Mar Geol 138:119–126. doi:10.1016/S0025-3227(97)00007-8

Sagarin RD, Gaines SD (2002) The "abundant centre" distribution: to what extent is it a biogeographical rule? Ecol Lett 5:137–147. doi:10.1046/j.1461-0248.2002.00297.x

Sandom CJ, Ejrnæs R, Hansen MDD, Svenning JC (2014) High herbivore density associated with vegetation diversity in interglacial ecosystems. Proc Natl Acad Sci USA 111:4162–4167. doi:10.1073/pnas.1311014111

Sasic M, Mihoci I, Kucinic M (2013) Crveni popis danjih leptira Hrvatske. Drzavni zavod za zastitu prirode, Ministarstvo zastite okolisa i prirode, Zagreb. http://www.dzzp.hr/dokumenti_upload/20130909/dzzp201309091608230.pdf. Accessed 6 Feb 2017

Shreeve TG, Dennis RLH, Roy DB, Moss D (2001) An ecological classification of British butterflies: ecological attributes and biotope occupancy. J Insect Conserv 5:145–161. doi:10.1023/A:1017556113534

Slamova I, Klecka J, Konvicka M (2011) Diurnal behavior and habitat preferences of *Erebia aethiops*, an Aberrant Lowland Species of a Mountain Butterfly Clade. J Insect Behav 24:230–246. doi:10.1007/s10905-010-9250-8

Slancarova J, Vrba P, Platek M, Zapletal M, Spitzer L, Konvicka M (2015) Co-occurrence of three Aristolochia-feeding Papilionids (*Archon apollinus*, *Zerynthia polyxena* and *Zerynthia cerisy*) in Greek Thrace. J Nat Hist 49:1825–1848. doi:10.1080/00222933.2015.1006281

Slancarova J, Bartonova A, Zapletal M, Kotilinek M, Fric ZF, Micevski N, Kati V, Konvicka M (2016) Life history traits reflect changes in mediterranean butterfly communities due to forest encroachment. PLoS ONE 11:e0152026. doi:10.1371/journal.pone.0152026

Stuhldreher G, Fartmann T (2014) When habitat management can be a bad thing: effects of habitat quality, isolation and climate on a declining grassland butterfly. J Insect Conserv 18:965–979. doi:10.1007/s10841-014-9704-y

Svenning J-C, Pedersen PBM, Donlan J, Ejrnaesd R, Faurbya S, Galettie M, Hansen DM, Sandel B, Sandom CJ, Terborgh JW, Vera FWM (2015)

Science for a wilder Anthropocene: synthesis and future directions for trophic rewilding research. Proc Natl Acad Sci USA 113:898–906. doi:10.1073/pnas.1502556112

Ter Braak CJF, Šmilauer P (2012) CANOCO Reference Manual and User's Guide: Software for Ordination (version 5.0). Biometris, Wageningen

Thomas JA, Rose RJ, Clarke RT, Thomas CD, Webb NR (1999) Intraspecific variation in habitat availability among ectothermic animals near their climatic limits and their centres of range. Funct Ecol 13:55–64. doi:10.1046/j.1365-2435.1999.00008.x

Tolman T, Lewington R (2008) Collins butterfly guide: the Most complete field guide to the butterflies of Britain and Europe. Collins, London

Tshikolovets VV, Kosterin O, Gorbunov P, Yakovlev R (2016) The butterflies of Kazakhstan. Tshikolovets, Kiev

Tvrtković N, Verovnik R, Lovrenčić L, Vuković M, Šašić M (2015) New contributions to the butterfly fauna of Mt Velebit and the neighbouring area of Lika (Croatia). Nat Croat 24:281–292.

Van Andel TH, Tzedakis PC (1996) Palaeolithic landscapes of Europe and environs, 150,000–25,000 years ago: an overview. Quat Sci Rev 15:481–500. doi:10.1016/0277-3791(96)00028-5

van Strien AJ, van Swaay CAM, Kery M (2011) Metapopulation dynamics in the butterfly *Hipparchia semele* changed decades before occupancy declined in The Netherlands. Ecol Appl 21:2510–2520. doi:10.2307/41416675

Vermeire LT, Gillen RL (2001) Estimating herbage standing crop with visual obstruction in tallgrass prairie. J Range Manag 54:57–60. doi:10.2458/azu_jrm_v54i1_vermeire

CHAPTER II.

Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae)

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Chapter II.

Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of Proterebia afra (Lepidoptera: Nymphalidae: Satyrinae)

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Abstract

A characteristic butterfly of Asian steppes, *Proterebia afra* (Fabricius 1787), was studied in its two relic areas of occurrence within the Balkans—the Askion Mts (a single mountain massif in NW Greece) and Dalmatia (karstic inland S Croatia)—together with co-occurring butterfly communities during its early spring adult flight period. *P. afra* adults tolerate harsh continental conditions prevailing at its localities during its flight. Local densities were higher in the Askion Mts (up to 100 individuals per 50 m walk) than in Dalmatia (up to 30 individuals per 50 m). Within both areas of occurrence, it inhabits dry grasslands subject to intermediate grazing pressure that retains enough dry grass litter but still suppresses shrubs and trees. Hotter slopes are preferred in more northerly Dalmatia than in the more southerly Askion Mts. More co-occurring butterfly species and more Mediterranean species were recorded in the Askion Mts (69 vs 63; 23 vs 12), but more butterfly individuals and higher species' richness per transect were observed in Dalmatia. In ordination analyses, the main gradients organising the grassland butterfly communities distinguished between grassy plains and rocky slopes containing more woody plants. Given the distribution extents, local densities and present land use conditions, the Balkan *P. afra* is not currently endangered, but this may swiftly change with grazing abandonment, especially in Dalmatia, and some conservation actions should be considered in the near future.

Keywords

butterfly biology, continentality, glacial refugia, Quaternary, relict populations, species distribution modelling

Introduction

The Palaearctic steppes represent an enormously extensive biome, endangered owing to conversion of its fertile soils into arable land (Hoekstra *et al.*, 2005; Török *et al.*, 2016; Wesche *et al.*, 2016). The history of this biome started in Eurasia at the Eocene–Oligocene transition (Zachos *et al.*, 2001; Liu *et al.*, 2009) with the start of the uplift of the Tibetan Plateau, the accumulation of landmasses in the Northern Hemisphere (Ruddiman & Kutzbach, 1989; Ramstein *et al.*, 1997) and the expansion of grasses (Jacobs *et al.*, 1999), and is culminating with the Quaternary glacial cycles. The increased aridity, hence continentality, and the exposure of the continental shelf areas as a result of the reduction in sea levels (Ramstein *et al.*, 1997; Nicholas & Chivas, 2014), combined with grazing by megaherbivores (Fortelius *et al.*, 2006, Kahlke, 2014), contributed to the vast distribution of steppes, first reaching Western and Mediterranean Europe in the Miocene (Ribera & Blasco-Zumeta, 1998), and culminating during the Quaternary glacials (Magyari *et al.*, 2008; Álvarez-Lao & García, 2011; Panagiotopoulos *et al.*, 2014). During the considerably shorter (often only 10 kyr each) interglacials, the steppes diminished in size, but the effect was partly buffered by the activity of megaherbivores (Sandom *et al.*, 2014). In the Holocene, only azonal patches were left in Europe, usually termed steppe-like grasslands (for review, see Wesche *et al.*, 2016).

The Quaternary has been an age of permanent changes, with immense effects on existing biota (de Lattin, 1967). Using fossil records, current distributions and genetic markers, many authors have documented two types of biotas in the western Palaearctic. The warm-adapted or Mediterranean biota benefited from interglacial climates and retreated to southern refugia during cold periods (Hewitt, 1999; Seddon *et al.*, 2001; Habel *et al.*, 2011; Korabek *et al.*, 2018), whereas the cold-adapted biota displayed the opposite pattern (Schmitt & Hewitt, 2004; Mardulyn *et al.*, 2009; Smith *et al.*, 2017). Many exceptions add to the complexity of the system (Stewart & Lister, 2001; Schönswetter *et al.*, 2005; Haubrich & Schmitt, 2007; Schmitt & Varga, 2012; Kramp *et al.*, 2016). However, given that the geographical extension of steppes had been influenced by changing aridity, an additional, continental fauna was postulated. Its elements should expand westwards during glacials and display diminishing genetic diversity from the east to the west (Schmitt, 2007; Stewart *et al.*, 2010). Hence, steppe species are assumed to benefit from glacial climates, expanding along the steppe belt, and retreating into arid refugia during interglacials. Kajtoch *et al.*(2016) presented evidence that Eurasian steppe species tend to survive in several separate refugia across their range during interglacials, rather than displaying clear east–west expansions and contractions.

One typical element of the Palaearctic steppes is the Satyrine butterfly *Proterebia afra* (Fabricius, 1787). This species develops on coarse steppe grasses and forms a single adult generation annually in the spring (Bartonova *et al.*, 2017). It inhabits a few isolated areas of steppe-like grasslands in the Balkans (north-western Greece, Askion Mountains; north-eastern Greece, a small population in the Nestos river canyon; and Croatian Dalmatia, from the Velebit Mountains in the north to the Biokovo Mountains in the south and the Dinaric Alps in the east). Apart from the Balkans, it basically inhabits two latitudinal belts: (1) the northern continental (i. e., zonal) steppes (isolated populations in Crimea, N Caucasus, Krasnodar and Dagestan, and more continuous in Volgograd region, southern Ural, Kazakhstan and parts of Kyrgyzstan); and (2) the southern xeric mountain steppes (Turkey, S Caucasus, N Iran). The habitat exploitation also changes within the distributional range. It inhabits core steppe areas in Kazakhstan and survives on slopes of riverine terraces along the Volga and Don rivers in those mostly farmed parts of its distribution. In the South, it inhabits mountain slopes from lowland semi-barren grounds (as e.g. in the Kacheti region in Georgia) up to ~3000 m a.s.l. (Hesselbarth *et al.*, 1995; Tshikolovets *et al.*, 2014), shifting its seasonal flight period with altitude. Its current distribution makes *P. afra* a perfect model for a steppe species, which should respond to Quaternary climate changes across its range.

In this paper, we use mitochondrial DNA (mtDNA), nuclear DNA (nuDNA), amplified fragment length polymorphism (AFLP) and species distribution models (SDMs). Our aims are as follows: (1) to elucidate the phylogeography of the species throughout its distributional range; (2) to delimitate the climatically suitable areas for the species in recent conditions and the Last Glacial Maximum; and (3) to compare the results of the different markers and distribution models with published evidence of the Quaternary ecosystems dynamics to achieve a comprehensive understanding of the range dynamics of the species.

Material and methods

Sampling and molecular data

We obtained 182 samples of *P. afra* from nine countries and 41 sites merged into 15 populations (Table 1; Supporting Information, Table S1, including GenBank accession numbers). DNA was extracted from the legs using the Genomic DNA Mini Kit – Tissue (Geneaid), following the manufacturer's protocols.

One fast mitochondrial gene (cytochrome *c* oxidase subunit I; *COI*) and one conservative nuclear gene (arginine kinase; *ArgKin*) were amplified by polymerase chain reactions (PCRs) in 20 µL volume (10 µL PPP Mastermix Top-Bio, 6.9 µL PCR H_2O, 0.8 + 0.8 µL primers and 1.5 µL DNA). Two forward–reverse primer pairs were used for *COI*, LCO/HCO and Tonya/Hobbes (Monteiro & Pierce, 2001), and one pair for *ArgKin*, ArginineF/ArginineR (Wahlberg & Wheat, 2008). The thermal cycling profile was 95 °C for 5 min, 94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s, for 36 cycles, and final extension at 72 °C for 10 min. Sequencing was provided by Macrogen Inc. on ABI3730XL DNA analysers. Sequences were checked manually and aligned in Geneious v.8.0.5 (Kearse *et al.*, 2012).

Amplified fragment length polymorphism analysis followed the protocol of Vos *et al.*(1995). First, during the restriction–ligation step, genomic DNA was digested with two restriction enzymes (EcoRI and MseI), and specific double-stranded adapters were annealed to the ends of the fragments, in 10 µL volume for 16 h at 21 °C in a thermocycler. Second, a two-step PCR reduced the number of fragments. Preselective amplification was performed with primers with one selective base (EcoRI primer E and MseI primer M) and selective amplification with fluorescence

Table 1.
Specification of *Proterebia afra* populations used for its phylogeographic study, country and area of origin, and numbers of samples obtained for cytochrome *c* oxidase subunit I (*COI*), arginine kinase (*ArgKin*) sequences and amplified fragment length polymorphism (AFLP) markers MseI-CGC/EcoRI-AAA and MseI-CCT/EcoRI-AGG

	Population	**Country**	**Area**	**COI**	**ArgKin**	**AFLP**
	total			**182**	**128**	**117**
1	EI1	Iran	Eastern Alborz	25	23	22
2	EI2	Iran	Kopet Dag	2	1	2
3	CI	Iran	Alborz, Zanyan and env.	6	4	5
4	WI	Iran, Azerbaijan	Western and Eastern Azerbaijan, Talysh Mts.	4	2	2
5	GE	Georgia	Kacheti	8	8	8
6	AR	Armenia	Arailer Mt.	15	4	6
7	TU	Turkey	Anatolia and Taurus Mts.	10	5	5
8	GR1	Greece	Askion Mts.	36	35	22
9	GR2	Greece	Thrace, Nestos canyon	2	1	2
10	CR	Croatia	Dalmatia	30	29	26
11	RU1	Russia	Saratov and Volgograd prov.	18	4	6
12	RU2	Russia	Southern Ural, Orenburg prov.	6	2	2
13	CM	Russia	Crimea	7	1	3
14	DG	Russia	Dagestan prov.	3	0	3
15	KZ	Kazakhstan	Eastern Kazakhstan	10	9	3

labelled primers with two additional selective bases (EcoRI primer E+2 and MseI primer M+2). Two selective primer combinations were used: MseI-CGC with EcoRI-AAA (dye 6-FAM) and MseI-CCT with EcoRI-AGG (dye VIC). Genotyping was provided by Macrogen Inc. on an ABI3730XL capillary analyser with the 600 LIZ internal size standard marker (600 bp). Data were aligned in GeneMapper 5 (Applied Biosystems). Fragments < 60 bp were discarded. All fragments were evaluated visually; problematic peaks were either placed in the proper bin or removed. Markers were combined in a binary matrix; only samples with > 30 fragments were used.

Molecular analyses: sequence data

For haplotype reconstruction, *ArgKin* sequences were phased in DnaSP v.5. A statistical parsimony algorithm was used to reconstruct TCS haplotype networks (Clement *et al.*, 2000) in popart (Leigh & Bryant, 2015). To group populations, we computed the spatial analysis of molecular variance (SAMOVA) in spads v.1.0 (Dellicour & Mardulyn, 2014), for clusters $K = 2–14$, 5000 iterations, ten repetitions; the proportion of total genetic variance among groups (Φ_{CT}) was used to identify the best number of clusters. For the descriptive statistics, we used the software Arlequin v.3.11 (Excoffier *et al.*, 2007) and DnaSP v.5 (Librado & Rozas, 2009) (Table 2). To detect evidence of natural selection or population expansions, we used Tajima's *D*and Fu's F_s neutrality tests. To assess genetic divergence among haplotype clades, we calculated pairwise Φ_{ST}, a fixation index considering allele frequencies. Mantel tests to detect correlation between genetic and geographical distances were conducted in the Excel add-in GeneAlEx v.6.5 (Peakall & Smouse, 2006, 2012), at the individual level for the whole dataset, and for southern (EI1, EI2, CI, WI, GE, AR, TU, GR1, GR2 and CR) and northern (RU1, RU2, CM, DG, KZ) latitudes separately to specify whether they have diverging patterns. To estimate genetic distances among individuals for Mantel tests, we used Kimura's two-parameters distance (K80) in the R package 'ape' (Paradis *et al.*, 2004).

Substitution models were selected by jModelTest (Posada, 2008): HKY+I for *COI* and GTR+G+I for *ArgKin*. For phylogeny and divergence

Table 2.

Population genetic statistics of cytochrome *c* oxidase subunit I (*COI*) and arginine kinase gene (*ArgKin*) for 15 *Proterebia afra* populations in the Palaearctic steppes, computed in DnaSP and Arlequin

		N	S	h	H_p	H_d	Π	**Tajima's D**		**Fu's Fs**	
	COI	**182**	**99**	**67**		**0.960**	**0.021**	**-0.464**	**n.s.**	**-14.485**	*******
1	EI1	25	21	5	5	0.300	0.003	**-2.132**	*	0.669	n.s.
2	EI2	2	6	2	2	1.000	0.008	NA		NA	
3	CI	6	4	3	2	0.733	0.003	0.355	n.s.	0.669	n.s.
4	WI	4	11	3	1	0.833	0.008	0.584	n.s.	1.835	n.s.
5	GE	8	6	6	5	0.929	0.002	-1.045	n.s.	-2.489	n.s.
6	AR	15	0	1	1	0.000	0.000	NA		NA	
7	TU	10	12	6	6	0.778	0.004	-0.788	n.s.	-1.417	n.s.
8	GR1	36	8	8	8	0.805	0.002	-0.207	n.s.	-1.334	n.s.
9	GR2	2	0	1	1	0.000	0.000	NA		NA	
10	CR	30	25	12	12	0.798	0.003	**-2.229**	**	**-4.674**	**
11	RU1	18	18	13	8	0.941	0.006	-0.701	n.s.	**-4.341**	**
12	RU2	6	6	4	1	0.867	0.004	0.611	n.s.	-0.808	n.s.
13	CM	7	0	1	1	0.000	0.000	NA		NA	
14	DG	3	7	3	2	1.000	0.006	NA		NA	
15	KZ	10	12	8	6	0.956	0.004	-1.074	n.s.	**-3.034**	*
	ArgKin	**256**	**37**	**67**		**0.963**	**0.010**	**-0.881**	**n.s.**	**-56.763**	*******
1	EI1	46	14	16	14	0.926	0.006	-0.483	n.s.	**-12.674**	***
2	EI2	2	5	2	0	1.000	0.011	NA		NA	
3	CI	8	6	5	2	0.786	0.005	0.303	n.s.	-0.700	n.s.
4	WI	4	5	3	2	0.833	0.005	-0.797	n.s.	0.461	n.s.
5	GE	16	8	5	2	0.800	0.006	0.502	n.s.	1.510	n.s.
6	AR	8	6	3	0	0.714	0.006	0.718	n.s.	1.079	n.s.
7	TU	10	10	7	4	0.911	0.008	0.026	n.s.	-1.590	n.s.
8	GR1	70	13	18	8	0.880	0.007	0.629	n.s.	**-9.621**	***
9	GR2	2	1	2	2	1.000	0.002	NA		NA	
10	CR	58	12	13	10	0.827	0.008	0.987	n.s.	**-4.627**	***
11	RU1	8	5	5	1	0.786	0.005	0.466	n.s.	-1.637	n.s.
12	RU2	4	5	4	1	1.000	0.006	-0.213	n.s.	-1.414	n.s.
13	CM	2	4	2	1	1.000	0.009	NA		NA	
14	DG	0	NA	NA	NA	NA	NA	NA		NA	
15	KZ	18	9	8	3	0.752	0.005	-0.274	n.s.	-2.506	

ArgKin was phased for the analyses; therefore, the total number of individuals is $N/2$.
h, number of haplotypes; H_d, haplotype diversity; H_p, number of private haplotypes; N, number of sequences analysed; n.s., not significant; S, number of segregating sites; Π, nucleotide diversity; NA, not applicable computation.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

time, we used BEAST v.1.8.4 (Drummond *et al.*, 2012) to construct an ultrametric tree from haplotypes, under coalescent constant size tree prior, with 100000000 generations and four runs. As outgroups and as molecular clock calibration points, we used related Asiatic Satyrinae of the Ypthimina clade, under normal tree priors and setting the divergence time of *Proterebia* to the most recent common ancestor as (mean ± SD) 33 ± 3.3 Mya with *Paralasa* Moore, 1893, 21 ± 2.1 Mya with *Loxerebia* Watkins, 1925, and 15 ± 1.5 Mya with *Callerebia* Buttler, 1867 (Pena *et al.*, 2011). We adopted the uncorrelated lognormal relaxed clock and lognormal ucld.mean because we assume the origin of fossils, which had been used for original tree calibration, before their fossilization. The convergence of the runs was checked in Tracer v.1.6 (Rambaut *et al.*, 2014). Results were summarized onto a single target tree.

To estimate the ancestral range for each node of the mitochondrial tree, we used the software RASP (Yu *et al.*, 2015), which uses the R package BioGeoBEARS (Matzke, 2012, 2013). The framework compares alternative models of past geographical range estimation based on the Akaike information criterion (AIC): dispersal–extinction–cladogenesis (DEC), dispersal–vicariance analysis (DIVALIKE) and Bayesian inference for discrete areas (BAYAREALIKE). We combined the populations into seven areas based on geographical proximity: (1) EI1 + EI2; (2) CI + WI + AR + GE; (3) TU; (4) GR1 + GR2; (5) CR; (6) RU1 + RU2 + CM + DG; and (7) KZ. The range constraint (the number of areas the ancestral population was allowed to occupy) was set as two. The ancestral range was calculated for each node.

Molecular analyses: AFLP data

For the descriptive statistics, we used the R script aflpdat (Ehrich, 2006) and AFLP-SURV v.1.0 (Vekemans, 2002) (Table 3). To estimate genetic divergence among populations, we calculated pairwise Φ_{PT}, a fixation index for dominant markers, which we used for principal coordinates analysis (PCoA). A neighbour-joining tree was constructed from Nei's genetic distances in PHYLIP v.3.695 (Felsenstein, 2005), with 10000 bootstraps. Mantel tests were calculated at the individual level and for

Table 3.
Population genetic statistics of amplified fragment length polymorphism (AFLP) markers (MseI-CGC/EcoRI-AAA and MseI-CCT/EcoRI-AGG) for 15 *Proterebia afra* populations in the Palaearctic steppes, computed by aflpdat and AFLP-SURV

		N	**Loci**	**Mean loci ± SD**	**min-max**	**polym. loci**	**Rarity index (*DW*)**	**Private fragments**	**Nei's gene diversity**
	total	**117**	**475**	**63 ± 19**	**31-114**				
1	EI1	22	303	81 ± 14	50-106	188	1443.9	22	0.179
2	EI2	2	96	59 ± 6	54-63	96	584.1	1	0.158
3	CI	5	141	52 ± 9	38-62	141	544.4	2	0.129
4	WI	2	83	48 ± 12	39-56	83	521.8	3	0.149
5	GE	8	198	73 ± 12	52-93	198	1252.0	19	0.150
6	AR	6	121	40 ± 10	32-58	121	266.5	0	0.102
7	TU	5	136	44 ± 8	36-57	136	336.5	2	0.129
8	GR1	22	311	73 ± 16	40-114	167	1158.6	27	0.169
9	GR2	2	115	79 ± 6	74-83	115	1000.3	1	0.154
10	CR	26	263	59 ± 14	40-97	141	658.9	19	0.123
11	RU1	6	118	39 ± 5	31-46	118	276.5	1	0.096
12	RU2	2	49	32 ± 1	31-33	49	227.6	1	0.072
13	CM	3	98	50 ± 15	33-60	98	401.7	0	0.115
14	DG	3	96	50 ± 11	38-60	96	342.3	0	0.114
15	KZ	3	89	47 ± 8	40-55	89	437.6	1	0.104

DW, frequency down-weighed marker value according to Schönswetter and Tribsch (2005), an index showing the population rarity, based on mean fragment occurrences (Ehrich, 2006) minimizing the effect of uneven sampling.

northern and southern latitudes separately, from pairwise genetic distances.

To investigate the genetic structure of the data, we ran ParallelStructure (Besnier & Glover, 2013) under the admixture model, with allele frequencies correlated, 500000 burn-in and 1000000 generations, for K = 1–17, with ten iterations each. To estimate the number of clusters, we used Kestimator, an R script developed by Puechmaille (2016), to count approximated likelihood of the data [ln Pr($X|K$)], ΔK, posterior probability (PP) and the new estimators MaxMeaK and MedMeaK, better evaluating the number of clusters under uneven sampling. The last four of these estimators were corrected for spurious clusters, i.e. those which never achieve 70% ancestry in any individual. We used the CLUMPAC server (Kopelman *et al.*, 2015) to align the iterations.

We used the CIPRES Science Gateway (Miller *et al.*, 2010) for BEAST and ParallelStructure computations.

Species distribution modelling

To investigate climatically suitable areas for the species, we used SDM. We supplemented our own records with published data (Lukhtanov & Lukhtanov, 1994; Hesselbarth *et al.*, 1995; Tshikolovets *et al.*, 2009, 2014, 2016; Tikhonov *et al.*, 2014). We used georeferenced records, data with position estimable from maps or published locality descriptions. To avoid oversampling of European localities, only eight records per country were used. Duplicates were omitted. This resulted in a total of 244 records covering the whole species range.

We obtained information on the present climate and the climate during the Last Glacial Maximum (LGM; 22000 years ago) as the 19 BIOCLIM variables (BIO1–BIO19) described by the community climate system model (CCSM), the Worldclim 1.4 database (Hijmans *et al.*, 2005), at 2.5 arc min spatial resolution. We restricted the area to the Palaearctic realm (30–70°N, −10 to 150°E).

We calculated the SDMs using MaxEnt 3.4.1 (Phillips *et al.*, 2018) via the R environment using packages ‘dismo’ and ‘raster’ (Hijmans *et al.*, 2011; Hijmans, 2016). Given that the distribution records tend to be biased towards the accessible locations (cf. Fourcade *et al.*, 2014), we used bias background correction for road density [Center for International Earth Science Information Network (CIESIN) Columbia University & Information Technology Outreach Services (ITOS) University of Georgia, 2013], which we rasterized by QGIS v.2.14.3 (QGIS Development Team, 2018). We tested the records against 10000 random background points selected by the script ‘sampleRast’ (available at https://rdrr.io/github/adamlilith/enmSdm/man/sampleRast.html; accessed 6 July 2018). For the two values intrinsic for MaxEnt estimates, regularization multiplier (RM) and the response of feature classes (FC), we found the best values using the ENMeval package (Muscarella *et al.*, 2014). We selected the variables with lowest AICc (AIC corrected for small samples) values after $K - 1$ jackknife data partitioning. We entered the arguments with the lowest AICc values into MaxEnt.

We measured the relative importance of the BIOCLIM variables using the jacknife test and excluded the variables with negative gain or gain close to zero expressed as the difference between full model training gain and the gain of the model without the variable(s).

Results

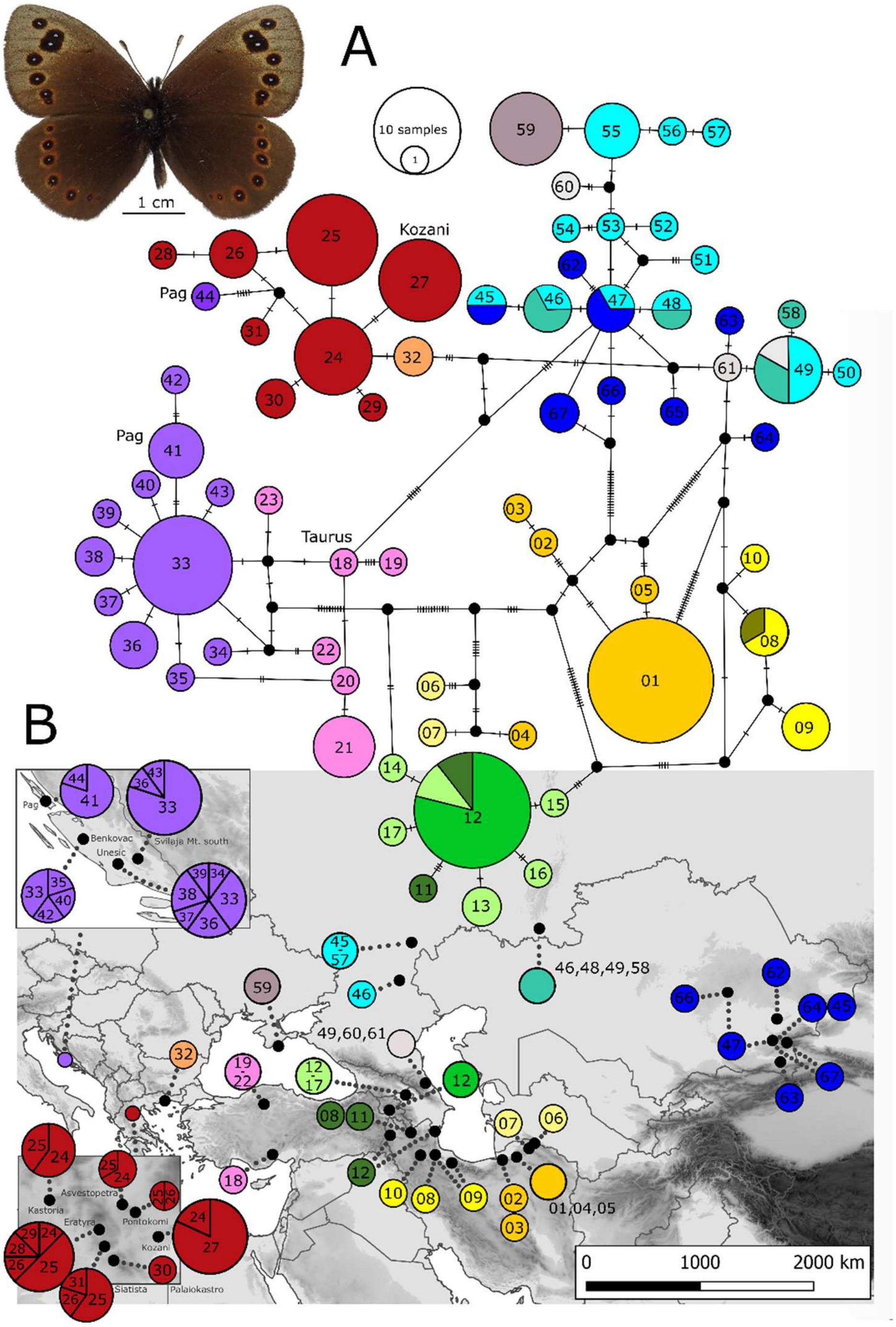
Sequence data: COI

We obtained 912 bp *COI* sequences for 182 individuals and identified 67 haplotypes (Fig. 1A, B). The haplotype network formed a circle. The samples north of the Greater Caucasus formed one haplogroup, with 24 closely related haplotypes (hereinafter ‘northern lineage’). Another haplogroup was formed by the samples from the Lesser Caucasus surroundings (WI, GE and AR). All other populations consisted of private haplotypes. Populations AR and CM were monomorphic. Turkish haplotypes were closely related to the Dalmatian lineage, and Greek haplotypes to the northern one. One Dalmatian sample was closely related to GR1. In SAMOVA, Φ_{CT} reached a plateau at $K = 9$ ($\Phi_{CT} = 0.85$) and increased further to $K = 14$ ($\Phi_{CT} = 0.87$), separating all populations except for AR + GE (see Supporting Information, Table S2A). The largest variance increases were for $K = 5$ ($\Phi_{CT} = 0.74$), distinguishing five lineages: (1) EI1 + EI2; (2) CI + WI + GE + AR; (3) TU + CR; (4) GR1 + GR2; and (5) the northern lineage.

The total haplotype diversity was high ($H_d = 0.96$) (Table 2). Significant negative values of Tajima's D for EI1 and CR indicate either population expansions or genetic selections. The significant Fu's F_s for the dataset could point to a historically rapid growth from a population with a small, effective population size. Fu's F_s showed an excess of haplotypes in CR, RU1 and KZ. Pairwise Φ_{ST} values were high among populations (> 0.7), except for the Lesser Caucasus and the northern lineage (see Supporting Information, Table S3). In the Mantel test, the genetic and the geographical matrices were positively correlated ($Rxy = 0.391$, $P = 0.01$, $y = 3 \times 10^{-6}x + 0.0123$, $R^2 = 0.1528$), more so in the southern ($Rxy = 0.767$, $P = 0.01$, $y = 7 \times 10^{-6}x + 0.0078$, $R^2 = 0.589$) than in the northern latitudes ($Rxy = 0.179$, $P = 0.01$, $y = 4 \times 10^{-7}x + 0.0039$, $R^2 = 0.0319$).

The BEAST tree recognized haplotypes from the eastern Alborz Mountains and Kopet Dag as the most basal, with the age of the split 5 Mya (±2 Mya 95% highest posterior density; Fig. 1C), at the beginning of the Pliocene. The second split separated the samples originating south of the Greater Caucasus ~4 Mya. The remaining single lineage split ~2.6 Mya into a branch containing Turkish and Dalmatian haplotypes and a branch containing Greek and northern haplotypes. Within the northern lineage, the analysis distinguished the haplotypes from eastern Kazakhstan and those from the Volga region. Southern Ural and Dagestan samples occurred on both branches.

The best-performing ancestral area range estimation model was DEC (AIC = 119), followed by DIVALIKE (AIC = 127) and BAYAREALIKE (AIC = 160). According to the DEC model, the most likely origin of extant populations is the area south of the Greater Caucasus and mountains of northern Iran (AB) (Fig. 1C). Afterwards, the species expanded to Turkey (BC), leaving the isolated populations in NE Iran (A). Then it expanded to Greece (CD) or, alternatively, to Russia (CF). The Russian–Greek branch was established (DF), and the two areas were separated afterwards. From Russia, the species expanded to Kazakhstan (FG). The Turkish population extended its range towards Croatia (CE).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Kastoria
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Eratyra
Pontokomi
Kozani
Siatista
Palaiokastro
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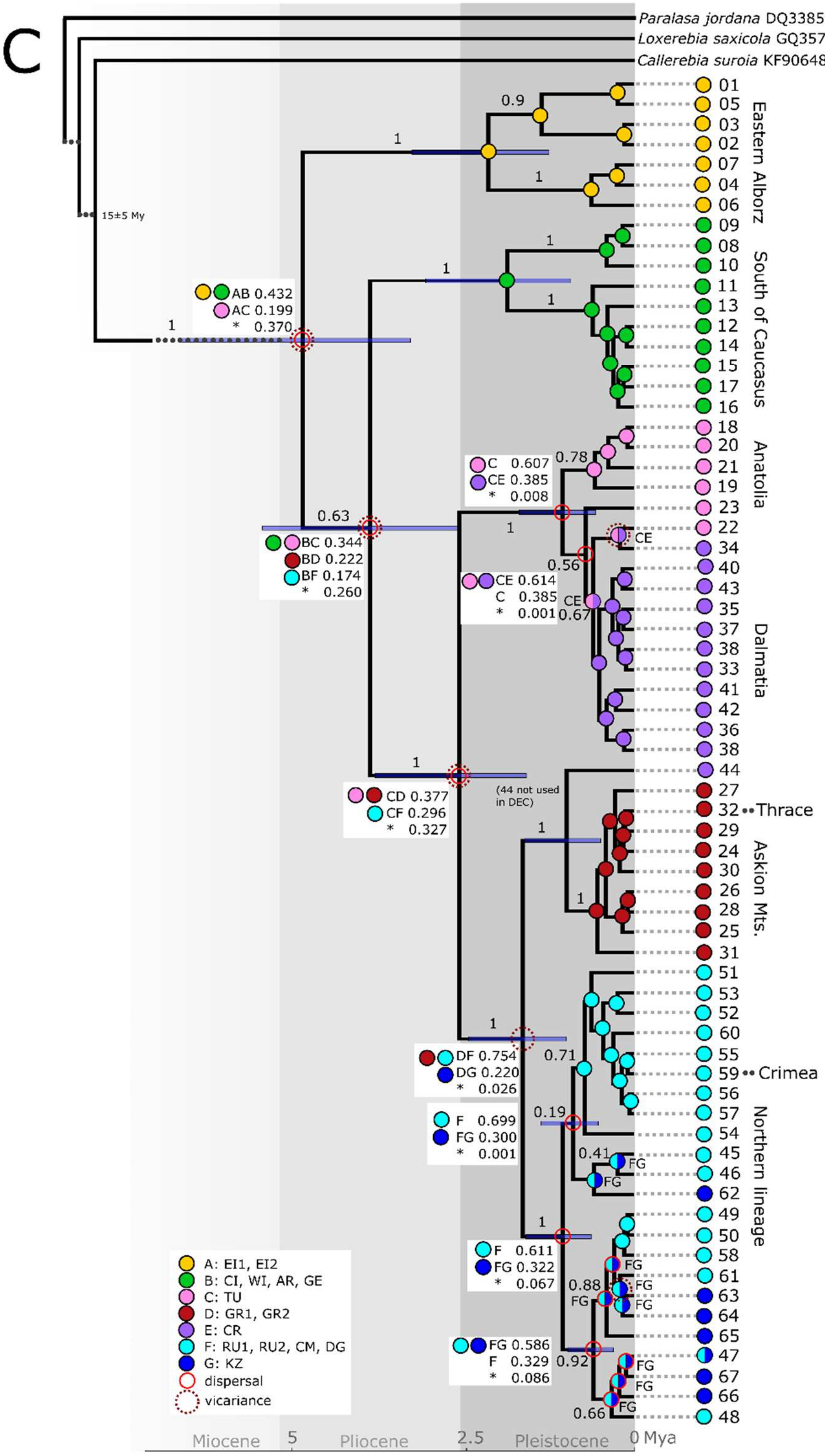
C
Paralasa jordana DQ338597
Loxerebia saxicola GQ357214
Callerebia suroia KF906483
15±5 My
Eastern Alborz
South of Caucasus
Anatolia
Dalmatia
Thrace
Askion Mts.
Crimea
Northern lineage
AB 0.432
AC 0.199
* 0.370
BC 0.344
BD 0.222
BF 0.174
* 0.260
CD 0.377
CF 0.296
* 0.327
C 0.607
CE 0.385
* 0.008
CE 0.614
C 0.385
* 0.001
(44 not used in DEC)
DF 0.754
DG 0.220
* 0.026
F 0.699
FG 0.300
* 0.001
F 0.611
FG 0.322
* 0.067
FG 0.586
F 0.329
* 0.086
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A: EI1, EI2
B: CI, WI, AR, GE
C: TU
D: GR1, GR2
E: CR
F: RU1, RU2, CM, DG
G: KZ
dispersal
vicariance
Miocene
5
Pliocene
2.5
Pleistocene
0 Mya

Figure 1.

Mitochondrial phylogeographic patterns of *Proterebia afra* in the Palaearctic steppes, based on 912 bp of the cytochrome *c* oxidase subunit I (*COI*). **A**, TCS haplotype network showing the relationships of the 67 haplotypes detected. Mutations are shown as black dots and hatch marks. The colours correspond to the map. **B**, haplotypes distributed on the map. **C**, Bayesian tree computed in BEAST v.1.8.4 from individual haplotypes. The molecular clock was calibrated by diversification from outgroups (Pena *et al.*, 2011). Node bars display 95% highest posterior density interval of the molecular clock. Branch labels show posterior probabilities. Nodes contain information on ancestral range estimation based on a dispersal–extinction–cladogenesis (DEC) model computed in BioGeoBEARS (Matzke, 2012).

Sequence data: **ArgKin**

We obtained 510 bp *ArgKin* sequences (128 individuals, 67 haplotypes; Fig. 2A). The haplotypes of the eastern Alborz Mountains (EI1, EI2) were well distinguished. The rest formed a single haplogroup, but the region south of the Greater Caucasus contained mostly separate haplotypes; the same applied for GR2. The widespread haplotypes comprised samples from AR, TU, RU1, RU2, GR and CR. In SAMOVA, the highest Φ_{CT} was for $K = 2$ ($\Phi_{CT} = 0.337$), again separating EI1 + EI2 from the rest (see Supporting Information, Table S2B). The haplotype diversity was high ($H_d = 0.96$) (Table 2). Fu's F_s showed negative significant results for the dataset, and for EI1, GR1 and CR. Values of pairwise Φ_{ST} were low, higher only for EI1, EI2 and GR2 if compared with the remaining populations (Supporting Information, Table S3). The Mantel test was not significant ($Rxy = -0.019$, $P = 0.16$). The Bayesian tree topology was consistent with the network (Fig. 2B).

Amplified fragment length polymorphism data

The two primer combinations yielded results for 117 individuals, with a total of 475 polymorphic peaks (Table 3; Supporting Information, Table S4).

Population statistics (*N* of polymorphic loci, *N* of private fragments; Table 3) corresponded to the uneven sampling. The *DW* index was highest (> 1000) in EI1, GE, GR1 and GR2; it was lowest (< 300) in AR, RU1 and RU2. Nei's gene diversity was highest (> 0.150) in EI1, EI2, GE, GR1 and GR2; it was lowest (< 0.100) in RU1 and RU2.

The overall Φ_{PT} was 0.20. Stronger differentiations in pairwise Φ_{PT} (> 0.2; Supporting Information, Table S3) applied to EI1, EI2, GR2 and CR, compared with all other populations; a weaker differentiation was found within AR, TU, RU1, RU2 and KZ. Principal coordinates analysis (Fig. 3A, axes 1 and 2) showed that populations alongside the south of the Caspian Sea (EI1, EI2, CI, WI and GE) and GR2 were highly differentiated from each other and from the rest. The neighbour-joining tree (Fig. 3B) corresponded to the geographical distribution of the populations (except for GR2), mostly with a weak branch support. The Balkan populations GR1 and CR were identified as sisters (87% bootstrap support) and placed among the northern populations. Mantel tests showed a positive correlation for the dataset ($Rxy = 0.256$, $P = 0.01$, $y = 0.0043x + 76.046$, $R^2 = 0.0658$) and an even stronger correlation for southern latitudes ($Rxy = 0.445$, $P = 0.01$, $y = 0.0072x + 75.794$, $R^2 = 0.1979$), but no correlation for the northern latitudes ($Rxy = 0.182$, $P = 0.07$, $y = 0.0016x + 48.515$, $R^2 = 0.033$).

In ParallelStructure, the best $\Delta K = 2$, again separating EI1 and EI2 from the rest; ln $\Pr(X|K) = 5$, corrPP = 5, assigning separate clusters for the following: (1) EI1 and EI2; (2) GE and CI; (3) GR1 and GR2; (4) CR; (5) a widespread cluster: WI, AR, TU, RU1, RU2, CM and DG; and (6) MedMeaK and MaxMeaK, additionally distinguishing two Greek clusters (Fig. 4; Supporting Information, Fig. S1). Both CI and WI were transitional between the Eastern Alborz Mountains, Kacheti and the widespread cluster. Some admixture of the widespread cluster existed in all populations. An admixture of the clusters assigned to Greece appeared in Anatolia, Crimea and Dagestan. In different individuals of GR1, three distinctive clusters prevailed: the one assigned mostly to GR1, the widespread one and the one of GR2.

Species distribution modelling

The best RM was according to AICc = 2 and the most parsimonious function of FC was a hinge feature (H) (Supporting Information, Table S5). The average area under the curve over the ten replicate runs was 0.916 (± 0.002 SD), showing a good fit for the model. The selected most important BIOCLIM variables after the jackknife procedure, and the respective percentages of explained variation, were as follows: BIO18 (precipitation of warmest quarter), 35.9%; BIO5 (maximum temperature of warmest month), 18.8%; BIO19 (precipitation of coldest quarter), 14.5%; BIO16 (precipitation of wettest quarter), 13.0%; BIO15 (precipitation seasonality), 10.3%; and BIO8 (mean temperature of wettest quarter), 7.5%. Based on the selection of variables, the distribution of *P. afra* appears to be controlled by precipitation.

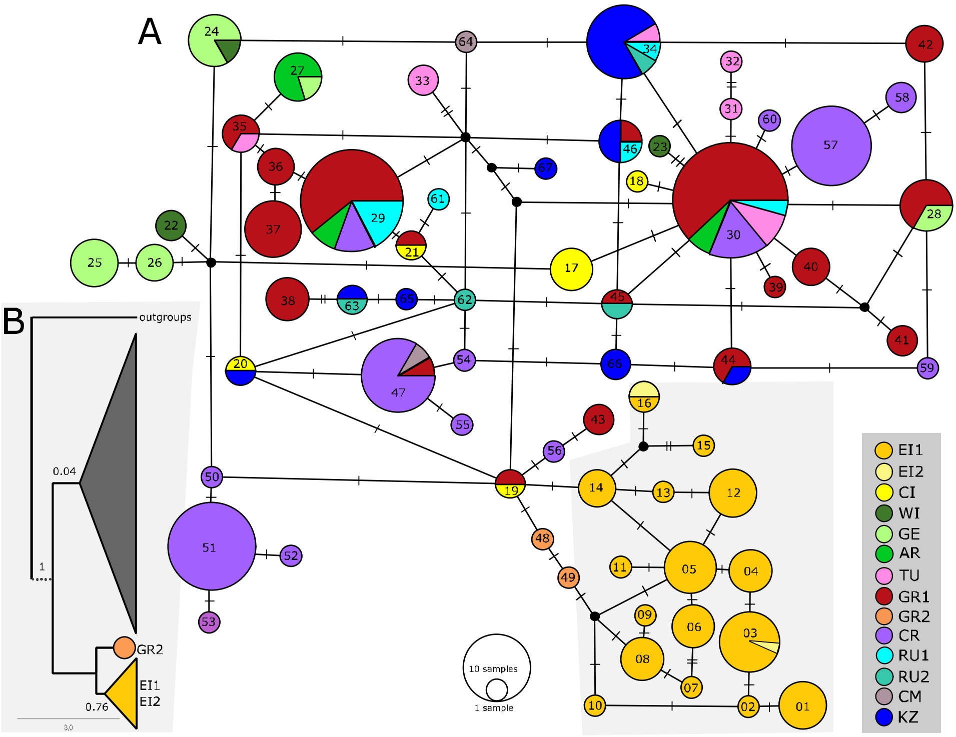

Figure 2.
Nuclear phylogeographic patterns of *Proterebia afra* in the Palaearctic steppes based on 510 bp of the arginine kinase gene. **A**, TCS haplotype networks. The gene was phased for the analysis. Mutations are shown as black dots and hatch marks. **B**, collapsed Bayesian tree computed in BEAST v.1.8.4 from sequences. Branch labels show posterior probabilities.

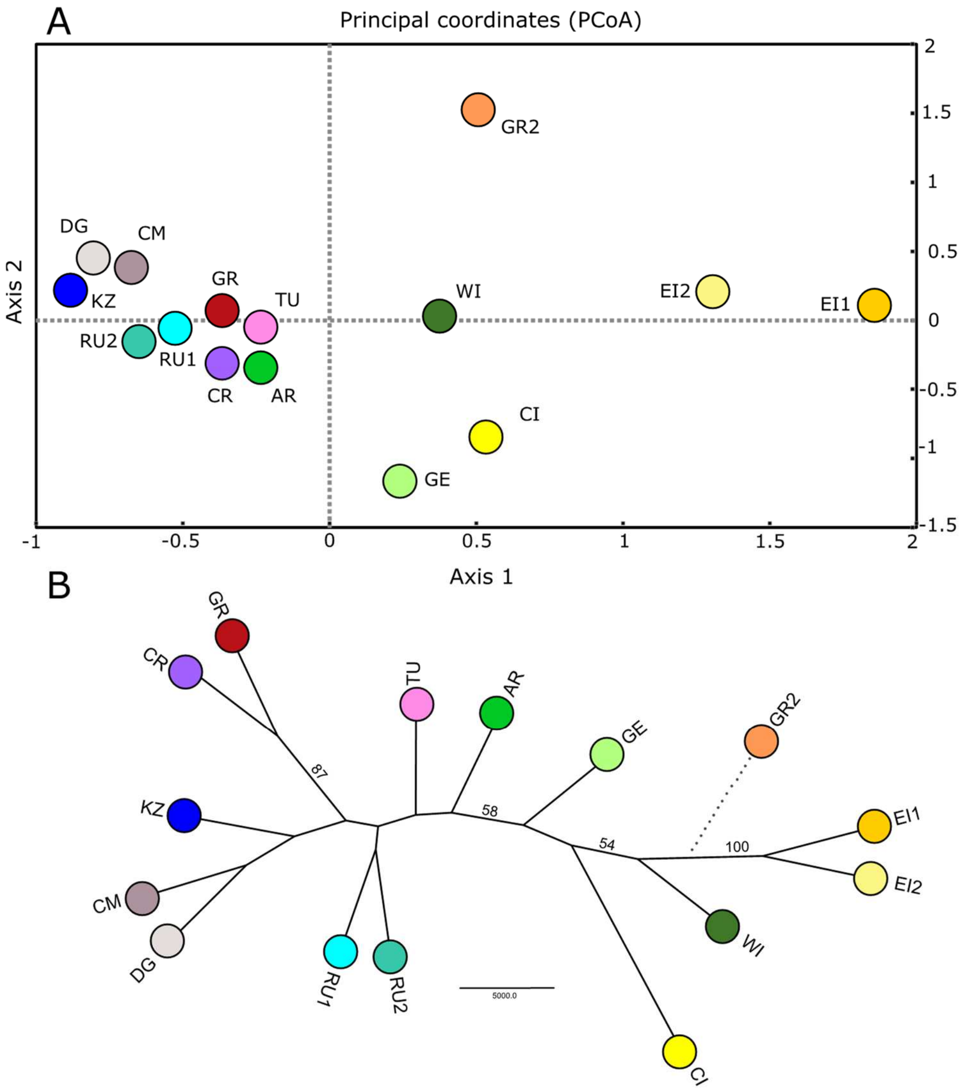

Figure 3.
Relationships among 15 *Proterebia afra* populations of the Palaearctic steppes revealed by amplified fragment length polymorphism (AFLP) markers. **A**, principal coordinates analysis (PCoA), axis 1 and 2. Explained variation of axes 1–3: 32, 19 and 13% of the total, respectively, with eigenvalues 8.853, 5.088 and 3.706. **B**, neighbour-joining tree, with 10000 bootstraps. Bootstrap supports > 50% are displayed.

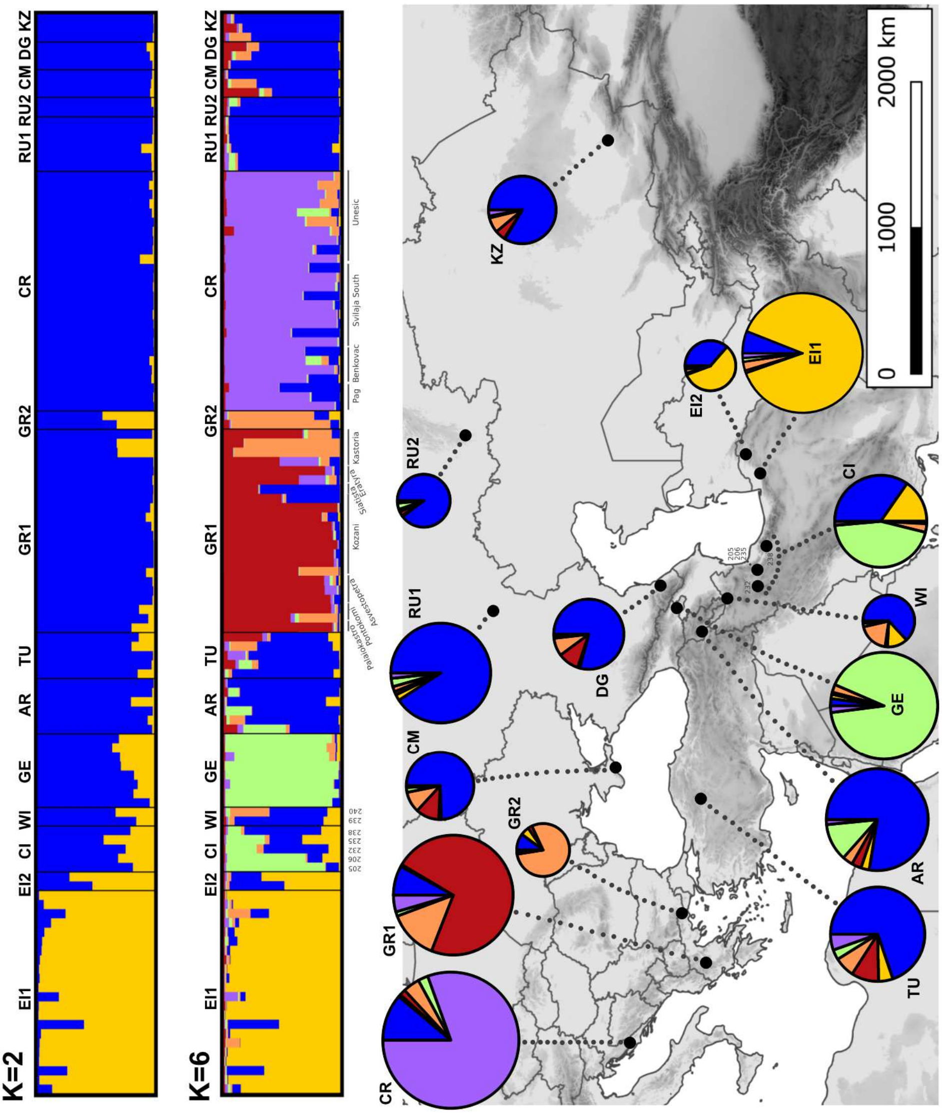

Figure 4.
ParallelStructure diagrams of 117 *Proterebia afra* samples of the Palaearctic steppes based on amplified fragment length polymorphism (AFLP) markers. The displayed $K = 2$ accords to best ΔK and $K = 6$ to the estimators MedMeaK and MaxMeaK of Puechmaille (2016), controlled for clusters that never achieve ancestry < 0.7 in any sample. The map shows the proportions of each of the six clusters in different populations.

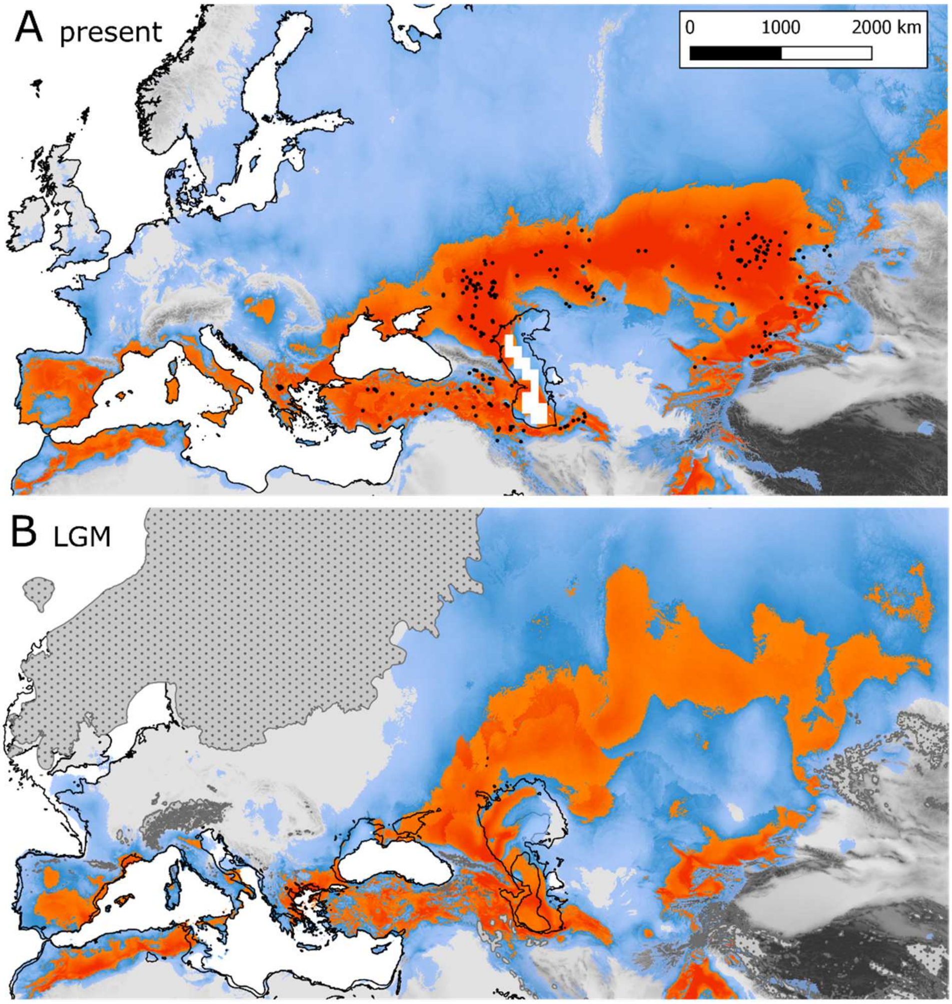

Figure 5.
Species distribution models showing the climatically suitable areas for *Proterebia afra* in the present conditions (A) and the Last Glacial Maximum (LGM; ~22000 years ago; B). MaxEnt computations are based on six BIOCLIM variables from the Community Climate System Model, Worldclim v.1.4, at 2.5 arc min spatial resolution. The blue colours show the suitability from 0.025 (the average minimal training presence threshold, including the least suitable training record) and red colours from 0.314 (the average ten percentile training presence threshold, suitability of 90% of the training data). The records used for model training are shown as black dots. The LGM glacial sheet (dotted) was adopted from Ehlers *et al.* (2011).

The present model corresponds to the present distribution, except for predicting wider occurrence within the Balkans and presence in western parts of the Mediterranean and in the Panonian basin, areas which the species apparently never colonized (Fig. 5). The model for the LGM showed a northward shift of the continuous Central Asiatic distribution belt, and isolation of the Tien Shan and Pamir Mountains foothills. In the south, the inhabited range has shifted downhill, but not southwards. In Dalmatia, the suitable habitats disappeared, but habitable patches remained in parts of the Adriatic basin currently below sea level. Even in the present climate, the connection of Dalmatia with the rest of the Balkans seems climatically limited.

Discussion

Based on mitochondrial, nuclear and genome-wide markers and species distribution modelling, we assessed the biogeographical patterns of the Palaearctic steppe butterfly *P. afra*. All the markers support an ancestral range stretching from the mountain steppes of north-eastern Iran to the southern Caucasus (Fig. 6). In this mountainous area, the most ancient genetic split occurred at the beginning of the Pliocene. A western lineage originating from the split expanded to Anatolia during the mid-Pliocene and then to the rest of the extant range. The colonization of the continental steppes, i.e. the largest part of the extant range, took place even later.

Given that the dates for the splits are based on calibration points much further in the past than the patterns discussed here, they should be taken with caution. Even if correct for the mutational events, time-delayed lineage sorting may attribute inappropriate dates to past events.

Origin in the western Asiatic mountains

Within the ancestral *P. afra* range in northern Iran, the genetically most distinct populations inhabit the eastern Alborz and Kopet Dag Mountains. The area of the Karakum and Kyzylkum deserts, separating these populations from the present distribution in Central Asia (Fig. 5), probably acted as a permanent barrier during the entire Plio-Pleistocene, because the area had been arid since the late Miocene and, additionally, Karakum was flooded during the Pliocene (cf. Graham *et al.*, 2012). However, in AFLP, the north-eastern Iranian samples in Kopet Dag show some admixture with the widespread cluster, suggesting limited and relatively recent gene flow from Central Asia, perhaps occurring along the foothills of the Central Asiatic mountains.

The mountains of the southern part of the range (i.e. Anatolia, Lesser Caucasus, Zagros, Alborz and Kopet Dag) harbour phylogenetically ancient populations showing high spatial differentiation and haplotype turnover, plus partial isolation in the AFLP markers. The mountainous area probably structured the populations, limiting their interconnection during entire glacial cycles. This is supported by the weaker correlations between geography and genetic distances for the lineage inhabiting northern lowlands than for the southern lineage inhabiting mostly mountains, found for two of the three markers.

In these mountains, the SDM supports downhill shifts of the climatic optima of the species during glacial periods and uphill shifts under the current climate. The southern Caucasus region, although acting as a long-term refugium for woody plants, was drier during glacials, harbouring mosaics of grasslands and woodlands (Denk *et al.*, 2001). The postglacial colonization of high altitudes (and herewith the frequent founder effect) might explain the impoverishment of the mountain population of Arailer Mountain (Armenia). In contrast, the geographically proximate lowland Kacheti population (Georgia) is diverse and distinctive in AFLP markers. A similar pattern of downhill shifts during glacials is well documented for cold-adapted grassland species from the Alps (e.g. Schönswetter *et al.*, 2005; Haubrich & Schmitt, 2007; Alvarez *et al.*, 2012); however, these shifts were mostly temperature driven, whereas humidity was the probable driver in *P. afra*.

The southern Balkan (Greek) populations might be derived directly from Anatolian ancestors at the Plio-Pleistocene transition, later becoming the source of the entire northern lineage, or the northern lineage is directly derived from Anatolia with a later connection with Greece. The latter scenario is the more likely one for the following reasons: (1) the internal structures in the *COI* gene are older in the northern lineage than in Greece

(Fig. 1C); and (2) a closer relatedness exists between the Anatolian and the northern lineage than with the Greek one, as revealed by ParallelStructure (Fig. 4).

Dynamics in Europe

Within Europe, many grassland butterflies display the pattern of postglacial expansion from southern refugia [e.g. *Melanargia galathea* (Linnaeus, 1758) (Habel *et al.*, 2011), *Polyommatus coridon* (Poda, 1761) (Kühne *et al.*, 2017) or the *Maniola* spp. Schrank, 1801 (Kreuzinger *et al.*, 2015)]. Phylogeographic differences between such butterflies and *P. afra* underline the difference between European semi-natural grasslands and the continental steppe elements. The wide distribution of steppe habitats and elements at the Balkan Peninsula during glacials is well recognized (Krystufek *et al.*, 2009; Athanassiou, 2012; Feoktistova *et al.*, 2017). Our SDMs recognized the northern Aegean and south-western Black Sea regions as climatically suitable during both the LGM and at present, allowing a long-term persistence of *P. afra* without postglacial expansion.

For the Dalmatian population, the SDMs indicated limited connection with other populations over time. Its origin, however, remains unresolved. The mtDNA suggests a mid- to late Pleistocene expansion from Anatolia, whereas AFLP points to a common origin with the southern Balkans. This discordance might result from introgression by a different lineage or from mitochondrial lineage replacement, or the two markers might mirror processes at different time scales, with AFLP showing more recent events than mtDNA (Toews & Brelsford, 2012). The mtDNA signal of the Dalmatian population suggests a recent demographic expansion. The SDM predicts that the habitable area dimished during the LGM (Fig. 5B), which was unlikely to benefit the population during glacial climates. However, the admixture of the widespread AFLP cluster within the Balkan samples indicates repeated contact with other populations.

Biogeography of the northern steppes

In the northern part of the range of *P. afra*, the populations between eastern Kazakhstan and the Volga region, including the isolated Dagestan and Crimea, form a group of closely related mtDNA haplotypes, some of them shared across distant areas. They also belong to a single AFLP cluster. This suggests a wide extant distribution without dispersal limitation and recent range expansion according to the neutrality tests. The Crimean population is impoverished and derived from south-western Russian samples (RU1) in mtDNA and contains some admixture with the two Greek clusters in AFLP. The widespread mitochondrial cluster might have been sub-structured between the east and the west, with Crimean and Dagestanian sub-refugia. The genetic poverty of Crimea does not necessarily result from a distinct glacial refugium but might alternatively be the product of a recent (perhaps postglacial) isolation.

Under the LGM climate, *P. afra* retained a contiguous distribution belt stretching from the Black Sea to the foothills of the Central Asiatic mountains. Suitable climatic conditions also persisted in Crimea, between the northern Caucasus foothills and the Caspian Sea coast and on the northern Tian Shan and Pamir foothills. Both genetic markers and the modelling thus agree that *P. afra* occurred, for the entire Quarternary, in a wide contiguous steppe belt, only avoiding northern and probably too cold latitudes and dry deserts, in a similar manner to other steppe species (Krystufek *et al.*, 2009; Zinenko *et al.*, 2015; Feoktistova *et al.*, 2017).

A widely distributed northern lineage was also observed in the common hamster, *Cricetus cricetus* Linneaus, 1758 (Feoktistova *et al.*, 2017). This rodent is a typical element of the mammoth steppe, and its glacial range also included the southern steppes (Anatolia, southern Caucasus), where it became extinct during the Holocene. The species complex of meadow and steppe vipers, *Vipera ursinii* (Bonaparte, 1835) and *Vipera renardi* (Christoph, 1861) (Zinenko *et al.*, 2015) splits into a lineage expanding from Anatolia to the Balkans, and a lineage crossing the Caucasus and expanding towards the Eurasian lowland steppes. The first fossils of *V. renardi* in the northern steppes are dated 0.8–0.9 and 0.5–0.6 Mya, in agreement with the first split within the northern *P. afra* lineage.

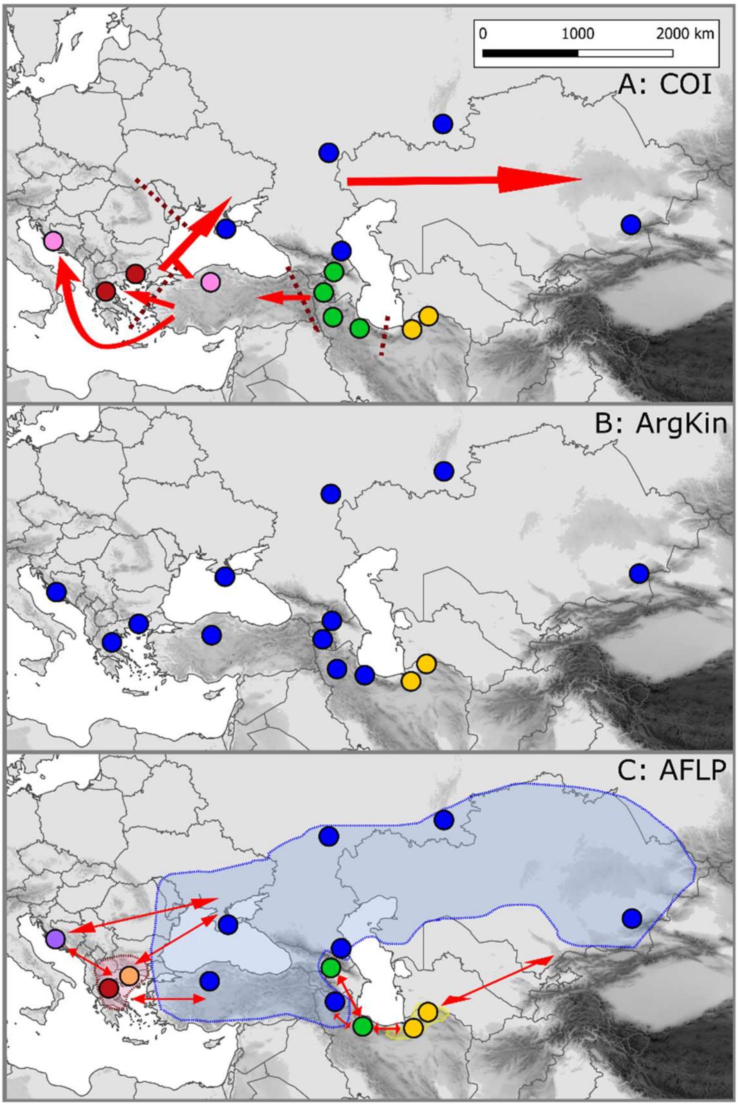

Figure 6.
Phylogeographic patterns of *Proterebia afra* displayed on distribution maps. **A**, five mitochondrial lineages (cytochrome *c* oxidase subunit I, *COI*) revealed by spatial analysis of molecular variance (SAMOVA) and major dispersals and vicariances based on the dispersal–extinction–cladogenesis (DEC) model of ancestral area estimation. **B**, two lineages revealed by nuclear arginin kinase gene (*ArgKin*). **C**, prevailing cluster in each of the populations based on structure analysis of amplified fragment length polymorphism (AFLP) markers and their major admixtures.

Conclusion

Contrary to the expectation proposed for steppe species by Stewart *et al.* (2010), *P. afra* does not display a longitudinal gradient of diminishing genetic diversity. A pattern of genetically distinct and internally uniform units, resulting from a long-term permanence in refugia and observed for some steppe species in western parts of their ranges (Kajtoch *et al.*, 2014, 2016), applies only to the westernmost (Balkan Peninsula) and the southern (Anatolia, south of the Greater Caucasus, northern Iran) parts of the distribution of *P. afra*. The butterfly also deviates from warm-adapted Mediterranean elements as follows: (1) it is associated with *Festuca ovina* and *Stipa*spp. grasslands (Bartonova *et al.*, 2017), which were widely distributed in more arid periods (Tarasov *et al.*, 2000); (2) its genetic structure resembles those of steppe animals for which fossil records exist (Zinenko *et al.*, 2015; Feoktistova *et al.*, 2017) and which were associated with the glacial mammoth steppe faunas (cf. Krystufek *et al.*, 2009); and (3) during the LGM, it retained contiguous distribution in the northern Asiatic steppes, without retreating to the Levant or North Africa (examples of retreating insects: Habel *et al.*, 2011; Tóth *et al.*, 2017).

It follows that *P. afra* displays a continuous, temporally rather stable distribution and uniform genetic structure across central Eurasian steppes, while forming differentiated relic populations in southern Asiatic mountains and arid parts of Mediterranean Europe. Based on this model, some of the patterns reported for steppe species with ranges structured in similar manner in studies restricted to Europe, such as strong differentiation of populations (Kajtoch *et al.*, 2016), might be artefacts of incomplete range sampling, or range margins phenomena (Sexton *et al.*, 2009).

So far, only very few Palaearctic steppe insects have been studied across their entire ranges, applying extensive sampling similar to ours. Nonetheless, a recent phylogegraphical study of *Melanargia russiae* (Esper, 1783), a species with a wide distribution in central Palaerctic steppes and insular distribution in southern Europe, retrieved western differentiation of lineages and undifferentiated eastern lineage (Dinca *et al.*, 2018). Even some non-steppe grassland butterflies appear to have extensive eastern lineage [e.g. *Melitaea cinxia* (Linnaeus, 1758)

(Wahlberg & Saccheri, 2007); *Melitaea ornata* Christoph, 1893 (Tóth *et al.*, 2017)]. It should be admitted that *P. afra* represents a phylogenetically isolated monotypic genus, whereas many insects occurring both in the Central Asiatic steppes and the Mediterranean, Anatolian and Central Asiatic mountains ('ponto-alpine' distribution) belong to species-rich genera; examples include *Agrodiaetus* Hübner, 1822 and *Pseudochazara* de Lesse, 1951 butterflies, or many Noctuidae moths (Varga, 1996).

Although the distribution of *P. afra* is large, on the continental scale, different populations vary in conservation priorities. The Greek and Dalmatian populations inhabit restricted areas deviating from their surroundings by their relatively arid continental climate and grassland maintenance by grazing (Bartonova *et al.*, 2017). It is possible that past human land use shaped the present distribution of *P. afra* in the Southern Balkans jointly with climatic conditions (cf. Slancarova *et al.*, 2016). This might also be the case for the western parts of the northern lineage (south of European Russia), where *P. afra* mainly inhabits steppe remnants on river valley slopes (cf. distribution maps of Lukhtanov & Lukhtanov, 1994; Tikhonov *et al.*, 2014). A historical extinction is reported from the surroundings of Odesa (Ukraine) (Kudrna *et al.*, 2015).

The Eurasian steppes have existed as a wide belt throughout the 'ice age' stages. Their extent and vegetation underwent major changes with the decline of the megafauna in the early Holocene (Lorenzen *et al.*, 2011), the subsequent activity of pastoral cultures (Warmuth *et al.*, 2012) and recent intensive farming in areas such as the northern Black Sea coast or the lowlands north of the Caucasus. For a steppe butterfly, the activity of grazing animals probably influenced the vegetation dynamics in the plains of Eastern Europe, perhaps buffering the effects of climate.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site

Figure S1. Kestimator (Puechmaille, 2016) evaluation of ParallelStructure runs on amplified fragment length polymorphism (AFLP) data of the Palaearctic steppe butterfly *Proterebia afra*. The results of the estimators ln $Pr(X|K)$, ΔK, posterior probability (PP) and the estimators MaxMeaK and MedMeaK (better evaluating the number of clusters under uneven sampling) are depicted, as are their corrections for spurious clusters with different thresholds.

Table S1. Samples of *Proterebia afra* used for its phylogeographic study within the Palaearctic steppes and GenBank accession codes of the samples.

Table S2. Results of spatial analysis of molecular variance (SAMOVA) analyses (in spads, with 5000 iterations, ten runs) for *Proterebia afra* populations over the Palaearctic steppes, for cytochrome *c* oxidase subunit I (*COI*; 15 populations; A) and arginine kinase gene (*ArgKin*; 14 populations; B). K, number of clusters; Φ_{CT}, fixation index.

Table S3. Pairwise Φ_{ST} values of 15 *Proterebia afra* populations within the Palaearctic steppes computed in Arlequin, for cytochrome *c* oxidase subunit I (*COI*; A) and phased arginine kinase gene (*ArgKin*; B); C, pairwise Φ_{PT} values for amplified fragment length polymorphism (AFLP) markers computed by genalex. Non-significant ($P > 0.05$) values are shaded.

Table S4. Binary amplified fragment length polymorphism (AFLP) matrix of 117 *Proterebia afra* samples from the Palaearctic steppes obtained with two selective primers, MseI-CGC with EcoRI-AAA (dye 6-FAM) and MseI-CCT with EcoRI-AGG (dye VIC).

Table S5. Comparisons of regularization multiplier (RM) parameter (A) and different feature classes (FC; B) of MaxEnt models by ENMeval R package using the Akaike information criterion corrected for small samples (AICc), used for calculations of spatial distribution models of the Palaearctic steppe butterfly *Proterebia afra* (the lowest AICc values indicate the best models). C, relative gain of the MaxEnt model after removing each of the variables. Only variables with positive gain > 0.01 were used

References

Alvarez N, Manel S, Schmitt T. 2012. Contrasting diffusion of Quaternary gene pools across Europe: the case of the arctic–alpine *Gentiana nivalis* L. (Gentianaceae). *Flora - Morphology, Distribution, Functional Ecology of Plants* 207: 408–413.

Álvarez-Lao DJ, García N. 2011. Geographical distribution of Pleistocene cold-adapted large mammal faunas in the Iberian Peninsula. *Quaternary International* 233: 159–170.

Athanassiou A. 2012. A skeleton of *Mammuthus trogontherii* (Proboscidea, Elephantidae) from NW Peloponnese, Greece. *Quaternary International* 255: 9–28.

Bartonova A, Kolar V, Maresova J, Sasic M, Slancarová J, Suchacek P, Konvicka M. 2017. Isolated Asian steppe element in the Balkans: habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities. *Journal of Insect Conservation* 21: 559–571.

Besnier F, Glover KA. 2013. ParallelStructure: a R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. *PLoS One* 8: e70651.

Center for International Earth Science Information Network (CIESIN) Columbia University & Information Technology Outreach Services (ITOS) University of Georgia. 2013. *Global roads open access data set, version 1 (gROADSv1)*. Palisades: NASA Socioeconomic Data and Applications Center (SEDAC). Available at: http://sedac.ciesin.columbia.edu/data/set/groads-global-roads-open-access-v1 (accessed 7 July 2018).

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.

Dellicour S, Mardulyn P. 2014. spads 1.0: a toolbox to perform spatial analyses on DNA sequence data sets. *Molecular Ecology Resources* 14: 647–651.

Denk T, Frotzler N, Davitashvili N. 2001. Vegetational patterns and distribution of relict taxa in humid temperate forests and wetlands of Georgia (Transcaucasia). *Biological Journal of the Linnean Society* 72: 287–332.

Dincă V, Bálint Z, Vodă R, Dapporto L, Hebert PDN, Vila R. 2018. Use of genetic, climatic, and microbiological data to inform reintroduction of a regionally extinct butterfly. *Conservation Biology* 32: 828–837.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.

Ehlers J, Gibbard PL, Hughes PD. 2011. *Quaternary glaciations - extent and chronology* , Vol. 15. Amsterdam: Elsevier.

Ehrich D. 2006. aflpdat: a collection ofr functions for convenient handling of AFLP data. *Molecular Ecology Notes* 6: 603–604.

Excoffier L, Laval G, Schneider S. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

Felsenstein J. 2005. *PHYLIP (phylogeny inference package) version 3.6*. Distributed by the author. Seattle: Department of Genome Sciences, University of Washington.

Feoktistova NY, Meschersky IG, Bogomolov PL, Sayan AS, Poplavskaya NS, Surov AV. 2017. Phylogeographic structure of the common hamster (*Cricetus cricetus* L.): late Pleistocene connections between Caucasus and Western European populations. *PLoS One* 12: e0187527.

Fortelius M, Eronen J, Liu L, Pushkina D, Tesakov A, Vislobokova I, Zhang Z. 2006. Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 238: 219–227.

Fourcade Y, Engler JO, Rödder D, Secondi J. 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PLoS One* 9: e97122.

Graham MR, Oláh-Hemmings V, Fet V. 2012. Phylogeography of co-distributed dune scorpions identifies the Amu Darya River as a long-standing component of Central Asian biogeography. *Zoology in the Middle East* 55: 95–110.

Habel JC, Lens L, Rödder D, Schmitt T. 2011. From Africa to Europe and back: refugia and range shifts cause high genetic differentiation in the Marbled White butterfly *Melanargia galathea*. *BMC Evolutionary Biology* 11: 215.

Haubrich K, Schmitt T. 2007. Cryptic differentiation in alpine-endemic, high-altitude butterflies reveals down-slope glacial refugia. *Molecular Ecology* 16: 3643–3658.

Hesselbarth G, Oorschot van H, Wagener S. 1995. *Die Tagfalter der Türkei unter Berücksichtigung der angrenzenden Länder*. Keltern: Goecke & Evers.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87–112.

Hijmans RJ. 2016. *Raster: geographic data analysis and modeling. R package version 2.5-8.* Available at: https://rdrr.io/cran/raster/ (accessed 1 February 2018).

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hijmans RJ, Phillips S, Leathwick J, Elith J. 2011. *Dismo: species distribution modeling . R package version 1.1-4.* Available at: https://rdrr.io/cran/dismo/ (accessed 3 March 2018).

Hoekstra JM, Boucher TM, Ricketts TH, Roberts C. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology Letters* 8: 23–29.

Jacobs BF, Kingston JD, Jacobs LL. 1999. The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Garden* 86: 590–643.

Kahlke RD. 2014. The origin of Eurasian mammoth faunas (*Mammuthus–Coelodonta* Faunal Complex). *Quaternary Science Reviews* 96: 32–49.

Kajtoch Ł, Cieślak E, Varga Z, Paul W, Mazur MA, Sramkó G, Kubisz D. 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. *Biodiversity and Conservation* 25: 2309–2339.

Kajtoch Ł, Kubisz D, Gutowski JM, Babik W. 2014. Evolutionary units of *Coraebus elatus* (Coleoptera: Buprestidae) in central and eastern Europe – implications for origin and conservation. *Insect Conservation and Diversity* 7: 41–54.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15: 1179–1191.

Korabek O, Petrusek A, Jurickova L. 2018. Glacial refugia and postglacial spread of an iconic large European land snail, *Helix pomatia* (Pulmonata: Helicidae). *Biological Journal of the Linnean Society* 123: 218–234.

Kramp K, Cizek O, Madeira PM, Ramos AA, Konvicka M, Castilho R, Schmitt T. 2016. Genetic implications of phylogeographical patterns in the conservation of the boreal wetland butterfly *Colias palaeno* (Pieridae). *Biological Journal of the Linnean Society* 119: 1068–1081.

Kreuzinger AJ, Fiedler K, Letsch H, Grill A. 2015. Tracing the radiation of Maniola (Nymphalidae) butterflies: new insights from phylogeography hint at one single incompletely differentiated species complex. *Ecology and Evolution* 5: 46–58.

Krystufek B, Bryja J, Buzan EV. 2009. Mitochondrial phylogeography of the European ground squirrel, *Spermophilus citellus*, yields evidence on refugia for steppic taxa in the Southern Balkans. *Heredity* 103: 129–135.

Kudrna O, Pennerstorfer J, Lux K. 2015. *Distribution atlas of European butterflies and skippers*. Schwanfeld: Wissenschaftlicher Verlag PEKS e.K.

Kühne G, Kosuch J, Hochkirch A, Schmitt T. 2017. Extra-mediterranean glacial refugia in a mediterranean faunal element: the phylogeography of the chalk-hill blue *Polyommatus coridon* (Lepidoptera, Lycaenidae). *Scientific Reports* 7: 43533.

de Lattin G. 1967. *Grundriss der Zoogeographie*. Stuttgart: G. Fischer.

Leigh JW, Bryant D. 2015. popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.

Liu Z, Pagani M, Zinniker D, Deconto R, Huber M, Brinkhuis H, Shah SR, Leckie RM, Pearson A. 2009. Global cooling during the Eocene-Oligocene climate transition. *Science* 323: 1187–1190.

Lorenzen ED, Nogués-Bravo D, Orlando L, Weinstock J, Binladen J, Marske KA, Ugan A, Borregaard MK, Gilbert MTP, Nielsen R, Ho SYW, Goebel T, Graf KE, Byers D, Stenderup JT, Rasmussen M, Campos PF, Leonard JA, Koepfli KP, Froese D, Zazula G, Stafford TW, Aaris-Sørensen K, Batra P, Haywood AM, Singarayer JS, Valdes PJ, Boeskorov G, Burns JA, Davydov SP, Haile J, Jenkins DL, Kosintsev P, Kuznetsova T, Lai X, Martin LD, McDonald HG, Mol D, Meldgaard M, Munch K, Stephan E, Sablin M, Sommer RS, Sipko T, Scott E, Suchard MA, Tikhonov A, Willerslev R, Wayne RK, Cooper A, Hofreiter M, Sher A, Shapiro B, Rahbek C, Willerslev E. 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature* 479: 359–364.

Lukhtanov V, Lukhtanov AG. 1994. *Die Tagfalter nordwestasiens: (Lepidoptera, Diurna)*. Marktleuthen: V. Eitschberger.

Magyari EK, Chapman JC, Gaydarska B, Marinova E, Deli T, Huntley JP, Allen JRM, Huntley B. 2008. The ‘oriental’ component of the Balkan flora: evidence of presence on the Thracian Plain during the Weichselian late-glacial. *Journal of Biogeography* 35: 865–883.

Mardulyn P, Mikhailov YE, Pasteels JM. 2009. Testing phylogeographic hypotheses in a Euro-Siberian cold-adapted leaf beetle with coalescent simulations. *Evolution* 63: 2717–2729.

Matzke NJ. 2012. Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis (DEC) analyses. *Frontiers of Biogeography* 4: 210.

Matzke N. 2013. *BioGeoBEARS: biogeography with Bayesian (and likelihood) evolutionary analysis in R scripts*. Berkeley: University of California.

Miller MA, Pfeiffer W, Schwartz T. 2010. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. 2010 Gateway Computing Environments Workshop (GCE), November 14; New Orleans, LA: 1–8.

Monteiro A, Pierce NE. 2001. Phylogeny of Bicyclus (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1α gene sequences. *Molecular Phylogenetics and Evolution* 18: 264–281.

Muscarella R, Galante PJ, Soley-Guardia M, Boria RA, Kass JM, Uriarte M., Anderson RP. 2014. ENMeval: an R package for conducting spatially independent evaluations and estimating optimal model complexity forMaxent ecological niche models. *Methods in Ecology and Evolution* 5: 1198–1205.

Nicholas WA, Chivas AR. 2014. Late Quaternary sea-level change on the Black Sea shelves. In: Chiocci FL, ChivasAR, eds. *Continental shelves of the world: their evolution during the last glacio-eustatic cycle*. Bath: Geological Society Publishing House, 199–212.

Panagiotopoulos K, Böhm A, Leng MJ, Wagner B, Schäbitz F. 2014. Climate variability over the last 92 ka in SW Balkans from analysis of sediments from lake Prespa. *Climate of the Past* 10: 643–660.

Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.

Peakall R, Smouse PE. 2006. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537–2539.

Pearce J, Ferrier S. 2000. An evaluation of alternative algorithms for fitting species distribution models using logistic regression. *Ecological Modelling* 128: 127–147.

Pena C, Nylin S, Wahlberg N. 2011. The radiation of satyrini butterflies (Nymphalidae: Satyrinae): a challenge for phylogenetic methods. *Zoological Journal of the Linnean Society* 161: 64–87.

Phillips SJ, Dudík M, Schapire RE. 2018. *Maxent software for modeling species niches and distributions (version 3.4.1)*. Available at: http://biodiversityinformatics.amnh.org/open_source/maxent/ (accessed 7 July 2018).

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.

Puechmaille SJ. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16: 608–627.

QGIS Development Team. 2018. *QGIS Geographic information system.* Open source geospatial foundation project. Available at: http://qgis.osgeo.org (accessed 5 March 2018).

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. *Tracer v1.6.* Available at: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 2 January 2018).

Ramstein G, Fluteau F, Besse J, Joussaume S. 1997. Effect of orogeny, plate motion and land–sea distribution on Eurasian climate change over the past 30 million years. *Nature* 386: 788–795.

Ribera I, Blasco-Zumeta J. 1998. Biogeographical links between steppe insects in the Monegros region (Aragon, NE Spain), the eastern Mediterranean, and central Asia. *Journal of Biogeography* 25: 969–986.

Ruddiman WF, Kutzbach JE. 1989. Forcing of late Cenozoic Northern hemisphere climate by plateau uplift in southern Asia and the American west. *Journal of Geophysical Research: Atmospheres* 94: 18409–18427.

Sandom CJ, Ejrnæs R, Hansen MDD, Svenning J-C. 2014. High herbivore density associated with vegetation diversity in interglacial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 111: 4162–4167.

Schmitt T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology* 4: 11.

Schmitt T, Hewitt GM. 2004. Molecular biogeography of the arctic-alpine disjunct burnet moth species *Zygaena exulans* (Zygaenidae, Lepidoptera) in the Pyrenees and Alps. *Journal of Biogeography* 31: 885–893.

Schmitt T, Varga Z. 2012. Extra-Mediterranean refugia: the rule and not the exception? *Frontiers in Zoology* 9: 22.

Schönswetter P, Stehlik I, Holderegger R, Tribsch A. 2005. Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology* 14: 3547–3555.

Schönswetter P, Tribsch A. 2005. Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon* 54: 725–732.

Seddon JM, Santucci F, Reeve NJ, Hewitt GM. 2001. DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. *Molecular Ecology* 10: 2187–2198.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ. 2009. Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics* 40: 415–436.

Slancarova J, Bartonova A, Zapletal M, Kotilinek M, Faltynek Fric Z, Micevski N, Kati V, Konvicka M. 2016. Life history traits reflect changes in Mediterranean butterfly communities due to forest encroachment. *PLoS One* 11: e0152026.

Smith S, Sandoval-Castellanos E, Lagerholm VK, Napierala H, Sablin M, Von Seth J, Fladerer FA, Germonpré M, Wojtal P, Miller R, Stewart JR, Dalén L. 2017. Nonreceding hare lines: genetic continuity since the Late Pleistocene in European mountain hares (Lepus timidus). *Biological Journal of the Linnean Society* 120: 891–908.

Stewart JR, Lister AM. 2001. Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology & Evolution* 16: 608–613.

Stewart JR, Lister AM, Barnes I, Dalén L. 2010. Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* 277: 661–671.

Tarasov PE, Volkova VS, Webb T, Guiot J, Andreev AA, Bezusko LG, Bezusko TV, Bykova GV, Dorofeyuk NI, Kvavadze EV, Osipova IM, Panova NK, Sevastyanov DV. 2000. Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from Northern Eurasia. *Journal of Biogeography* 27: 609–620.

Tikhonov VV, Stradomsky BV, Kuznetsov GV, Andreev SA. 2014. *Butterflies of the Caucasus and the South of Russia.* Available at: http://www.babochki-kavkaza.ru (accessed 10 January 2018).

Toews DP, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* 21: 3907–3930.

Török P, Ambarlı D, Kamp J, Wesche K, Dengler J. 2016. Step(pe) up! raising the profile of the Palaearctic natural grasslands. *Biodiversity and Conservation* 25: 2187–2195.

Tóth JP, Varga Z, Verovnik R, Wahlberg N, Váradi A, Bereczki J. 2017. Mito-nuclear discordance helps to reveal the phylogeographic patterns of *Melitaea* *ornata* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society* 121: 267–281.

Tshikolovets VV, Kosterin O, Gorbunov P, Yakovlev R. 2016. *The butterflies of Kazakhstan*. Kiev: Tshikolovets.

Tshikolovets VV, Naderi A, Eckweiler W. 2014. *The butterflies of Iran and Iraq*. Pardubice: Tshikolovets.

Tshikolovets VV, Yakovlev RV, Kosterin O. 2009. *The butterflies of Altai, Sayans and Tuva (South Siberia)*. Kiev: Tshikolovets.

Varga, Z. 1996. Biogeography and evolution of oreal Lepidoptera in the Palaearctic. *Acta Zoologica Academiae Scientiarum Hungaricae* 42: 289–330.

Vekemans X. 2002. *A program for genetic diversity analysis with AFLP (and RAPD) population data*. Brussels: Laboratoire de Génétique et Ecologie Végétal, Université Libre.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

Wahlberg N, Saccheri I. 2007. The effects of Pleistocene glaciations on the phylogeography of *Melitaea cinxia* (Lepidoptera: Nymphalidae). *European Journal of Entomology* 104: 675–684.

Wahlberg N, Wheat CW. 2008. Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of Lepidoptera. *Systematic Biology* 57: 231–242.

Warmuth V, Eriksson A, Bower MA, Barker G, Barrett E, Hanks BK, Li S, Lomitashvili D, Ochir-Goryaeva M, Sizonov GV, Soyonov V, Manica A. 2012. Reconstructing the origin and spread of horse domestication in the Eurasian steppe. *Proceedings of the National Academy of Sciences of the United States of America* 109: 8202–8206.

Wesche K, Ambarlı D, Kamp J, Török P, Treiber J, Dengler J. 2016. The Palaearctic steppe biome: a new synthesis. *Biodiversity and Conservation* 25: 2197–2231.

Yu Y, Harris AJ, Blair C, He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution* 87: 46–49.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292: 686–693.

Zinenko O, Stümpel N, Mazanaeva L, Bakiev A, Shiryaev K, Pavlov A, Kotenko T, Kukushkin O, Chikin Y, Duisebayeva T, Nilson G, Orlov NL, Tuniyev S, Ananjeva NB, Murphy RW, Joger U. 2015. Mitochondrial phylogeny shows multiple independent ecological transitions and northern dispersion despite of Pleistocene glaciations in meadow and steppe vipers (*Vipera ursinii* and *Vipera renardi*). *Molecular Phylogenetics and Evolution* 84: 85–100.

CHAPTER III.

Recently lost connectivity in the Western Palaearctic steppes: the case of a scarce specialist butterfly

Manuscript

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Recently lost connectivity in the Western Palaearctic steppes: the case of a scarce specialist butterfly

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Abstract

Palaearctic steppes evolved under the Cenozoic cold arid climate and megaherbivore pressure. A large portion of the biome persisted into the Holocene, but has recently been subjected to human land use alternation. *Pseudophilotes bavius* (Lepidoptera: Lycaenidae) is a steppe specialist sporadically distributed in Eastern Europe (from Transylvania to Southern Ural), the Balkans, the South-Eastern Mediterranean and Irano-Anatolian regions. We used samples covering the distribution area to assess the phylogeography and population genetics of the species based on five genes (cytochrome c oxidase subunit 1, arginine kinase, sarco/endoplasmic reticulum calcium ATPase gene, elongation factor 1 alpha, wingless). The basal lineages are situated in the Irano-Anatolian region. The species radiated to the Balkans and Eastern European steppes probably when the climatic conditions became suitable after the mid-Pleistocene transition and mammoth steppe establishment. In these parts of the range the species survived both glacial and interglacial periods and did not retreat to refugia. In the Balkans, the populations are distinct and have probably been isolated for a longer time, given the diversity of Balkan habitats. In Eastern Europe, the population connectivity had to exist until recently, since the species' life history is unlikely to allow long-distance dispersal. The populations could have become fragmented with the conversion of steppes into cropland during the past centuries. Three different *Wolbachia* alleles were discovered in *P. bavius*, but they do not seem to contribute to the mitochondrial patterns in the butterfly. Future conservation will require proper knowledge on its distribution and habitat needs. Management of inhabited sites should respect historical disturbance-succession dynamics.

Keywords

Pseudophilotes bavius, Rubrapterus, habitat fragmentation, phylogeography, conservation genetics, *Wolbachia*

Introduction

The continental climate, the aridity of the ice ages, the expansion of grasses, the mighty Pleistocene megafauna, and specific human life strategies, all formed and maintained the Palaearctic steppes (Guthrie 1989; Wesche et al. 2016). The biome originated at the beginning of the Oligocene (Bredenkamp et al. 2002) and its extent culminated with the establishment of the long, 100 thousand years' (ky), glacial cycles of the Middle and Late Pleistocene (after the mid-Pleistocene transition, about 780 thousand years ago (kya); Ruddiman et al., 1989; Lisiecki and Raymo, 2007) when it formed the mammoth steppe (Kahlke 2014). The megafauna likely maintained the grasslands even during interglacial periods (Zimov et al. 2012; Sandom et al. 2014). However, the Holocene extirpation of the large herbivores caused retreats of the open habitats and forest (re)establishment in the marginal areas, though never completely (Pokorný et al. 2015; Feurdean et al. 2018). During the previous century, farming has developed from small scale to a large-scale industrial enterprise, while nomadic pastoralism disappeared from many areas. Since often existing on fertile dark soils (chernozems), steppes were converted to arable land wherever possible in the Western Palaearctic (Török et al. 2016).

The wide steppe biome stretches southwards to the grasslands of the Southern Palaearctic. Apart from disjunct distribution in the Mediterranean area, these grasslands are widely present in Anatolia, Transcaucasia and the mountains of Northern Iran. These Irano-Anatolian grasslands are climatically similar to the more northerly situated Middle Asian steppes, together referred to as the Irano-Turanian region, and distinguished by enhanced continentality from the adjoining Mediterranean region (Djamali et al. 2012b; Wesche et al. 2016). The (forest) steppes are developed on mountain slopes with transition into semi-desert in the lowlands. Here too, the steppes dominated ice-age landscapes (Djamali et al. 2012a) and supported large herbivores (Albayrak and Lister 2012). Basal lineages of some widely distributed species have been discovered in the region (Zinenko et al. 2015; Bartonova et al. 2018; Lebedev et al. 2018). In Turkey, about 44% of pristine steppes have been converted into croplands (Ambarlı et al. 2016).

In disjunct patchy form, the steppes also occur in the Balkan Peninsula. The peninsula has a complex geomorphology and climate, and its interglacial vegetation consists of evergreen Mediterranean, temperate, alpine and continental elements (Polunin 1980). Such diversity survived the glacial periods in situ (Kryštufek et al. 2009; Panagiotopoulos et al. 2014; Papadopoulou et al. 2018), but in different ratios and spatial distribution than today, with a higher representation of *Artemisia* L. and Chenopodiaceae steppes (Elenga et al. 2000; Pross et al. 2015). The extent of glaciation was wide in the Balkans during glacial maxima (Hughes et al. 2011; Pavlopoulos et al. 2018), and both the temperate and evergreen vegetation retreated (Urban and Fuchs 2005; Pross et al. 2015). The Pleistocene mammalian fauna of the Balkans contained numerous steppe elements, both large (Athanassiou 2012) and small (Kryštufek et al. 2009). The expansion of the Anatolian and Pontic elements to the Balkans was facilitated during the glacials (Magyari et al. 2008). During the last century, many open habitats were lost due to successional development, linked with rural depopulation (Debussche et al. 1999; Sirami et al. 2010) and causing changes in the faunal compositions (Herrando et al. 2016; Slancarova et al. 2016).

Here we aim to shed more light on the patterns of Palearctic steppe species' history. We present a biogeographic study of a scarcely distributed Palaearctic steppe element, the Lycaenid butterfly *Pseudophilotes bavius* (Eversmann, 1832), potentially suffering from steppe alternation. Its distribution includes the Balkan Peninsula (including its southern, Mediterranean climate regions), Caucasus, and Irano-Anatolian regions, i.e. three of Myers' 25 biodiversity hotspots, where the remarkable diversity in a relatively small area is suffering from habitat loss (Myers et al. 2000). The species is listed in the EU Habitat Directive (Annexes II and IV of Council Directive 92/43/EEC). It is considered as "Least Concern" in the recent IUCN evaluation of Mediterranean butterflies' status (Numa 2016), but could be more endangered than this category suggests (van Swaay et al. 2012). Across the range, the populations are reportedly localised and isolated (ten Hagen 1996; Tshikolovets et al. 2014; Dincă V. 2011; Tikhonov et al. 2014).

Todisco et al. (2018), in a study focused on a congeneric species, *Pseudophilotes barbagiae* De Prins & Poorten, 1982, reported some diversity in the mitochondrial gene cytochrome c oxidase subunit 1 for *P. bavius* samples from the Balkans and Eastern Europe. Focusing here on *P. bavius*, we add substantially more samples from the southern and eastern parts of its range, presuming that these areas may play a significant role in the biogeographic history of the species. So far, only a few widely distributed Palaearctic steppe species have been studied across comprehensive parts of their ranges (Zinenko et al. 2015; Feoktistova et al. 2017; Bartonova et al. 2018; Lebedev et al. 2018). We used five genetic markers (cytochrome c oxidase subunit 1, arginine kinase, sarco/endoplasmic reticulum calcium ATPase gene, elongation factor 1 alpha, wingless), phylogenetic and population genetic metrics, and hypothesise that (1) the basal lineages of the species are situated in the Irano-Anatolian mountains, and either (2) the populations from the Balkans and Carpathians are relict and will consist of distinct genetic units (Kajtoch et al. 2016) or (3) the isolation of the Eastern European and Balkan populations could be relatively new (i.e. since the beginning of Holocene, or even later), and the structure will be rather shallow. We also inspected the presence of *Wolbachia*, the maternally transmitted intracellular bacteria, associated with selectively driven sweeps or paraphyly in mitochondrial DNA (Hurst and Jiggins 2005).

Materials and methods

Study species

The genus *Pseudophilotes* Beuret, 1958 is of Palaearctic origin and its representatives inhabit open grassy habitats, both lowland and alpine. *P. bavius* inhabits Irano-Turanian and Pontic areas, reaching the Balkans and inner Carpathian arch (Figure 1a, b). Specifically, it is present in Northern Iran, Turkey, Syria, Transcaucasia, Caucasus, Western Kazakhstan, Southern Urals, Saratov, the lower Volga and surroundings, Eastern Ukraine, Crimea, Odessa surroundings, Transylvania, Dobrogea in Eastern Romania, Treska valley in North Macedonia, Northern and South-Eastern Bulgaria, North-Western Greece and the Peloponnese (Hesselbarth et al.

1995; ten Hagen 1996; Pamperis 1997; Dincă V. 2011; Tikhonov et al. 2014; Tshikolovets et al. 2014; Belik 2016; Švara et al. 2016; Kolev 2017; Rákosy and Weidlich 2017). North African *Pseudophilotes fatma* (Oberthür, 1890) is frequently regarded as a subspecies of *P. bavius* (Tolman and Lewington 2008), but we will keep the names separate for the purposes of this study (cf. Numa, 2016; Todisco et al., 2018).

The larvae of *P. bavius* feed on flowers, less often on leaves and stems, of steppe and mountainous species of *Salvia* L. They are occasionally visited by ants (Crișan et al. 2011; Bury and Savchuk 2015). The pupa hibernates. Adults form one generation annually, from mid-April to June. A partial second generation occurs in the southern localities between July and September (Hesselbarth et al. 1995). A study of the Transylvanian population revealed that the dispersal of the species is limited because of its short adult life span (3.4 days) and low mobility (under 30 metres per day) (Crişan et al. 2014). The biotopes, as referred to in literature, include steppes, calcareous grounds, sheltered rocky slopes, dry river terraces. The species is potentially highly dependent on microhabitat (Tolman 1992) but its proper requirements have not been widely evaluated (cf. Német et al., 2016). In Askion, Greece, the adults chose partially sheltered places with some shrubs on steppe slopes (Figure 3c in Bartoňová et al., 2017).

Specimen sequencing

We sequenced 49 tissue samples of *Pseudophilotes bavius* and mined 26 samples (including *P. fatma*) from the BOLD (Ratnasingham and Hebert, 2007) and GenBank databases. Four samples overlapped. The total dataset consisted of 71 samples from 17 localities in 11 countries (Table 1, Supplementary Table 1).

DNA was extracted using the Genomic DNA Mini Kit – Tissue (Geneaid) following the manufacturer's protocols. Using Polymerase Chain Reactions (PCR), we amplified cytochrome c oxidase subunit 1 gene (*COI*) from the mitochondrial genome; and arginine kinase (*ArgKin*), elongation factor 1 alpha (*EF-1α*), sarco/endoplasmic reticulum calcium ATPase gene (*Ca-ATPase*), and *wingless* from the nuclear genome (primers: (Monteiro and Pierce, 2001; Wahlberg and Wheat, 2008;

Wahlberg et al., 2016). For *COI*, we used primer pairs LCO/HCO (*COI*-1st) and Jerry/Pat (*COI*-2nd); and LCO/K699, Ron/HCO and Tonya/Hobbes in case of fragmented DNA. For *EF-1α*, we used primer pairs Starsky/Monica (EF-1α-1st) and AlF/ EFrcM4 (EF-1α-2nd). All forward primers had T7promoter and reverse T3 universal tails attached. We prepared PCR in 12.5 µl volume (6.25 µl Bioline 2x MyTaq HS Red Mix, 4 µl PCR H2O, 0.625 + 0.625 µl primers; 1 µl DNA). The thermal cycling profile was 95 °C for 5 min; then 40 cycles of 94 °C for 30 s, 50 °C (*COI, wingless,* AIF/EFrcM4) / 55 °C (*ArgKin*, *Ca-ATPase,* Starsky/Monica) for 30 s, 72 °C for 90 s; and final extension 72 °C for 10 min. PCR products were cleaned with enzymes FastAP and ExoI (Thermofisher) and sequenced in one direction in Macrogen Inc. on ABI3730XL DNA analysers. Sequences were checked visually, aligned in Geneious v. 8.0.5 (Kearse et al., 2012) and organized in the VoSeq database (Peña and Malm, 2012).

Molecular analyses

We used TCS statistical parsimony algorithm (Clement et al., 2000) in program POPART (Leigh and Bryant, 2015) to construct the haplotype network from the first part of *COI* (the barcode; 67 samples), because of BOLD sequences inclusion, and from the longer *COI* (*COI*-long, including the barcode and *COI*-2nd), available for 45 samples. Statistics describing population diversity were inferred from the barcode in DNASP v. 5 (Librado and Rozas, 2009).

We investigated the level of differentiation among populations using pairwise Jost's (Jost, 2008) in R package 'mmod 1.3.2' (Winter, 2012), which averages the genetic differences between individual samples in population pairs. To inspect the mutual positions of individual samples, we computed Principal coordinate analysis (PCoA) in R package 'ape' (Paradis et al., 2004) from Kimura's two-parameters distances (K80) with pairwise deletion for missing data handling.

We further used the distance matrix to test for isolation by distance via Mantel test in R package 'vegan' (Oksanen et al., 2019), excluding *fatma.* To explore the distribution of variability, we computed analyses of molecular variance (AMOVA) in R package 'poppr 2.8.1' with *quasieuclid* correction, *farthest_neighbor* algorithm, and *mean* correction for missing data (Kamvar et al., 2014), excluding *fatma*.

Jost's D, PCoA, AMOVA and Mantel test were computed for the concatenated dataset, and individually for nuDNA and mtDNA. Moreover, PCoA and AMOVA were conducted for the concatenated dataset after exclusion of the most genetically distant samples (Armenia, Turkey, Iran, *fatma*). From analyses of the concatenated and nuclear datasets, the samples with less than two successfully amplified genes were excluded (Supplementary Table 1).

Bayesian trees were inferred in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003). MrBayes was run for 10 million MCMC generations, sampled every 5000 generation, with temperature = 0.2, four simultaneous chains, four independent runs. The first 10% of trees were discarded as burn-in. The convergence of the four runs was checked visually by the *sump* command according to the log likelihood and high effective sample sizes. The trees from the four runs were summarized under the 50% majority-rule consensus. The analyses were run for the concatenated, mitochondrial and nuclear datasets separately. We used samples of *Pseudophilotes vicrama* (Moore, 1865) and *P. baton* (Bergsträsser, 1779) as outgroups (Supplementary Table 1). Prior to MrBayes analyses, the best partition schemes and substitution models were evaluated by PartitionFinder 2.1.1 (Lanfear et al., 2017) based on the lowest BIC.

Wolbachia ***presence***

We checked the presence of *Wolbachia* in the *P. bavius* DNA samples by amplifying the *Wolbachia* surface protein gene (*wsp*) with the primer pair 81F and 691R (Braig et al., 1998) with universal tails, with the same protocols as for the host, increasing the amount of DNA in the PCR reaction to 3 µl, 55 °C annealing. We run 2 µl of the PCR product on 1.5% agarose gels. The positive samples were sequenced and the *wsp* strains were defined using the reference sequences of the Wolbachia MLST database (Baldo et al., 2006; https://pubmlst.org/wolbachia/).

Table 1. Origin of *Pseudophilotes bavius* samples used for its phylogeographic study and numbers of sequences obtained for each of the five genes, including sequences from databases, and *Wolbachia* screening. See Supplementary Table 1 for details.

	Country: specification	Population code	*COI*-1st/2nd	*ArgKin*	*Ca-ATPase*	*Wingless*	*EF-1α-1st/2nd*	*wsp* tested/positive	*wsp* allele
1	Kazakhstan	Aktolagay (KZ)	4/4	2	4	4	4/0	4/0	
2	Russia	Dagestan (RU)	1/1	0	1	0	0	1/1	294
3	Russia	Southern Ural (RU)	4/4	4	4	4	2/1	4/0	
4	Russia	Lower Volga (RU)	2/1	0	0	1	2/1	?	
5	Russia	Crimea	9/5	0	5	3	2/0	4/0	
6	Ukraine	Zaporizhia (UA)	2/2	0	2	0	1/0	2/0	
7	Ukraine	Odessa (UA)	2/2	2	2	2	2	2/0	
8	Romania	Dobrogea (RO)	3/2	2	2	2	2/3	2/0	
9	Romania	Transylvania (RO)	9/3	3	3	4	4/4	3/0	
10	Greece: Askion Mts.	Askion (GR)	15/12	12	12	11	9/11	12/12	61
11	Greece	Peloponnese (GR)	2/2	2	1	3	2/2	2/2	61
12	Bulgaria: Sakar Mts.	Sakar (BG)	3/3	3	3	3	3/1	3/3	61
13	North Macedonia: Treska valley	Treska (MK)	3/1	1	1	1	1	1/0	
14	Armenia: Vedi	Armenia	1/1	1	1	1	1/0	1/0	
15	Turkey: Mersin, Antalya, Ankara	Turkey	4/3	2	3	3	3/2	3/2	639
16	Iran: Qazvin, Mazandaran	Iran	3/2	2	3	1	2/1	2/0	
17	Morocco: Ifrane, *P. fatma*	Morocco - *fatma*	4/0	0	0	3	3/3	?	

Results

Our concatenated dataset contained 4155 base pairs: 1475 bp of *COI* (699 bp the barcode) and 2680 bp of the four nuclear genes (596 bp *ArgKin*, 444 bp *Ca-ATPase*, 1240 bp *EF1-alpha*, 400 bp *wingless*) (Supplementary Table 1, 2).

We identified 19 haplotypes in the barcode region of *COI* (607 bp after exclusion of sites containing some missing data; Figure 1C). The samples from the Balkans, Carpathians, Ukraine, Russia (including Dagestan) and Kazakhstan were closely related, with the majority of samples forming one frequent haplotype (H14). The samples from the southern parts of the range (Turkey, Iran, Armenia) were distinguished in two distinct lineages, with *P. fatma* related to Turkey and Armenia. Using the *COI*-long (1038 bp after missing data exclusion; Figure 1D), we again identified 19 haplotypes. The analysis further distinguished samples from Sakar (BG) by four mutations and from Zaporizhia by two mutations from the frequent haplotype Ha7, and connected the southern samples into one lineage (*fatma* samples were not available for this analysis).

The southern populations (*fatma*, Iran, Turkey) harbour the highest mitochondrial genetic diversity (Table 2). Furthermore, populations from Southern Ural, Crimea and Odessa were diverse. Romanian populations (Table 2) are also relatively diverse, which is apparent from the networks. Contrarily, the populations from Aktolagay, Zaporizhia, Askion, Peloponnese, Sakar and Treska were monomorphic.

Jost's D distinguished *fatma* and Iran from all other populations in all three datasets (Table 3, Supplementary Table 3). In the concatenated dataset, the differentiation of Dobrogea, Peloponnese, Sakar, Treska, Armenia and Turkey was higher. On the other hand, the differentiation among populations from Russia and Ukraine was rather low. PCoA of the concatenated dataset (Figure 2) distinguished *fatma* from *bavius* along the first axis and samples from Iran, Turkey and Armenia along the second axis. After exclusion of these samples, the first axis distinguished Sakar and Treska samples and the axis the second Peloponnese samples. PCoA for the mitochondrial and concatenated datasets were analogous (Supplementary Figure 1A). In the PCoA for the nuclear genes, the first axis again distinguished *fatma* from *bavius,* which formed a single cluster, with the Iranian and Treska samples on one margin of the second axis (Supplementary Figure 1B).

The Mantel test showed significant correlation between genetic and geographic distances for the concatenated dataset (correlation coefficient r= 0.225, p=0.002) but with a rather flat slope, indicating shallow isolation by distance (Supplementary Figure 2). The correlation was significant both for mtDNA (r= 0.147, p=0.047) and for nuDNA (r= 0.157, p=0.013). AMOVA for the concatenated dataset distributed 46.3% of the explained variability within populations and 53.7% among populations; after exclusion of distant samples, 59.4% and 40.6%, respectively. The variance was distributed in mtDNA as 30.7% vs 69.3%, and in nuDNA as 66.0% vs 34.0%.

The best substitution model was Tamura-Nei with invariable sites and gamma distribution (TRN+I+G) for the concatenated dataset, TRN+I+G for mitochondrial, and TRN+G for nuclear, all without partitioning. MrBayes, however, does not contain a TRN model, so the models were set as general time reversible (GTR) with proper rate variation models.

The Bayesian majority-rule consensus tree of the concatenated dataset (Figure 3) showed *fatma* as sister to *bavius*. The samples from Iran formed a basal lineage within *bavius*. Subsequently, the Turkish and Armenian samples separated. The samples from the remaining populations formed a single radiated lineage. Apart from the undistinguishable majority of the samples, a further seven monophyletic groups were recognized within this lineage: Zaporizhia; two samples from Askion; Peloponnese; Sakar; Treska + one Askion sample on the base; part of Transylvania; and Dobrogea + part of Southern Ural + one sample from Crimea. The mitochondrial tree (Supplemenary Figure 3A) resolved the two basal branches (Iran and Turkey + Armenia) as sister, but with a low posterior probability. It recognized five separated lineages within the widely distributed one (Zaporizhia; Peloponnese; Sakar; Treska; and part of Transylvania. The nuclear tree (Supplementary Figure 3B) revealed Iran as basal to the rest; the second split separated one Aktolagay sample. Within the widely distributed lineage, nuDNA recognized part of Aktolagay; one

Table 2. Descriptive population statistics in *Pseudophilotes bavius* and *P. fatma* barcode (669 bp of cytochrome c oxidase subunit 1 gene, 607 bp after exclusion of sites with missing data). Number of sequences (N), haplotypes and unique haplotypes are shown also for *COI*-long (1475 bp, 1038 bp after missing data exclusion). NE = not evaluated. Tajima's D was never significant.

	Number of sequences *COI*/*COI*-long	haplotypes *COI*/*COI*-long	unique haplotypes *COI*/*COI*-long	haplotype diversity (H_d)	polymorfic sites (S)	nucleotide diversity (π)	average number of nucleotide differences (k)	Tajima's D
All	**67/43**	**19/19**		**0.720**	**32**	**0.00633**	**3.84**	**-1.488**
Aktolagay (KZ)	4/4	1/1	0/0	0	0	0	0	NE
Dagestan (RU)	1/0	1/NE	1/NE					
Southern Ural (RU)	4/4	3/4	1/2	0.833	2	0.00160	1	-0.710
Lower Volga (RU)	NE	NE	NE	NE	NE	NE	NE	NE
Crimea	8/4	4/3	2/1	0.643	3	0.00122	0.75	-1.448
Zaporizhia (UA)	2/2	1/1	0/1	0	0	0	0	NE
Odessa (UA)	2/2	2/2	2/2	1	3	0.00478	3	NE
Dobrogea (RO)	3/2	2/2	1/1	0.667	2	0.00216	1.33	-1.489
Transylvania (RO)	9/3	2/2	1/1	0.556	2	0.00087	0.56	1.401
Askion (GR)	15/12	1/1	0/0	0	0	0	0	NE
Peloponnese (GR)	2/2	1/2	1/2	0	0	0	0	NE
Sakar (BG)	3/3	1/1	0/1	0	0	0	0	NE
Treska (MK)	3/1	1/1	1/1	0	0	0	0	NE
Armenia	1/1	1/1	0	NE	NE	NE	NE	NE
Turkey	4/3	3/3	2/2	0.833	4	0.00403	2.50	1.365
Iran	2/2	2/2	2/2	1	1	0.00159	1	NE
Morocco - *fatma*	4/0	2/NE	2/NE	0.667	4	0.00405	2.67	2.080

Table 3. Differentiation among populations of *Pseudophilotes bavius* and *P. fatma* (Jost's D, concatenated dataset of five genes).

	Aktolagay (KZ)	Dagestan (RU)	Southern Ural (RU)	Lower Volga (RU)	Crimea	Zaporizhia (UA)	Odessa (UA)	Dobrogea (RO)	Transylvania (RO)	Askion (GR)	Peloponnese (GR)	Sakar (BG)	Treska (MK)	Armenia	Turkey	Iran	Morocco - *fatma*
Aktolagay (KZ)	0																
Dagestan (RU)	0.0252	0															
Southern Ural (RU)	0.0199	0.0385	0														
Lower Volga (RU)	0.0461	0.0331	0.1237	0													
Crimea	0.0000	0.0246	0.0096	0.0214	0												
Zaporizhia (UA)	0.0371	0.0256	0.0412	0.0374	0.0333	0											
Odessa (UA)	0.0580	0.0335	0.0509	0.0577	0.0166	0.0427	0										
Dobrogea (RO)	0.0435	0.0579	0.0267	0.1262	0.0312	0.0826	0.0659	0									
Transylvania (RO)	0.0446	0.0208	0.0866	0.0330	0.0194	0.0324	0.0728	0.1110	0								
Askion (GR)	0.0353	0.0239	0.0666	0.0596	0.0095	0.0299	0.0385	0.0894	0.0526	0							
Peloponnese (GR)	0.0521	0.0732	0.1176	0.0561	0.0433	0.0764	0.0972	0.1546	0.0725	0.0553	0						
Sakar (BG)	0.1155	0.0488	0.1605	0.0600	0.0546	0.0911	0.0851	0.1692	0.1112	0.0820	0.1250	0					
Treska (MK)	0.1512	0.1220	0.1485	0.0773	0.1137	0.1176	0.1010	0.1222	0.1572	0.1179	0.1667	0.1448	0				
Armenia	0.1677	0.1707	0.1741	0.2136	0.1761	0.2388	0.2010	0.1909	0.1617	0.1742	0.1996	0.2226	0.2784	0			
Turkey	0.1957	0.1750	0.2224	0.2045	0.1694	0.2292	0.1735	0.2349	0.2067	0.1783	0.2174	0.1969	0.2384	0.0314	0		
Iran	0.2776	0.1803	0.3123	0.2611	0.2490	0.2605	0.2614	0.3176	0.2853	0.2612	0.2939	0.2596	0.2727	0.2072	0.2083	0	
Morocco - *fatma*	0.4004	0.4673	0.424	0.4388	0.4041	0.3846	0.4522	0.4328	0.4441	0.4493	0.4541	0.4364	0.4293	0.4022	0.4356	0.4657	0

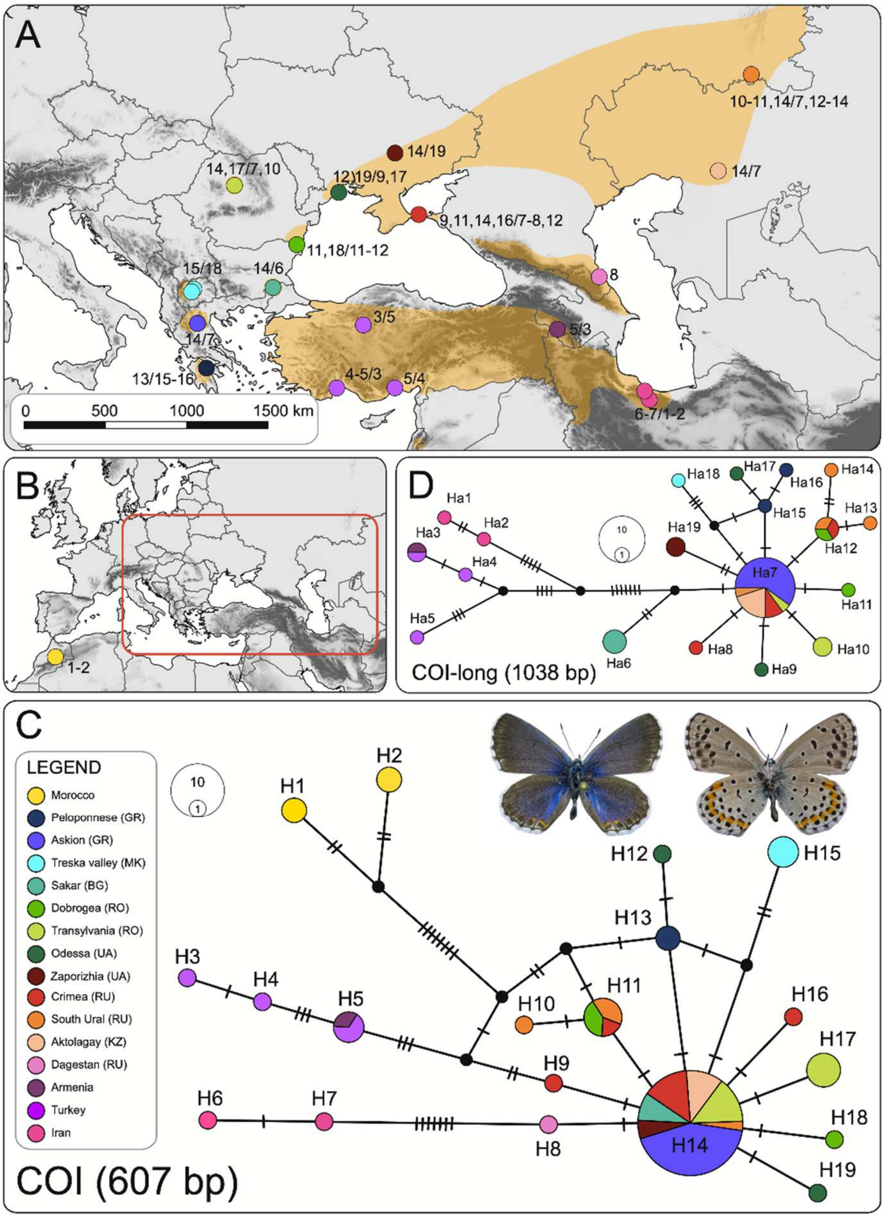

Figure 1. A. Distribution of the steppe butterfly *Pseudophilotes bavius* assumed from literature (orange shading). Points show origin of samples used in cytochrome c oxidase subunit 1 (*COI*) haplotype networks and numbers of haplotypes in *COI*/*COI*-long; **B.** Location of study area on the map of Europe (red rectangle) and locality of *P. fatma*; **C.** TCS Haplotype network of *COI* (barcode); **D.** TCS Haplotype network of *COI*-long.

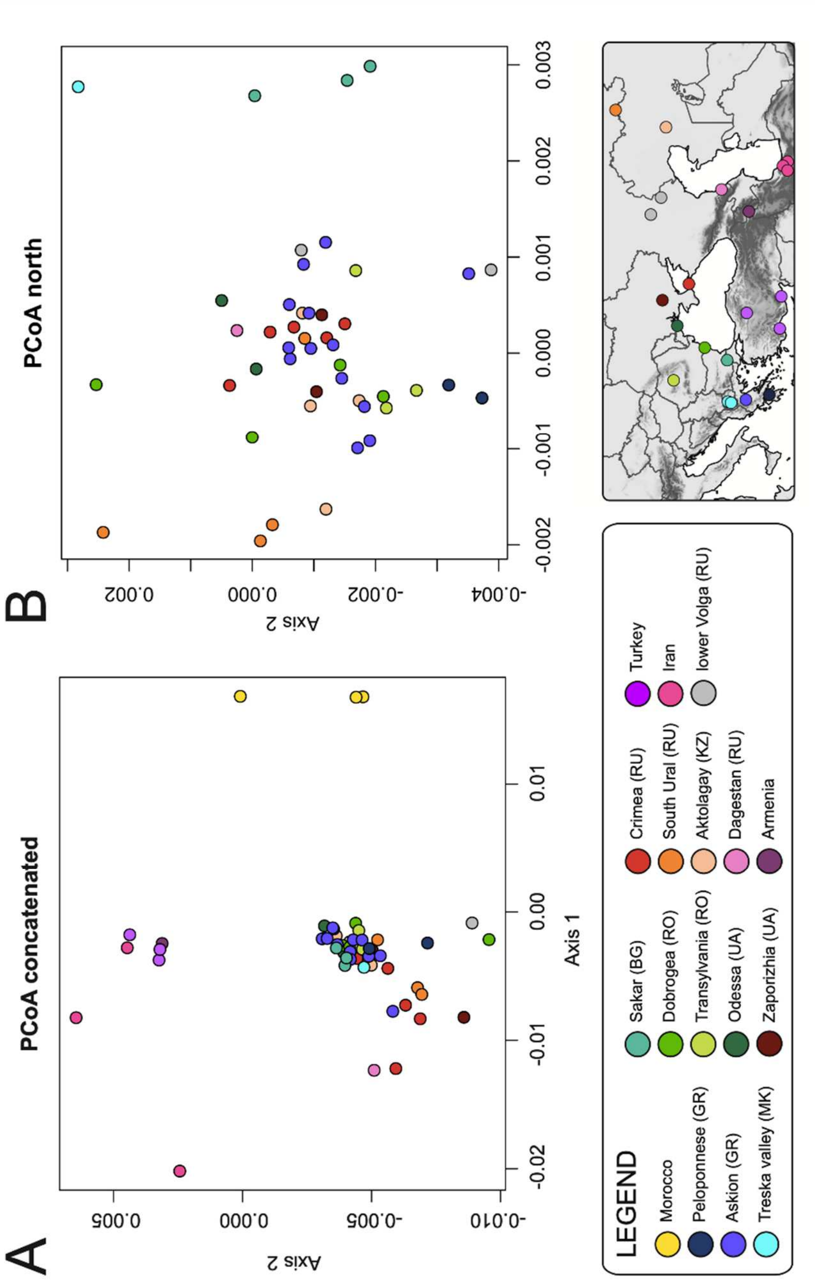

Figure 2. Analyses of Principal Coordinates (PCoA) in the steppe butterfly *Pseudophilotes bavius*. **A.** PCoA for concatenated dataset (5 genes); **B.** PCoA for concatenated dataset concatenated after exclusion of *fatma*, Iran, Turkey and Armenia.

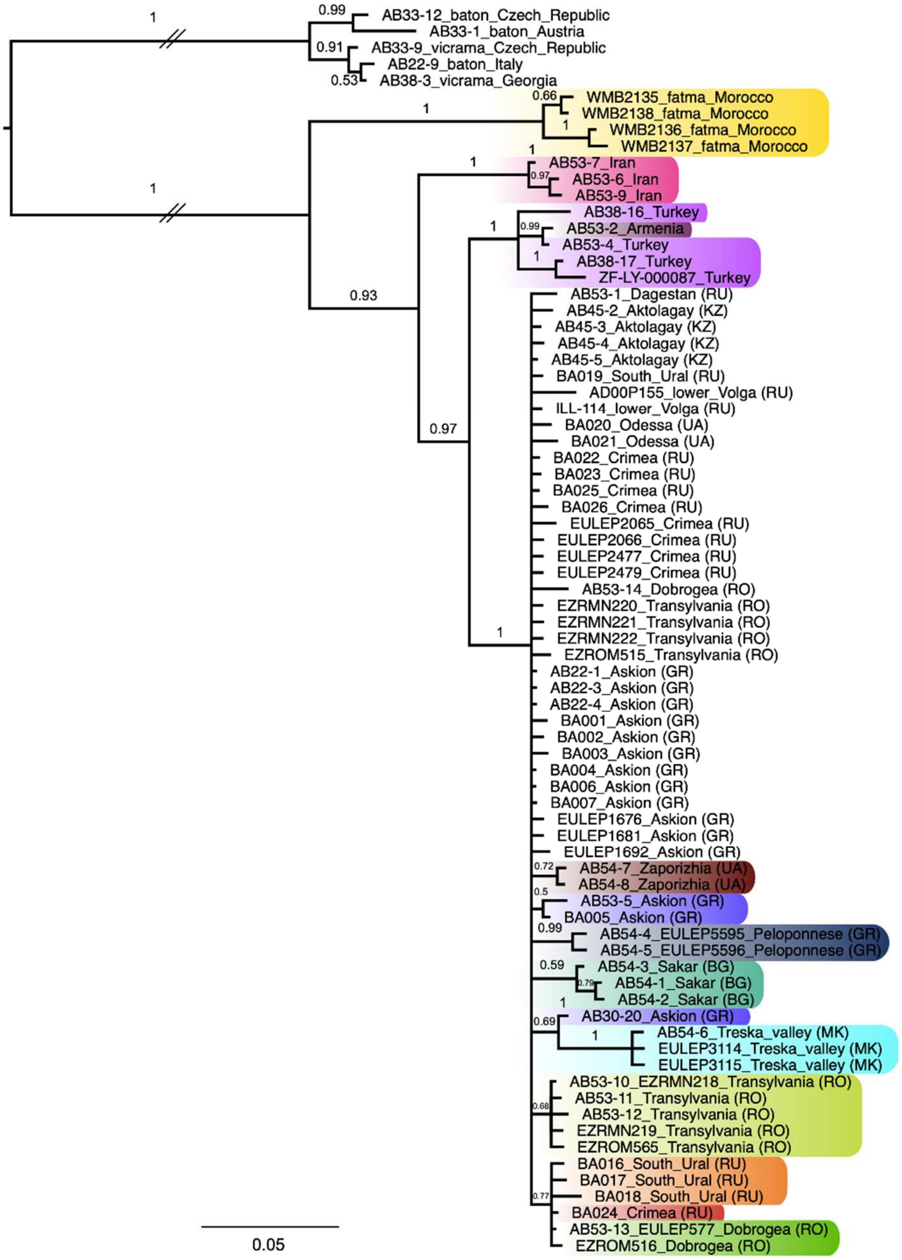

Figure 3. Bayesian Majority-rule consensus tree (MrBayes) of concatenated dataset (5 genes) in the steppe butterfly *Pseudophilotes bavius*. Branch labes show posterior probabilities.

sample from Askion + Treska; Peloponnese; two Askion samples + one from Southern Ural; one from the lower Volga + two Transylvanian; and Sakar.

Wolbachia ***presence***

From the 49 *Pseudophilotes bavius* samples tested for *Wolbachia* presence, 20 were positive. The *Wolbachia* MLST database revealed three different alleles within the species. Specifically, the Askion samples were all positive, the *Wolbachia* sequences matched *wsp* allele 61. The same allele is present in the Peloponnese (both tested samples positive) and in Sakar (all three positive). The two samples from Arslankoy, Turkey, were positive, and their *wsp* sequence matched allele 639 with one and two differences, respectively. The single Dagestan sample was positive, with *wsp* allele 294. All other tested samples were negative on gel (Supplementary Table 1).

Discussion

We assessed the phylogeographic and population history of a Palaearctic steppe Lycaenid butterfly *Pseudophilotes bavius*, including its relative *P. fatma* from North Africa.

In agreement with our predictions, the basal lineage of *P. bavius* is situated in Northern Iran, the Alborz Mountains near the Caspian Sea southern coast. The second split is between the samples from Turkey and Armenia and the remaining distribution area. These two basal lineages are sister in the faster gene, *COI*. Nuclear DNA distinguished only Iran and not Turkey with Armenia.

The "coherence" of the species is visible from PCoA for the nuclear dataset when compared to *P. fatma* (Supplementary Figure 1B). The study on diversification of the genus *Pseudophilotes* (Todisco et al., 2018), using the molecular clock calibrated by the mutation rate for arthropod mtDNA (Brower, 1994), placed the first split between the ancestor of *fatma*+*bavius* and the other representatives of the genus into the Messinian Period, when the Mediterranean sea was desiccated and subsequently refilled (Krijgsman et al., 1999). The split of *fatma* and *bavius* could be therefore younger, about 1.76 million years ago (Mya) (confidence interval: 3-0.7 My). The common ancestor of *P. bavius* and *P. fatma* could have lived along the southern Mediterranean coast. Whenever the African summer monsoon expanded northwards during the Plio/Pleistocene, savannah vegetation spread over the Sahara (Larrasoaña et al., 2003) and the Mediterranean evergreen belt alongside the African coast extended its width (Larrasoaña et al., 2013). Moreover, after the mid-Pleistocene transition, this climatic variability increased (Larrasoaña et al., 2003). The Syrian *P. bavius* (ten Hagen, 1996), not represented in our samples, could be an ancient lineage of such distribution.

There are more instances of the ancient lineages of steppe species situated in the Irano-Anatolian region. The steppe butterfly *Proterebia afra* (Fabricius, 1787), whose distribution range mainly corresponds to the range of *P. bavius*, has ancient lineages situated in the mountains of Northern Iran, Lesser Caucasus and Turkey (Bartonova et al., 2018). This area also harbours the basal lineages of vipers of the *Vipera renardi* (Christoph, 1861) complex (Zinenko et al., 2015), of the western mitochondrial lineage of the hamster *Cricetulus migratorius* (Pallas, 1773) (Lebedev et al., 2018), and of the steppe-Siberian vole *Microtus mystacinus* De Filippi, 1865 (Mahmoudi et al., 2017).

After the mid-Pleistocene transition to long 100 ky glacial cycles and water retention in ice, the Eurasian continent experienced increased aridification accompanied by the restructuring of mammalian faunas (Kahlke et al., 2011). Between 480 and 400 kya, the cold mammoth steppe established itself in Eurasia, inhabited by a combination of steppe and tundra elements (Kahlke, 2014). Under such conditions, *P. bavius* could have spread from the southern parts of its range, forming a widespread lineage reaching from the southern Balkans through the Carpathian basin to Southern Ural (Figure 3). Results of AMOVA showed that in this lineage the within population diversity is higher than the among population diversity.

The Greater Caucasus Mountains have formed a significant barrier. Our sample from Dagestan, i.e. the Caucasus northern slope, displays affinities to the widespread lineage, although connected to the Iranian samples in the haplotype network (Figure 1C).

The most frequently detected haplotype H14 (Ha7) is distributed in Aktolagay (Kazakhstan), Southern Ural (Russia), Crimea, Transylvania (Romania), and Askion (Greece) (but the latter is separated by one mutation in Todisco et al., 2018); the second widely located haplotype H11 (Ha12) is shared by Dobrogea (Romania), Southern Ural and Crimea (Figure 1C, d), indicating the connectivity of the populations. The populations in Eastern Europe (both Romanian, Odessa, Crimea) and Southern Ural thus contain both unique mitochondrial haplotypes as well as the widely distributed ones. The presence of unique haplotypes in almost all Eastern European populations points to *in situ* survival of the whole glacial cycles without retreat to refugia (Schmitt and Varga, 2012; Kajtoch et al., 2016).

The Transylvanian population is isolated by the Carpathian arch. The expansion of the species from the easterly located contiguous steppes (and not long-ago disconnected communication of the populations) could have been through the Iron Gates gorge on the river Danube. The Danube basin was arid and covered with steppes on loess soils during glacials, initially alternating with humid condition, and persisting also during the interglacials after the mid-Pleistocene transition, providing a stable migratory corridor (Fitzsimmons et al., 2012). A connection between a Pannonian basin population and populations east of the Carpathians was found for *Melanargia russiae* (Esper, 1783), another steppe butterfly with a widely distributed eastern genetic lineage (Dincă et al., 2018).

On the other hand, the populations in the Balkans are distinctive, rather uniform in mtDNA, forming unique haplotypes and distinctive also in nuclear DNA. This applies for the Peloponnese (Greece), Sakar (Bulgaria) and Treska (North Macedonia). The population from Askion (Greece) is intermediate: it is monomorphic in mtDNA with the widespread H14 (Ha7) and more diverse in nuDNA. This differentiation of populations probably mirrors the persisting vegetation and morphological diversity of the Balkan Peninsula, which could restrict dispersal of a steppe specialist if compared to the contiguous Eastern European steppes. The glacial maxima could have also limited this species dispersal, because of extensive mountain glaciations (Hughes et al., 2011; Pavlopoulos et al., 2018), and possibly restricted the species to partially sheltered gorges in the cold steppes. An analogous situation was observed in a steppe rodent, *Spermophilus citellus* (Linnaeus, 1766), which forms a radiated widespread lineage in the Danube region and on both western and eastern sides of the Carpathian arch, but multiple differentiated lineages in the Balkans (Říčanová et al., 2013), including a unique lineages in the Skopje surroundings in North Macedonia and on the Upper Thracian Plain.

Confronting the genetic patterns with the existing information on *P. bavius*'s life history, we recall that the butterfly has a narrow larval diet breadth, low mobility, small body size, low number of generations per year, short adult flight period, and an occasional tendency to occur in higher local densities (Tolman, 1992; Crişan et al., 2014; Tikhonov et al., 2014). Such a combination of traits is usually linked to narrow habitat specialisation in butterflies (Dapporto and Dennis, 2013; Bartonova et al., 2014). One trait distinguishing *P. bavius* from a habitat specialist profile is overwintering in the pupal stage, presumably allowing early season dispersal in habitat generalists (Boerschig et al., 2013; Bartonova et al., 2014). In *P. bavius*, overwintering in pupae may be an adaptation to the short humid spring followed by arid conditions in summer.

In terms of population genetics, specialist butterflies usually tend to form genetically differentiated but less diverse populations than generalists (Habel et al., 2013; Engler et al., 2014). Although the cited studies used much finer markers (allozymes, microsatellites) than we did, we detected the pattern of high differentiation and declined diversity in isolated populations inhabiting the Balkans. In contrast, the more northerly populations in Eastern Europe, despite including such isolated locations as Transylvania, display lower differentiation and higher diversity. A similar situation applied to a calcareous grassland specialist butterfly, *Polyommatus coridon* (Poda, 1761), in Germany where a low differentiation and a high genetic diversity in populations suggest a recently lost interconnection (Habel et al., 2015).

The history of the Eastern European steppes may explain the paradox of genetically little differentiated, although spatially highly isolated northern populations. The steppes of Eastern Europe retained high connectivity throughout most of the Holocene. The area was inhabited by nomadic peoples (e.g., the Kurgan cultures of the mid-Holocene, the Scythians and the Huns contemporary to European antiquity, the Pechenegs, Khazars or Cumans of the Middle Ages) (Gumilev 2008; Yunusbayev et al. 2015). It had been little cultivated due to its borderline position between clashing cultures (e.g., the Golden Horde and Crimean Khanate vs. Polish and Russian empires, or the Ottoman vs. Hungarian empires) (Sunderland 2004). The impacts of megafauna surviving until recently (e.g., the westernmost distribution of *Saiga tatarica* Linnaeus, 1766 in the 17–18th centuries were the Carpathian foothills; Sokolov and Zhirnov, 1998) combined with lifestock herding by nomads. The current pattern of land cultivation (e.g., up to 95% of original grassland area ploughed in Ukraine), likely isolating *P. bavius* habitats, is therefore very recent (Wesche et al. 2016). In South-Eastern Ukraine, many small localised *P. bavius* populations have already been lost (see distribution map in Akimov 2009), which gave the species its "vulnerable" status in the national Red Book. Interestingly, the Kurgan barrows of the ancient people in Ukraine act as a current refugia for steppe biota (Deák et al. 2016). In Ukrainian steppe plants, severe fragmentation but still high genetic diversity is documented (Hirsch et al. 2015; Dembicz et al. 2018). Contrarily, there are new records of *P. bavius* from the northernmost parts of the its range: the Saratov region (Belik 2016) and Bashkiria, where it was observed after 150 years' absence (Chelyabinsk Scientific and Educational Centre, 2016, http://www.kremlinrus.ru/news/143/53734/), accessed 27 March 2019). This could be connected to cropland abandonment following the collapse of the USSR, favouring returns of some species (cf. Kamp et al. 2018).

The populations in Askion, the Peloponnese, and Sakar share the same *Wolbachia* allele. *Wolbachia* is often associated with reduction of mtDNA diversity through its fast spread into a population, and could cause discordant patterns between mitochondrial and nuclear DNA (Hurst and Jiggins, 2005; Kodandaramaiah et al., 2013). However, *Wolbachia* is not always the cause of reduced mitochondrial diversity (cf. Bereczki et al., 2015). The *Wolbachia* positive populations of *P. bavius* from the Peloponnese and Sakar are distinctive also in nuDNA. The Askion population is not distinguishable from the Eastern European populations, but its mitochondrial impoverishment and nuclear diversity could be caused by intermediately long isolation, with the faster mitochondrial gene already losing variability. The presence of the same *Wolbachia* allele could although indicate that these Balkan populations are related to each other (cf. Tóth et al., 2017). The only tested sample from Treska was negative, but it may be derived from Askion in nuDNA (Figure 3, Supplementary Figure 3). Two more *Wolbachia* alleles were revealed in *P. bavius* – allele 294 in Dagestan and allele 639 in Arslankoy, Turkey.

Conservation implications

The genetic structure of *Pseudophilotes bavius*, a specialist steppe butterfly with presumably limited dispersal, mirrors the history of Western Palaearctic steppes. The basal lineages are situated in the Irano-Anatolian region and, when the climatic situation allowed, radiated into the Balkans and north-western steppes, where it survived both glacial and interglacial periods without retreating to refugia. In the Balkans, the surviving populations are distinct and have probably been isolated for a longer time. In Eastern Europe, the populations were likely interconnected until relatively recently, and have become fragmented with the conversion of steppes into arable land in the last centuries.

The isolated populations of *P. bavius* in Eastern Europe thus represent a case of a species with relatively recent isolation due to habitat loss over large scales. As reports from this part of the range agree that the distribution is extremely patchy, and the region is renowned for fertile soils, prospects of restoring connectivity among populations seem unrealistic. In such case, maximum efforts should be paid to conserving the occupied habitat patches, by providing appropriate habitat management if necessary. Regrettably, the knowledge of distribution extent remains fragmentary, as illustrated by the case of South-Eastern Bulgaria, where it was recorded in 2017 for the first time (Kolev, 2017). The same applies for precise habitat requirements, which likely vary in various parts of the range (e.g., lowland grassland in the Volga region, mountain gorges in Dagestan; cf. Tikhonov et al., 2014), and for land use patterns maintaining the habitats. The first step for efficient conservation thus should cover improved distribution mapping and habitat conditions description. In any case, future conservation management of inhabited sites should respect historical disturbance-succession dynamics, likely provided by historical land uses such as pastoralism, or such ecosystem engineers as large ungulates. Much of the biodiversity of the Western Palearctic steppes has already been lost, and the conservation community is committed to avoid further damage.

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Supplementary files

Supplementary files of this manuscript are deposited at https://docs.google.com/spreadsheets/d/1ONLv7auU2XYOlah8KhvCmTTZ4VuHNt9ibK7uB84ppgY/edit?usp=sharing

Supplementary Table 1. Samples of the steppe butterfly *Pseudophilotes bavius*, *P. fatma* and outgroups used in the study on *P. bavius* phylogeography, including information on sampling localities, voucher specimen location, genes successfully sequenced, GenBank codes, samples used in different analyses.

Supplementary Table 2. Alignment of the concatenated dataset of the steppe butterfly *Pseudophilotes bavius* in NEXUS format.

Supplementary Table 3. Jost's D differentiations among populations of the steppe butterfly *Pseudophilotes bavius*, with heatmap: **A.** concatenated dataset (5 genes); **B.** mtDNA; **C.** nuDNA.

Supplementary Figure 1. Analyses of Principal Coordinates (PCoA) in samples of the steppe butterfly *Pseudophilotes bavius*: **A.** mitochondrial DNA; **B.** nuclear DNA.

Supplementary Figure 2. Mantel test graph for concatenated dataset (5 genes) in the steppe butterfly *Pseudophilotes bavius*.

Supplementary Figure 3. Bayesian Majority-rule consensus tree (MrBayes) of **A.** mitochondrial DNA (*COI*, 1475 bp); **B.** nuclear DNA (4 genes, 2680 bp) in the steppe butterfly *Pseudophilotes bavius*. Branch labes show posterior probabilities.

References

Akimov, I.A., 2009. Chervona knyha Ukrayiny. Tvarynnyy svit [Red Book of Ukraine. Animals]. Hlobalkonsaltynh, Kyiv.

Albayrak, E., Lister, A.M., 2012. Dental remains of fossil elephants from Turkey. Quaternary International 276–277, 198–211.

Ambarlı, D., Zeydanlı, U.S., Balkız, Ö., Aslan, S., Karaçetin, E., Sözen, M., Ilgaz, Ç., Gürsoy Ergen, A., Lise, Y., Demirbaş Çağlayan, S., Welch, H.J., Welch, G., Turak, A.S., Bilgin, C.C., Özkil, A., Vural, M., 2016. An overview of biodiversity and conservation status of steppes of the Anatolian Biogeographical Region. Biodiversity and Conservation 25, 2491–2519.

Athanassiou, A., 2012. A skeleton of *Mammuthus trogontherii* (Proboscidea, Elephantidae) from NW Peloponnese, Greece. Quaternary International 255, 9–28.

Baldo, L., Dunning Hotopp, J.C., Jolley, K.A., Bordenstein, S.R., Biber, S.A., Choudhury, R.R., Hayashi, C., Maiden, M.C.J., Tettelin, H., Werren, J.H., 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Applied and Environmental Microbiology 72, 7098–7110.

Bartonova, A., Benes, J., Konvicka, M., 2014. Generalist-specialist continuum and life history traits of Central European butterflies (Lepidoptera) - are we missing a part of the picture? Eur. J. Entomol. 111, 543–553.

Bartoňová, A., Kolář, V., Marešová, J., Šašić, M., Šlancarová, J., Sucháček, P., Konvička, M., 2017. Isolated Asian steppe element in the Balkans: habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities. Journal of Insect Conservation 1–13.

Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867–884.

Belik, A.G., 2016. *Pseudophilotes bavius* (Eversmann, 1832) в фауне Саратовской области (Lepidoptera: Lycaenidae) [*Pseudophilotes bavius* (Eversmann, 1832) in the fauna of Saratov Province (Lepidoptera: Lycaenidae)]. Eversmannia 47–48, 105.

Bereczki, J., Rácz, R., Varga, Z., Tóth, J.P., 2015. Controversial patterns of *Wolbachia* infestation in the social parasitic *Maculinea* butterflies (Lepidoptera: Lycaenidae). Organisms Diversity & Evolution 15, 591–607.

Boerschig, C., Klein, A.-M., von Wehrden, H., Krauss, J., 2013. Traits of butterfly communities change from specialist to generalist characteristics with increasing land-use intensity. Basic and Applied Ecology 14, 547–554.

Braig, H.R., Zhou, W., Dobson, S.L., O'Neill, S.L., 1998. Cloning and Characterization of a gene encoding the Major Surface Protein of the bacterial endosymbiont *Wolbachia pipientis*. Journal of Bacteriology 180, 2373–2378.

Bredenkamp, G.J., Spada, F., Kazmierczak, E., 2002. On the origin of northern and southern hemisphere grasslands. Plant Ecology 163, 209–229.

Brower, A.V., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences of the United States of America 91, 6491–6495.

Bury, J., Savchuk, V., 2015. New data on the biology of ten lycaenid butterflies (Lepidoptera: Lycaenidae) of the genera *Tomares* Rambur, 1840, *Pseudophilotes* Beuret, 1958, *Polyommatus* Latreille, 1804, and *Plebejus* Kluk, 1780 from the Crimea and their attending ants (Hymenoptera: Formicidae). Acta entomologica silesiana 23, 1–16.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9, 1657–1659.

Crişan, A., Sitar, C., Craioveanu, C., Rákosy, L., 2011. The Protected Transylvanian Blue (*Pseudophilotes bavius hungarica*): new information on the morphology and biology. Nota Lepidopterologica 34, 163–168.

Crişan, A., Sitar, C., Craioveanu, M.C., Vizauer, T.-C., Rákosy, L., 2014. Multiannual population size estimates and mobility of the endemic *Pseudophilotes bavius hungarica* (Lepidoptera: Lycaenidae) from Transylvania (Romania). North-Western Journal of Zoology 10 (Supplement 1), S115–S124.

Dapporto, L., Dennis, R.L.H., 2013. The generalist-specialist continuum: Testing predictions for distribution and trends in British butterflies. Biological Conservation 157, 229–236.

Deák, B., Tóthmérész, B., Valkó, O., Sudnik-Wójcikowska, B., Moysiyenko, I.I., Bragina, T.M., Apostolova, I., Dembicz, I., Bykov, N.I., Török, P., 2016. Cultural monuments and nature conservation: a review of the role of kurgans in the conservation and restoration of steppe vegetation. Biodiversity and Conservation 25, 2473–2490.

Debussche, M., Lepart, J., Dervieux, A., 1999. Mediterranean landscape changes: evidence from old postcards. Global Ecology and Biogeography 8, 3–15.

Dembicz, I., Szczeparska, L., Moysiyenko, I.I., Wódkiewicz, M., 2018. High genetic diversity in fragmented Iris pumila L. populations in Ukrainian steppe enclaves. Basic and Applied Ecology, Plant population biology in a changing world 28, 37–47.

Dincă, V., Bálint, Z., Vodă, R., Dapporto, L., Hebert, P.D.N., Vila, R., 2018. Use of genetic, climatic, and microbiological data to inform reintroduction of a regionally extinct butterfly. Conservation Biology 32, 828–837.

Dincă V., C.S., 2011. Distribution and conservation status of *Pseudophilotes bavius* (Eversmann, 1832) (Lepidoptera: Lycaenidae) in Dobrogea (south-eastern Romania). Phegea 39, 59–67.

Djamali, M., Baumel, A., Brewer, S., Jackson, S.T., Kadereit, J.W., López-Vinyallonga, S., Mehregan, I., Shabanian, E., Simakova, A., 2012a. Ecological implications of *Cousinia* Cass. (Asteraceae) persistence through the last two glacial–interglacial cycles in the continental Middle East for the Irano-Turanian flora. Review of Palaeobotany and Palynology 172, 10–20.

Djamali, M., Brewer, S., Breckle, S.W., Jackson, S.T., 2012b. Climatic determinism in phytogeographic regionalization: A test from the Irano-Turanian region, SW and Central Asia. Flora - Morphology, Distribution, Functional Ecology of Plants 207, 237–249.

Elenga, H., Peyron, O., Bonnefille, R., Jolly, D., Cheddadi, R., Guiot, J., Andrieu, V., Bottema, S., Buchet, G., Beaulieu, J.-L.D., Hamilton, A.C., Maley, J., Marchant, R., Perez-Obiol, R., Reille, M., Riollet, G., Scott, L., Straka, H., Taylor, D., Campo, E.V., Vincens, A., Laarif, F., Jonson, H., 2000. Pollen-based biome reconstruction for southern Europe and Africa 18,000 yr bp. Journal of Biogeography 27, 621–634.

Engler, J.O., Balkenhol, N., Filz, K.J., Habel, J.C., Rödder, D., 2014. Comparative landscape genetics of three closely related sympatric Hesperid butterflies with diverging ecological traits. PLoS ONE 9, e106526.

Feoktistova, N.Y., Meschersky, I.G., Bogomolov, P.L., Sayan, A.S., Poplavskaya, N.S., Surov, A.V., 2017. Phylogeographic structure of the Common hamster (*Cricetus cricetus* L.): Late Pleistocene connections between Caucasus and Western European populations. PLoS ONE 12, e0187527.

Feurdean, A., Ruprecht, E., Molnár, Z., Hutchinson, S.M., Hickler, T., 2018. Biodiversity-rich European grasslands: Ancient, forgotten ecosystems. Biological Conservation 228, 224–232.

Fitzsimmons, K.E., Marković, S.B., Hambach, U., 2012. Pleistocene environmental dynamics recorded in the loess of the middle and lower Danube basin. Quaternary Science Reviews 41, 104–118.

Gumilev, L.N., 2008. Древняя Русь и Великая степь [Ancient Rus and Great Steppe]. Ayris-press, Moscow.

Guthrie, R.D., 1989. Frozen Fauna of the Mammoth Steppe: The Story of Blue Babe, 1 edition. ed. University of Chicago Press, Chicago.

Habel, J.C., Brückmann, S.V., Krauss, J., Schwarzer, J., Weig, A., Husemann, M., Steffan-Dewenter, I., 2015. Fragmentation genetics of the grassland butterfly *Polyommatus coridon*: Stable genetic diversity or extinction debt? Conservation Genetics 16, 549–558.

Habel, J.C., Roedder, D., Lens, L., Schmitt, T., 2013. The genetic signature of ecologically different grassland Lepidopterans. Biodiversity and Conservation 22, 2401–2411.

Herrando, S., Brotons, L., Anton, M., Páramo, F., Villero, D., Titeux, N., Quesada, J., Stefanescu, C., 2016. Assessing impacts of land abandonment on Mediterranean biodiversity using indicators based on bird and butterfly monitoring data. Environmental Conservation 43, 69–78.

Hesselbarth, G., Oorschot, H. van, Wagener, S., 1995. Die Tagfalter der Türkei unter Berücksichtigung der angrenzenden Länder [The butterflies of Turkey considering the neighboring countries]. Goecke & Evers, Keltern.

Hirsch, H., Wagner, V., Danihelka, J., Ruprecht, E., Sánchez-Gómez, P., Seifert, M., Hensen, I., 2015. High genetic diversity declines towards the geographic range periphery of *Adonis vernalis*, a Eurasian dry grassland plant. Plant Biology 17, 1233–1241.

Hughes, P.D., Woodward, J.C., van Calsteren, P.C., Thomas, L.E., 2011. The glacial history of the Dinaric Alps, Montenegro. Quaternary Science Reviews 30, 3393–3412.

Hurst, G.D.D., Jiggins, F.M., 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society B: Biological Sciences 272, 1525–1534.

Jost, L., 2008. G(ST) and its relatives do not measure differentiation. Molecular Ecology 17, 4015–4026.

Kahlke, R.-D., 2014. The origin of Eurasian Mammoth Faunas (*Mammuthus–Coelodonta* Faunal Complex). Quaternary Science Reviews 96, 32–49.

Kahlke, R.-D., García, N., Kostopoulos, D.S., Lacombat, F., Lister, A.M., Mazza, P.P.A., Spassov, N., Titov, V.V., 2011. Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe. Quaternary Science Reviews 30, 1368–1395.

Kajtoch, Ł., Cieślak, E., Varga, Z., Paul, W., Mazur, M.A., Sramkó, G., Kubisz, D., 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. Biodiversity and Conservation 25, 2309–2339.

Kamp, J., Reinhard, A., Frenzel, M., Kämpfer, S., Trappe, J., Hölzel, N., 2018. Farmland bird responses to land abandonment in Western Siberia. Agriculture, Ecosystems & Environment 268, 61–69.

Kamvar, Z.N., Tabima, J.F., Grunwald, N.J., 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2, e281.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Kodandaramaiah, U., Simonsen Thomas J., Bromilow Sean, Wahlberg Niklas, Sperling Felix, 2013. Deceptive single-locus taxonomy and phylogeography: *Wolbachia*-associated divergence in mitochondrial DNA is not reflected in morphology and nuclear markers in a butterfly species. Ecology and Evolution 3, 5167–5176.

Kolev, Z., 2017. *Rubrapterus bavius* (Eversmann, 1832), a butterfly genus and species new to Bulgaria (Insecta, Lepidoptera, Lycaenidae). ZooNotes 1–4.

Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. Nature 400, 652–655.

Kryštufek, B., Bryja, J., Bužan, E.V., 2009. Mitochondrial phylogeography of the European ground squirrel, *Spermophilus citellus*, yields evidence on refugia for steppic taxa in the southern Balkans. Heredity 103, 129–135.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. Molecular Biology and Evolution 34, 772–773.

Larrasoaña, J.C., Roberts, A.P., Rohling, E.J., 2013. Dynamics of Green Sahara periods and their role in Hominin evolution. PLoS ONE 8, e76514.

Larrasoaña, J.C., Roberts, A.P., Rohling, E.J., Winklhofer, M., Wehausen, R., 2003. Three million years of monsoon variability over the northern Sahara. Climate Dynamics 21, 689–698.

Lebedev, V., Poplavskaya, N., Bannikova, A., Ryurikov, G., Surov, A., 2018. Genetic differentiation in *Cricetulus migratorius* Pallas, 1773 (Rodentia, Cricetidae). Mammalian Biology 92, 115–119.

Leigh, J.W., Bryant, D., 2015. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6, 1110–1116.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Lisiecki, L.E., Raymo, M.E., 2007. Plio–Pleistocene climate evolution: trends and transitions in glacial cycle dynamics. Quaternary Science Reviews 26, 56–69.

Magyari, E.K., Chapman, J.C., Gaydarska, B., Marinova, E., Deli, T., Huntley, J.P., Allen, J.R.M., Huntley, B., 2008. The ‘oriental’ component of the Balkan flora: evidence of presence on the Thracian Plain during the Weichselian late-glacial. Journal of Biogeography 35, 865–883.

Mahmoudi, A., Darvish, J., Aliabadian, M., Yazdani, M.F., Kryštufek, B., 2017. New insight into the cradle of the grey voles (subgenus Microtus) inferred from mitochondrial cytochrome b sequences. Mammalia 81, 583–593.

Monteiro, A., Pierce, N.E., 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1alpha gene sequences. Mol. Phylogenet. Evol. 18, 264–281.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Német, E., Czekes, Z., Markó, B., Rákosy, L., 2016. Host plant preference in the protected myrmecophilous Transylvanian Blue (*Pseudophilotes bavius hungarica*) butterfly (Lepidoptera: Lycaenidae) and its relationship with potential ant partners. Journal of Insect Conservation 20, 765–772.

Numa, C., 2016. The status and distribution of Mediterranean butterflies, IUCN Library System. IUCN, Malaga, Spain.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Pamperis, L.N., 1997. The butterflies of Greece, 1 edition. ed. Bastas-Plessas, Athens, Greece.

Panagiotopoulos, K., Böhm, A., Leng, M.J., Wagner, B., Schäbitz, F., 2014. Climate variability over the last 92 ka in SW Balkans from analysis of sediments from Lake Prespa. Climate of the Past 10, 643–660.

Papadopoulou, P., Iliopoulos, G., Zidianakis, I., Tsoni, M., Koukouvelas, I., 2018. Vegetation and palaeoclimatic reconstruction of the Sousaki Basin (eastern Gulf of Corinth, Greece) during the Early Pleistocene - ScienceDirect. Quaternary International 476, 110–119.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20, 289–290.

Pavlopoulos, K., Leontaritis, A., Athanassas, C.D., Petrakou, C., Vandarakis, D., Nikolakopoulos, K., Stamatopoulos, L., Theodorakopoulou, K., 2018. Last glacial geomorphologic records in Mt Chelmos, North Peloponnesus, Greece. Journal of Mountain Science 15, 948–965.

Peña, C., Malm, T., 2012. VoSeq: A Voucher and DNA Sequence Web Application. PLoS ONE 7, e39071.

Pokorný, P., Chytrý, M., Juřičková, L., Sádlo, J., Novák, J., Ložek, V., 2015. Mid-Holocene bottleneck for central European dry grasslands: Did steppe survive the forest optimum in northern Bohemia, Czech Republic? The Holocene 25, 716–726.

Polunin, O., 1980. Flowers of Greece and the Balkans : a field guide. Oxford University Press, Oxford.

Pross, J., Koutsodendris, A., Christanis, K., Fischer, T., Fletcher, W.J., Hardiman, M., Kalaitzidis, S., Knipping, M., Kotthoff, U., Milner, A.M., Müller, U.C., Schmiedl, G., Siavalas, G., Tzedakis, P.C., Wulf, S., 2015. The 1.35-Ma-long terrestrial climate archive of Tenaghi Philippon, northeastern Greece: Evolution, exploration, and perspectives for future research. Newsletters on Stratigraphy 253–276.

Rákosy, L., Weidlich, M., 2017. *Rubrapterus bavius* from north-eastern Bulgaria and new data on its conservation status in Romania. Entomologica Romanica 21, 15–22.

Ratnasingham, S., Hebert, P.D.N., 2007. bold: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7, 355–364.

Říčanová, Š., Koshev, Y., Říčan, O., Ćosić, N., Ćirović, D., Sedláček, F., Bryja, J., 2013. Multilocus phylogeography of the European ground squirrel: cryptic interglacial refugia of continental climate in Europe. Molecular Ecology 22, 4256–4269.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ruddiman, W.F., Raymo, M.E., Martinson, D.G., Clement, B.M., Backman, J., 1989. Pleistocene evolution: Northern hemisphere ice sheets and North Atlantic Ocean. Paleoceanography 4, 353–412.

Sandom, C.J., Ejrnæs, R., Hansen, M.D.D., Svenning, J.-C., 2014. High herbivore density associated with vegetation diversity in interglacial ecosystems. Proceedings of the National Academy of Sciences of the United States of America 111, 4162–4167.

Schmitt, T., Varga, Z., 2012. Extra-Mediterranean refugia: The rule and not the exception? Frontiers in Zoology 9, 1–12.

Sirami, C., Nespoulous, A., Cheylan, J.-P., Marty, P., Hvenegaard, G.T., Geniez, P., Schatz, B., Martin, J.-L., 2010. Long-term anthropogenic and ecological dynamics of a Mediterranean landscape: Impacts on multiple taxa. Landscape and Urban Planning 96, 214–223.

Slancarova, J., Bartonova, A., Zapletal, M., Kotilinek, M., Fric, Z.F., Micevski, N., Kati, V., Konvicka, M., 2016. Life history traits reflect changes in Mediterranean butterfly communities due to forest encroachment. PLoS ONE 11, e0152026.

Sokolov, V.E., Zhirnov, L.V., 1998. Saigak: filogeniya, sistematika, ekologiya, okhrana i ispol'zovanie [The saiga antelope: phylogeny, systematics, ecology, conservation, and use]. Russian Academy of Sciences, Moscow.

Sunderland, W., 2004. Taming the Wild Field: Colonization and Empire on the Russian Steppe. Cornell University Press, New York.

Švara, V., Popović, M., Peternel, A., Radevski, Đ., Vukotić, K., Verovnik, R., 2016. Surveys of butterfly and skipper fauna in the southwestern part of the Republic of Macedonia (Lepidoptera: Papilionoidea & Hesperioidea). Natura Sloveniae 18, 23–37.

ten Hagen, W., 1996. Tagfalterbeobachtungen in Syrien und Jordanien (2. Beitrag) (Lepidoptera: Papilionoidea, Hesperioidea). Nachrichten des Entomologischen Vereins Apollo 17, 123–142.

Tikhonov, V.V., Stradomsky, B.V., Kuznetsov, G.V., Andreev, S.A., 2014. Butterflies of the Caucasus and the South of Russia. http://www.babochki-kavkaza.ru. Accessed 1 March 2019

Todisco, V., Grill, A., Fiedler, K., Gottsberger, B., Dincă, V., Vodă, R., Lukhtanov, V., Letsch, H., 2018. Molecular phylogeny of the Palaearctic butterfly genus *Pseudophilotes* (Lepidoptera: Lycaenidae) with focus on the Sardinian endemic P. barbagiae. BMC Zoology 3, 4.

Tolman, T., 1992. On the life history of *Pseudophilotes bavius* Eversmann, 1832 in S. Greece, its distribution in the Peloponnesos and a new record for N Greece Lepidoptera: Lycaenidae. Phegea 201, 35–39.

Tolman, T., Lewington, R., 2008. Collins butterfly guide: The Most Complete Field Guide to the Butterflies of Britain and Europe. Collins, London.

Török, P., Ambarlı, D., Kamp, J., Wesche, K., Dengler, J., 2016. Step(pe) up! Raising the profile of the Palaearctic natural grasslands. Biodiversity and Conservation 25, 2187–2195.

Tóth, J.P., Varga, Z., Verovnik, R., Wahlberg, N., Váradi, A., Bereczki, J., 2017. Mito-nuclear discordance helps to reveal the phylogeographic patterns of *Melitaea ornata* (Lepidoptera: Nymphalidae). Biological Journal of the Linnean Society 121, 267–281.

Tshikolovets, V.V., Naderi, A., Eckweiler, W., 2014. The Butterflies of Iran and Iraq. Tshikolovets Publications.

Urban, B., Fuchs, M., 2005. Late Pleistocene vegetation of the basin of Phlious, NE-Peloponnese, Greece. Review of Palaeobotany and Palynology 137, 15–29.

van Swaay, C., Collins, S., Dušej, G., Maes, D., Munguira, M.L., Rakosy, L., Ryrholm, N., Šašić, M., Settele, J., Thomas, J., Verovnik, R., Verstrael, T., Warren, M., Wiemers, M., Wynhoff, I., 2012. Dos and Don’ts for butterflies of the Habitats Directive of the European Union. Nature Conservation 1, 73–153.

Wahlberg, N., Peña, C., Ahola, M., Wheat, C.W., Rota, J., 2016. PCR primers for 30 novel gene regions in the nuclear genomes of Lepidoptera. ZooKeys 596, 129–141.

Wahlberg, N., Wheat, C.W., 2008. Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of lepidoptera. Systematic Biology 57, 231–242.

Wesche, K., Ambarlı, D., Kamp, J., Török, P., Treiber, J., Dengler, J., 2016. The Palaearctic steppe biome: a new synthesis. Biodiversity and Conservation 25, 2197–2231.

Winter, D., 2012. mmod: an R library for the calculation of population differentiation statistics. Molecular Ecology Resources 12, 1158–1160.

Yunusbayev, B., Metspalu, M., Metspalu, E., Valeev, A., Litvinov, S., Valiev, R., Akhmetova, V., Balanovska, E., Balanovsky, O., Turdikulova, S., Dalimova, D., Nymadawa, P., Bahmanimehr, A., Sahakyan, H., Tambets, K., Fedorova, S., Barashkov, N., Khidiyatova, I., Mihailov, E., Khusainova, R., Damba, L., Derenko, M., Malyarchuk, B., Osipova, L., Voevoda, M., Yepiskoposyan, L., Kivisild, T., Khusnutdinova, E., Villems, R., 2015. The Genetic Legacy of the Expansion of Turkic-Speaking Nomads across Eurasia. PLoS Genetics 11, e1005068.

Zimov, S.A., Zimov, N.S., Tikhonov, A.N., Chapin III, F.S., 2012. Mammoth steppe: a high-productivity phenomenon. Quaternary Science Reviews 57, 26–45.

Zinenko, O., Stümpel, N., Mazanaeva, L., Bakiev, A., Shiryaev, K., Pavlov, A., Kotenko, T., Kukushkin, O., Chikin, Y., Duisebayeva, T., Nilson, G., Orlov, N.L., Tuniyev, S., Ananjeva, N.B., Murphy, R.W., Joger, U., 2015. Mitochondrial phylogeny shows multiple independent ecological transitions and northern dispersion despite of Pleistocene glaciations in meadow and steppe vipers (*Vipera ursinii* and *Vipera renardi*). Molecular Phylogenetics and Evolution 84, 85–100.

SUMMARY

1. Summary of results

The Submediterranean steppe-like grasslands and Palaearctic steppes are subjects of recent conservation concerns (Török et al., 2016; Wesche et al., 2016). This thesis contributes to the knowledge on their history and present situation.

The first chapter assesses the current conditions of the two Balkan localities of the steppe butterfly *Proterebia afra* (Fabricius, 1787), the species' microhabitat selection and co-occurring butterfly communities. In exceptionally arid Askion Mountains (Greece), the species prefers grassy rocky plains with dry grass biomass, without shrubs or trees. In the area, many striking steppe or Mediterranean grasslands species co-exist: e.g., *Pseudophilotes bavius* (Eversmann, 1832), *Erynnis marloyi* (Boisduval, 1834), *Anthocharis gruneri* Herrich-Schäffer, 1851, and *Euchloe penia* (Freyer, 1851). In more northerly situated Dalmatia (Croatia), *P. afra* inhabits south-oriented rocky slopes. The butterfly community in the area contains some Mediterranean species (e.g., *Melanargia larissa* (Geyer, 1828), *Libythea celtis* (Laicharting, 1782), or *Pyrgus sidae* (Esper, 1784)) combined with Euro-Siberian butterflies, which occupy different habitats in Central European conditions (e.g., *Argynnis niobe* (Linnaeus, 1758), *Euphydryas aurinia* (Rottemburg, 1775)), pointing to a high diversity of the Dalmatian landscape. In both areas, *P. afra* was a dominant butterfly of the spring aspect. The species is not endangered in any of them, but the situation could change with land use alternation. Dalmatian grasslands are subject to land abandonment and following succession after the war in 1990s and even direct afforestation.

The second chapter reveals the biogeographic history of *P. afra*. The study is unique in terms that it covers the whole distribution area of the steppe species, the Irano-Anatolian region, lowland Palaearctic steppes and the Balkans. Based on genetic data, the ancestral range of the species is situated in the Irano-Anatolian region. It subsequently expanded to the Balkans and to the northern steppes (from Crimea, Volgograd in the west to eastern Kazakhstan in the east). This could have happened several glacial cycles ago, in middle Pleistocene. In the Irano-Anatolian region, the species probably diversified by uphill shifts in interglacials and downhill in glacials. In the northern steppes, it forms a widespread little differentiated lineage. According to the species distribution model, the species survived widely distributed in the northern part of its range also during glacial maxima. The Balkan populations also survived *in situ* and could have been partially connected to the northern steppes during the past.

Adding one more example of a steppe species history, the third chapter discloses phylogeography and population genetics of another steppe butterfly, *Pseudophilotes bavius*. This species inhabits the Irano-Anatolian steppes as well as has several isolated populations in the Balkans and in Eastern Europe, from Transylvania to Southern Urals and Western Kazakhstan. The basal lineages were again discovered in the Irano-Anatolian region. The species quickly radiated in the Balkans and Eastern Europe, possibly when the steppes became widespread in the middle Pleistocene. In the Balkans, the species is isolated and forms differentiated genetic lineages. On the other hand, in Eastern Europe, the populations are genetically more diverse and less differentiated. The populations are either still interconnected, or, more likely, have not been fragmented too long ago. The latter option is more plausible, because the species is a strict habitat specialist with limited dispersal ability. Possibly, its distribution was rather continuous until a recent past, owing to pastoral cultures inhabiting the vast plains of Eastern Europe, and a position of the area at a suture between competing cultures. For *P. bavius*, the present distribution patterns and population connectivity are uncertain in lowland steppes of South-Eastern Ukraine and southern Russia, where only a small fraction of steppes survives (Wesche et al., 2016). Analogous situation was found in Ukrainian steppe plants, where strong fragmentation but still a high genetic population diversity exist (Hirsch et al., 2015; Dembicz et al., 2018).

The second and third chapters provide evidence for survival of invertebrate steppe species in the relatively northerly situated areas also during the glacials. The steppe biome was the prevailing habitat of the Palaearctic region in Quaternary. It was inseparably connected to the mammalian megafauna roaming the vast areas. According to the fossil record, many small mammals and other vertebrates inhabited the biome, as well as fossilising invertebrates (Berman et al., 2011; Zinovyev et al., 2016;

Schweiger and Svenning, 2018). The butterfly fossil record is rare (cf. Espeland et al., 2018) and butterfly biogeographic history has to be estimated from the genetic information and climatic modelling.

The outdated perception of glacial ecosystems as a polar desert is challenged by studies on mammoth steppe fauna and flora (Zazula et al., 2003; Berman et al., 2011; Zimov et al., 2012; Chytrý et al., 2019). According to this evidence, the biome was diverse, highly productive and consisting of a finely grained habitat mosaic. The mammoth steppe hosted a combination of two open habitats mammalian faunas that originated in two centres – in the cold northern (e.g., *Rangifer tarandus* (Linnaeus, 1758)*, Ovibos moschatus* (Zimmermann, 1780)*, Alopex lagopus* (Linnaeus, 1758)) and in the southern continental (e.g., *Saiga borealis* Tschersky, 1876 /*Saiga tatarica* (Linnaeus, 1766), *Equus* L. sp., *Crocura crocura* (Erxleben, 1777)) environments (Kahlke, 2014). The same applies also for the small mammals (Erbajeva et al., 2011; Shchetnikov et al., 2016) and beetles (Zinovyev et al., 2016). Even though not preserved in the fossil record, it is highly plausible that the same applies also for butterflies. There are examples of butterflies, currently associated with various habitat types, which exist as differentiated populations in Western, Central or Southern Europe, and form widely distributed 'eastern' lineage (or lineages) with shallow genetic structure across a wide area in Eastern Europe and Asia (Wahlberg and Saccheri, 2013; Kodandaramaiah et al., 2013; Todisco et al., 2012; Bartonova et al., 2018; Maresova et al., 2019, J. Marešová, unpublished data). The survival of many butterfly species in wide areas of Eurasia could be the rule rather than the exception: the mammoth steppe in continental Eurasia could have buffered the climate and allow survival in wide areas. On the more oceanic margin of the continent (Western, Southern and Central Europe), species could have also persisted in many areas thank to a diverse habitat mosaic (Sandom et al., 2014).

The mammoth steppe disappeared in the course of Holocene, partly in connection with large megafauna extirpation (Guthrie, 2006), although remnants of the biome still exist in some regions owing to human land use patterns or continuous presence of feral ungulates (Ricankova et al., 2014; Chytrý et al., 2019). Even some "seminatural" grassland of Europe may in fact represent remnants of the ancient biome (Feurdean et al., 2018; Pokorný et al., 2015). Across Eurasia, wide areas of tundra and taiga (re-) established (cf. Zimov et al., 2012). The steppe areas, especially in the Western Palaearctic, were widely ploughed (Török et al., 2016; Wesche et al., 2016). At the end of the 20th century, a large portion of cropland was abandoned, which favours biodiversity first (Kamp et al., 2015, 2018), but the biotopes could degrade if not managed (Kämpf et al., 2016; Mathar et al., 2016).

Survival of many butterfly species thus could depend on dealing with recent habitat depletion and fragmentation rather than with Quaternary climate alternations. Recently, afforestation was proposed to mitigate the climate change (cf. Feurdean et al., 2018). This could be extremely dangerous for the grassland biota, adapted to open and semi open environments for several millions of years, and has to be considered with caution.

2. Future perspectives

The future research on Submediterranean grasslands and Palaearctic steppes should target proper habitat management. The necessary steps should include inventory of current state of the biome, human pressure, presence of ungulate ecosystem engineers, and comparative effects of wild, domestic and feral ungulates on associated biodiversity, including specialised insects.

More examples of phylogeography or population genetics of steppe, southern Submediterranean grasslands, as well as continental grassland butterflies *sensu* De Lattin (1967) should be added to complete the picture. Many grassland species were studied only in the European part of their range, but inclusion of Asiatic samples could reveal new patterns.

With the implementation of new generation methods, such as RAD sequencing (Baird et al., 2008; Peterson et al., 2012; Suchan et al., 2016), the amount of genetic knowledge will rapidly increase, but also the bioinformatic demands. The wider use of these methods in insect biogeography depend on the adjustment for low quality and fragmented DNA (Tin et al., 2014; Sproul and Maddison, 2017). In the case of insects, millions of specimens are deposited in private and museum collections. The collections harbour samples from historical localities, where the species no longer occur today. Phylogeography of a species should be considered in reintroduction efforts, where the historical population could have been adapted to specific climate (cf. Dincă et al., 2018).

3. References

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. PLoS ONE 3, e3376.

Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867–884.

Berman, D., Alfimov, A., Kuzmina, S., 2011. Invertebrates of the relict steppe ecosystems of Beringia, and the reconstruction of Pleistocene landscapes. Quaternary Science Reviews 30, 2200–2219.

Chytrý, M., Horsák, M., Danihelka, J., Ermakov, N., German, D.A., Hájek, M., Hájková, P., Kočí, M., Kubešová, S., Lustyk, P., Nekola, J.C., Pavelková Řičánková, V., Preislerová, Z., Resl, P., Valachovič, M., 2019. A modern analogue of the Pleistocene steppe-tundra ecosystem in southern Siberia. Boreas 48, 36–56.

de Lattin, G., 1967. Grundriss der Zoogeographie. VEB Gustav Fischer Verlag, Jena.

Dembicz, I., Szczeparska, L., Moysiyenko, I.I., Wódkiewicz, M., 2018. High genetic diversity in fragmented Iris pumila L. populations in Ukrainian steppe enclaves. Basic and Applied Ecology, Plant population biology in a changing world 28, 37–47.

Dincă, V., Bálint, Z., Vodă, R., Dapporto, L., Hebert, P.D.N., Vila, R., 2018. Use of genetic, climatic, and microbiological data to inform reintroduction of a regionally extinct butterfly. Conservation Biology 32, 828–837.

Erbajeva, M.A., Khenzykhenova, F.I., Alexeeva, N.V., 2011. Late Pleistocene and Holocene environmental peculiarity of the Baikalian region, based on mammal associations and deposits. Quaternary International 237, 39–44.

Espeland, M., Breinholt, J., Willmott, K.R., Warren, A.D., Vila, R., Toussaint, E.F.A., Maunsell, S.C., Aduse-Poku, K., Talavera, G., Eastwood, R., Jarzyna, M.A., Guralnick, R., Lohman, D.J., Pierce, N.E., Kawahara, A.Y., 2018. A Comprehensive and Dated Phylogenomic Analysis of Butterflies. Current Biology 28, 770-778.e5.

Feurdean, A., Ruprecht, E., Molnár, Z., Hutchinson, S.M., Hickler, T., 2018. Biodiversity-rich European grasslands: Ancient, forgotten ecosystems. Biological Conservation 228, 224–232.

Guthrie, R.D., 2006. New carbon dates link climatic change with human colonization and Pleistocene extinctions. Nature 441, 207–209.

Hirsch, H., Wagner, V., Danihelka, J., Ruprecht, E., Sánchez-Gómez, P., Seifert, M., Hensen, I., 2015. High genetic diversity declines towards the geographic range periphery of *Adonis vernalis*, a Eurasian dry grassland plant. Plant Biology 17, 1233–1241.

Kahlke, R.-D., 2014. The origin of Eurasian Mammoth Faunas (*Mammuthus–Coelodonta* Faunal Complex). Quaternary Science Reviews 96, 32–49.

Kamp, J., Reinhard, A., Frenzel, M., Kämpfer, S., Trappe, J., Hölzel, N., 2018. Farmland bird responses to land abandonment in Western Siberia. Agriculture, Ecosystems & Environment 268, 61–69.

Kamp, J., Urazaliev, R., Balmford, A., Donald, P.F., Green, R.E., Lamb, A.J., Phalan, B., 2015. Agricultural development and the conservation of avian biodiversity on the Eurasian steppes: a comparison of land-sparing and land-sharing approaches. Journal of Applied Ecology 52, 1578–1587.

Kämpf, I., Mathar, W., Kuzmin, I., Hölzel, N., Kiehl, K., 2016. Post-Soviet recovery of grassland vegetation on abandoned fields in the forest steppe zone of Western Siberia. Biodiversity and Conservation 25, 2563–2580.

Kodandaramaiah, U., Simonsen Thomas J., Bromilow Sean, Wahlberg Niklas, Sperling Felix, 2013. Deceptive single-locus taxonomy and phylogeography: *Wolbachia*-associated divergence in mitochondrial DNA is not reflected in morphology and nuclear markers in a butterfly species. Ecology and Evolution 3, 5167–5176.

Maresova, J., Habel, J.C., Neve, G., Sielezniew, M., Bartonova, A., Kostro-Ambroziak, A., Faltynek Fric, Z., 2019. Cross-continental phylogeography of two boreo-montane Nymphalid butterflies. PLoS ONE 14, e0214483.

Mathar, W.P., Kämpf, I., Kleinebecker, T., Kuzmin, I., Tolstikov, A., Tupitsin, S., Hölzel, N., 2016. Floristic diversity of meadow steppes in the Western Siberian Plain: effects of abiotic site conditions, management and landscape structure. Biodiversity and Conservation 25, 2361–2379.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE 7, e37135.

Pokorný, P., Chytrý, M., Juřičková, L., Sádlo, J., Novák, J., Ložek, V., 2015. Mid-Holocene bottleneck for central European dry grasslands: Did steppe survive the forest optimum in northern Bohemia, Czech Republic? The Holocene 25, 716–726.

Ricankova, V.P., Robovsky, J., Riegert, J., 2014. Ecological Structure of Recent and Last Glacial Mammalian Faunas in Northern Eurasia: The Case of Altai-Sayan Refugium. PLoS One 9, e85056.

Sandom, C.J., Ejrnæs, R., Hansen, M.D.D., Svenning, J.-C., 2014. High herbivore density associated with vegetation diversity in interglacial ecosystems. Proceedings of the National Academy of Sciences of the United States of America 111, 4162–4167.

Schweiger, A.H., Svenning, J.-C., 2018. Down-sizing of dung beetle assemblages over the last 53 000 years is consistent with a dominant effect of megafauna losses. Oikos 127, 1243–1250.

Shchetnikov, A.A., Bezrukova, E.V., Maksimov, F.E., Kuznetsov, V.Y., Filinov, I.A., 2016. Environmental and climate reconstructions of the Fore-Baikal area during MIS 5-1: Multiproxy record from terrestrial sediments of the Ust-Oda section (Siberia, Russia). Journal of Asian Earth Sciences 129, 220–230.

Sproul, J.S., Maddison, D.R., 2017. Sequencing historical specimens: successful preparation of small specimens with low amounts of degraded DNA. Molecular Ecology Resources 17, 1183–1201.

Suchan, T., Pitteloud, C., Gerasimova, N.S., Kostikova, A., Schmid, S., Arrigo, N., Pajkovic, M., Ronikier, M., Alvarez, N., 2016. Hybridization Capture Using RAD Probes (hyRAD), a New Tool for Performing Genomic Analyses on Collection Specimens. PLoS ONE 11, e0151651.

Tin, M.M.-Y., Economo, E.P., Mikheyev, A.S., 2014. Sequencing Degraded DNA from Non-Destructively Sampled Museum Specimens for RAD-Tagging and Low-Coverage Shotgun Phylogenetics. PLoS ONE 9, e96793.

Todisco, V., Gratton, P., Zakharov, E.V., Wheat, C.W., Sbordoni, V., Sperling, F.A.H., 2012. Mitochondrial phylogeography of the Holarctic *Parnassius phoebus* complex supports a recent refugial model for alpine butterflies. Journal of Biogeography 39, 1058–1072.

Török, P., Ambarlı, D., Kamp, J., Wesche, K., Dengler, J., 2016. Step(pe) up! Raising the profile of the Palaearctic natural grasslands. Biodiversity and Conservation 25, 2187–2195.

Wahlberg, N., Saccheri, I., 2013. The effects of Pleistocene glaciations on the phylogeography of *Melitaea cinxia* (Lepidoptera: Nymphalidae). European Journal of Entomology 104, 675–684.

Wesche, K., Ambarlı, D., Kamp, J., Török, P., Treiber, J., Dengler, J., 2016. The Palaearctic steppe biome: a new synthesis. Biodiversity and Conservation 25, 2197–2231.

Zazula, G.D., Froese, D.G., Schweger, C.E., Mathewes, R.W., Beaudoin, A.B., Telka, A.M., Harington, C.R., Westgate, J.A., 2003. Ice-age steppe vegetation in east Beringia - Tiny plant fossils indicate how this frozen region once sustained huge herds of mammals. Nature 423, 603–603.

Zimov, S.A., Zimov, N.S., Tikhonov, A.N., Chapin III, F.S., 2012. Mammoth steppe: a high-productivity phenomenon. Quaternary Science Reviews 57, 26–45.

Zinovyev, E.V., Dudko, R.Y., Gurina, A.A., Prokin, A.A., Mikhailov, Y.E., Tsepelev, K.A., Tshernyshev, S.E., Kireev, M.S., Kostyunin, A.E., Legalov, A.A., 2016. First records of sub-fossil insects from Quaternary deposits in the southeastern part of West Siberia, Russia. Quaternary International 420, 221–232

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Field experience

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Publications with impact factor (IF)

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Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2018. Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125, 867-884.

Potocky, P., **Bartonova, A.**, Benes, J., Zapletal, M., Konvicka, M., 2018. Life-history traits of Central European moths: gradients of variation and their association with rarity and threats. Insect Conservation and Diversity 11, 493-505.

Bartonova, A., Kolar, V., Maresova, J., Sasic, M., Slancarova, J., Suchacek, P., Konvicka, M., 2017. Isolated Asian steppe element in the Balkans: habitats of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae) and associated butterfly communities. Journal of Insect Conservation 21, 559-571.

Korb, S.K., Faltynek Fric, Z., **Bartonova, A.**, 2017. On the status and position of *Melitaea minerva* var. *palamedes* Groum-Grshimailo, 1890 (Lepidoptera: Nymphalidae). SHILAP Revista de Lepidopterologia 45, 17-22.

Vlasanek, P., **Bartonova, A.**, Marec, F., Konvicka, M., 2017. Elusive *Parnassius mnemosyne* (Linnaeus, 1758) larvae: habitat selection, sex determination and sex ratio (Lepidoptera: Papilionidae). SHILAP Revista de Lepidopterologia 45, 561-569.

Slancarova, J., **Bartonova, A.**, Zapletal, M., Kotilinek, M., Faltynek Fric, Z., Micevski, B., Kati, V., Konvicka, M., 2016. Life history traits reflect changes in Mediterranean butterfly communities due to forest encroachment. PLoS ONE 11, e0152026.

Bartonova, A., Benes, J., Faltynek Fric, Z, Chobot, K., Konvicka, M., 2016. How universal are reserve design rules? A test using butterflies and their life history traits. Ecography 39, 456-464.

Korb, S.K., Bolshakov, L.V., Faltynek Fric, Z., **Bartonova, A.**, 2016. Cluster biodiversity as a multidimensional structure evolution strategy: checkerspot butterflies of the group *Euphydryas aurinia* (Rottemburg, 1775) (Lepidoptera: Nymphalidae). Systematic Entomology 41, 441-457.

Bartonova, A., Benes, J., Konvicka, M., 2014. Generalist-specialist continuum and life history traits of Central European butterflies (Lepidoptera) – are we missing a part of the picture? European Journal of Entomology 111, 543-553.

Non-IF publications

Bartoňová, A., Faltýnek Fric, Z., Konvička, M., 2019. Příběh okáče *Proterebia afra* - eurasijského stepního motýla, který žil společně s mamuty. Živa 67, 35-37 [in Czech].

Konvička, M., Beneš, J., Spitzer, L., **Bartoňová, A.**, Zapletal, M., 2017. Management stanovišť ohrožených druhů denních a nočních motýlů v České republice. N – certifikovaná metodika. MŽP ČR. [in Czech].

Korb, S.K., Faltynek Fric, Z., **Bartonova, A.**, 2016. Phylogeography of *Koramius charltonius* (Gray, 1853) (Lepidoptera: Papilionidae): a case of too many poorly circumscribed subspecies. Nota Lepidopterologica 39, 169-191.

International conferences

Bartonova, A., Konvicka, M., Korb, S., Kramp, K., Schmitt, T., Faltynek Fric, Z., 2017. Twice to Europe and then north-eastwards: Phylogeography of *Proterebia afra* (*=phegea*) [oral presentation]. In: Sasic, M., Rota, J., Mihoci, I., 2017. Book of abstracts of the 20th European Congress of Lepidopterology. April 24th – April 30th 2017, Podgora, Croatia. Croatian Natural History Museum, Zagreb.

Bartonova, A., Faltynek Fric, Z., Konvicka, M., 2016. Steppe species under the glacial cycles: Phylogeography of *Proterebia afra* [poster]. In: WallisDeVries, M, Wynhoff, I., Van Swaay, C. (Eds) Future 4 Butterflies In Europe. Book of Abstracts. Dutch Butterfly Conservation (De Vlinderstichting). 31st of March to April 2nd 2016. Wageningen, Netherlands.

Bartonova, A., Faltynek Fric, Z., Suchacek, P., Kolar, V., Maresova, J., Slancarova, J., Zapletal, M., Sasic, M., Konvička, M., 2015. *Proterebia afra* – Relic of the glacial steppe in the Balkans? [oral presentation] In: XIXth European Congress of Lepidopterology. Book of Abstracts. September 27 – October 2 2015. Radebeul, Germany.

Bartonova, A., Konvicka, M., Benes, J., 2014. Life history traits of butterfly assemblages reflect topography and landscape context of Czech nature reserves [poster] In: 7th International Conference on the Biology of Butterflies. Book of Abstracts. August 11-14 2014. University of Turku, Finland.

Bartonova, A., Konvicka, M., Benes, J., 2012. EU-protected butterflies *Phengaris nausithous* and *Phengaris teleius*: situation in the Czech Republic [poster]. Wallis DeVries M., Wynhoff I., Van Swaay C. (Eds.): Future of Butterflies In Europe III. Book of abstracts. 29-31 March 2012. Wageningen, Netherlands

Bartonova, A., Vlasanek, P., Konvicka, M., Marec, F., 2012. Microhabitat Selection and Sex Determination in *Parnassius Mnemosyne* larvae [poster]. In: Wallis DeVries M., Wynhoff I., Van Swaay C. (Eds.): Future of Butterflies In Europe III. Book of abstracts. 29-31 March 2012. Wageningen, Netherlands

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