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ORIGINAL PAPER

Conserving Europe's most endangered butterfly: the Macedonian Grayling (*Pseudochazara cingovskii*)

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Abstract The Macedonian Grayling is listed as critically endangered in the recent IUCN Red List of European butterflies because of its extreme rarity and habitat loss due to quarrying. This categorisation was, however, based on rather limited knowledge on its actual distribution, population size and habitat requirements. In 2012, we conducted field surveys to acquire more information. We found the species at six new sites extending its known range of suitable habitat to just under 10 km^2. The daily population size was estimated using capture-mark-recapture method in the most densely populated part of the Pletvar pass site at more than 650 individuals. Adults proved to be extremely sedentary, not moving far even within the continuous habitat on the same slope. Oviposition was observed on dry plant material and in a rock crevice close to the potential larval host plant *Festuca* sp. Quarrying is confirmed to be the main threat to the habitat of the Macedonian Grayling with five out of seven populated sites containing active marble quarries. Due to the enlargement of the known area of occupancy, its threat status would now be estimated at endangered. Despite the restricted knowledge about its distribution and trends in the population size, the IUCN criteria proved to be applicable to determine the threat status of a rare and localized butterfly such as *Pseudochazara cingovskii*. Its original assessment of being called the most threatened butterfly in Europe resulted in immediate research project and subsequent actions that will undoubtedly help to conserve it in the future.

Keywords Lepidoptera · Conservation · Endemism · Red List · IUCN

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Introduction

Butterflies are known as useful indicators for biodiversity and conservation and are typically one of the best studied groups of insects both at the regional and national level (Maes and Van Dyck 2005; Thomas 2005; Settele et al. 2009). However, most of the actual conservation efforts in Europe are concentrated in north-west European countries where the declines in butterfly distribution and abundance were most severe (Warren et al. 2001; Maes and Van Dyck 2001). Threatened species from this region have been studied in detail regarding their metapopulation structure, habitat preferences and larval ecology, which has shown to be pivotal in successful habitat management (Thomas et al.

2011). Further south and east in Europe where butterfly diversity is much higher (Kudrna et al. 2011), autecological studies of threatened species are sparse and there is almost no active management of habitats of threatened species or management is inappropriate (Konvička et al. 2008). This is in striking contrast with the distribution of most threatened butterfly species in Europe (status: critically endangered, endangered and vulnerable) with almost two thirds of them with ranges limited to southern Europe (Van Swaay et al. 2010). Among these, many are island endemics, especially from Macronesia, while others are limited to the mountain ranges of the three large peninsulas and the Alps/Pyrennees (Kudrna et al. 2011).

In Europe, three species are listed as *critically endangered* (CR) according to IUCN criteria (IUCN 2011) pending urgent conservation measures to reverse their decline (Van Swaay et al. 2011): the Macedonian Grayling *Pseudochazara cingovskii* (Gross 1973), the Siberian Brown *Coenonympha phryne* (Pallas 1771) which only marginally reaches Europe in south-east Ukraine and the European part of Russia (Tshikolovets 2003) and the Madeiran large white *Pieris wollastoni* (Butler 1886) which was for the last time reliably observed in 1977 (Aguiar and Karsholt 2006) and is now presumably extinct. Possible causes of extinction are habitat loss, viral diseases introduced together with *Pieris rapae* (Linnaeus 1758) (Gardiner 2003) or introduced braconid wasp parasitoids (Lozan et al. 2008).

Here, we focus on the Macedonian Grayling, an endemic known from a single site described by Gross (1973) as a subspecies of *Pseudochazara sintensi* Staudinger, 1895. It was soon recognized as a separate species owing to its distinct wing colour pattern and androconial scales (Brown 1976; Gross 1978). It is confined to marble screes and rocks with sparse bushy vegetation on the slopes above the Pletvar pass in the central part of the Republic of Macedonia (Gross 1973). While larval stages and host plant utilization in captivity (*Deschampsia* sp., *Poa annua*, *Poa* sp.) are described in detail (Aussem and Hesselbarth 1980), nothing is known about larval ecology and oviposition behaviour in nature. The current threat status of this CR species is based on a combination of criteria B1ab(iii, v) + 2ab(iii, v) (see IUCN 2011) which refers to a small extent of occurrence (EOO, <100 km^2) and area of occupancy (AOO, <10 km^2), presence at only a single site and a continuing decline inferred from habitat loss due to marble excavation. The estimated range of the species is <1.5 km^2 (IUCN 2013). As geographic rarity is considered the single most important factor in all known extinctions in Europe (Fontaine et al. 2007) such a minute range together with habitat loss is the main conservation concern for the species.

In order to determine the present conservation status of the Macedonian Grayling, field surveys were conducted at the Pletvar site and its wider surroundings. Our aim was (1) to search for new populations of the species, (2) precisely delimit the distribution of adults and the potential habitat at Pletvar pass, (3) make indicative estimates of the population size at Pletvar, and (4) study the oviposition behaviour of the species. This information would allow a possible reassessment of the threat status and provide more detailed guidelines for the protection of the species and its habitats.

Materials and methods

Study area

The Pletvar pass is situated in the central part of the Republic of Macedonia. It represents the most important connection between Vardar valley and south western Macedonia with its two major towns Bitola and Prilep. It is also a border zone of limestone on the southern slopes of Mt. Kozjak to the north and the predominantly metamorphic rocky terrain to the south. Further limestone outcrops are situated in the wider surroundings of the pass, both towards the north-west and south-east. These were identified using Google Earth satellite images and were commonly marked by signs of quarrying activities. A total of 10 potentially suitable sites were selected and visited during the survey. Due to the predominantly pale rocky substrate and sparse vegetation, the potential habitat of the Macedonian Grayling was easily visible on satellite images allowing precise delineation of the habitat patches before the field surveys started (Fig. 1).

The central part of Macedonia has a moderate continental climate with cold and wet winters and dry and hot summers (Stojmilov 2001). Average annual temperature in nearby Prilep is 11–12 °C with 580 mm of rainfall (Lazarevski 1993). The vegetation is predominantly xerophilous with dry calcareous grasslands and dry scrublands, broad leaved deciduous forests (Querco–Ostryetum carpinifolia) and inland cliffs and exposed rocks (Micevski 2000).

Field surveys

Field surveys were conducted in the first half of July during the peak season for adults based on previous observations (Verovnik, personal observation). Each potential site was visited between 0800 and 1130 hours as well as from 1500 to 1730 hours, when adult butterflies are active. As recording the presence of adults was our main objective and not an estimation of the total number of butterflies present, only a part of the potential habitat of the species at a site was surveyed, with a minimum of 45 min spent for each survey at potential new sites. Within each survey area,

butterflies were counted to estimate adult abundance (see Table 1). The presence of other butterfly species was also noted at all sites.

At Pletvar Pass the entire potential habitat was searched for adults to determine the real extent of the species' distribution. In parallel, a mark-release-recapture survey (MRR) was done in the part of the Pletvar site with the visibly highest butterfly density. MRR was conducted on three consecutive days to allow for the calculation of a coarse daily population size using the Jolly–Seber approach (Jolly 1965) and the Lincoln–Petersen Index (Southwood and Henderson 2000). Marking of adults was conducted between 0730 and 1200 hours and continued from 1430 until 1730 hours from 7 to 9 July 2012. In order to estimate the adult's mobility, we divided the surveyed slope in three parts and marked the individuals with different letter codes depending on the site of the first capture (Fig. 2). Additionally, the next slope towards the east separated by approximately 100 m of unsuitable woodland habitat was checked for dispersing adults on the last day of

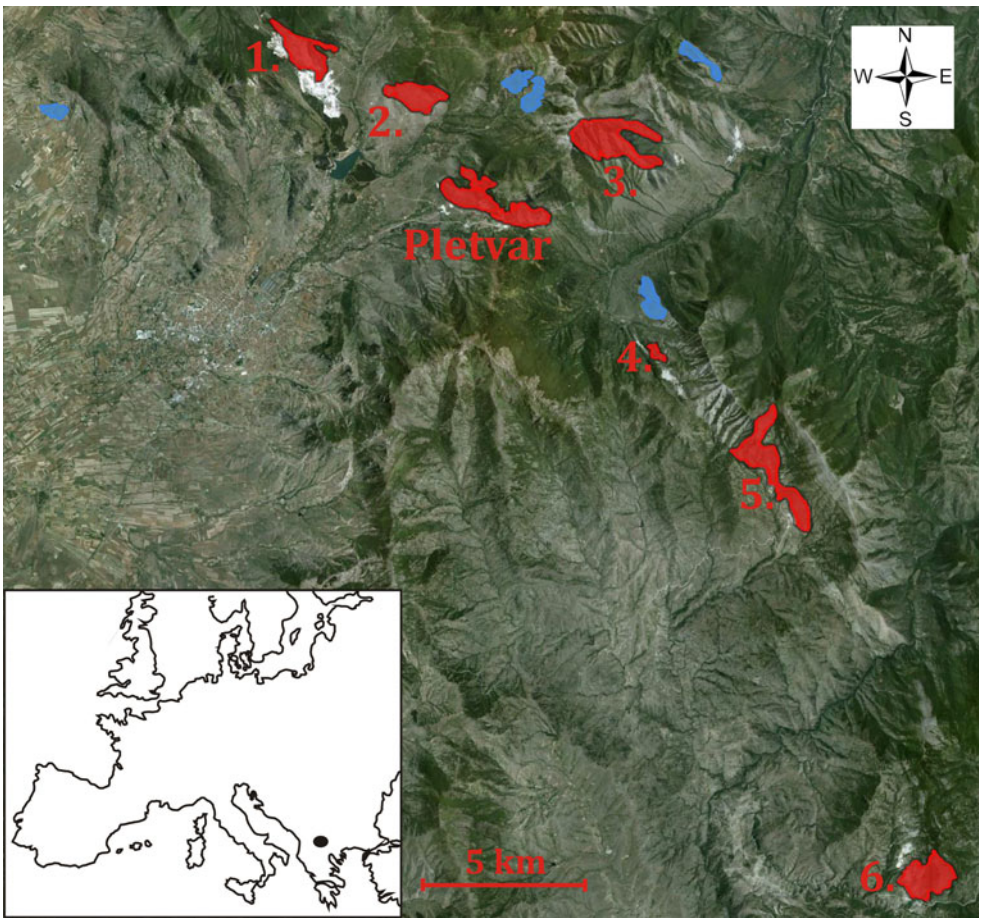

Fig. 1 The distribution and outline of potential suitable habitat of the surveyed sites in the central part of the Republic of Macedonia. The occupied sites are marked in *red* and numbered as in Table 1. Sites marked with *blue* were predicted to be suitable, but no adults were found there during our survey. Small map shows approximate position of the sampling area in Europe. (Color figure online)

Table 1 Sites with newly discovered populations of the Macedonian Grayling (*P. cingovskii*) in the central part of the Republic of Macedonia

Sites	Size (Ha)	Altitude span (m)	Quarrying	Adults observed	Distance to closest (km)	Distance to Pletvar (km)
1. Mt. Crvenica	116	880–1,220	Yes	20	1.90–Mt. Čave	4.98
2. Mt. Čave	96.2	900–1,180	No	50	1.90–Mt. Crvenica	2.16
3. Mt. Kozjak	203	920–1,700	Yes	8	2.20–Pletvar pass	2.2
4. Belovodica	14	920–1,080	No	3	3.42–Veprčani	4.95
5. Veprčani	187	900–1,440	Yes	2	3.42–Belovodica	8.95
6. Vitolište	152	710–1,170	Yes	4	10.6–Veprčani	22.79

The distances given are planimetric, measured using Google Earth

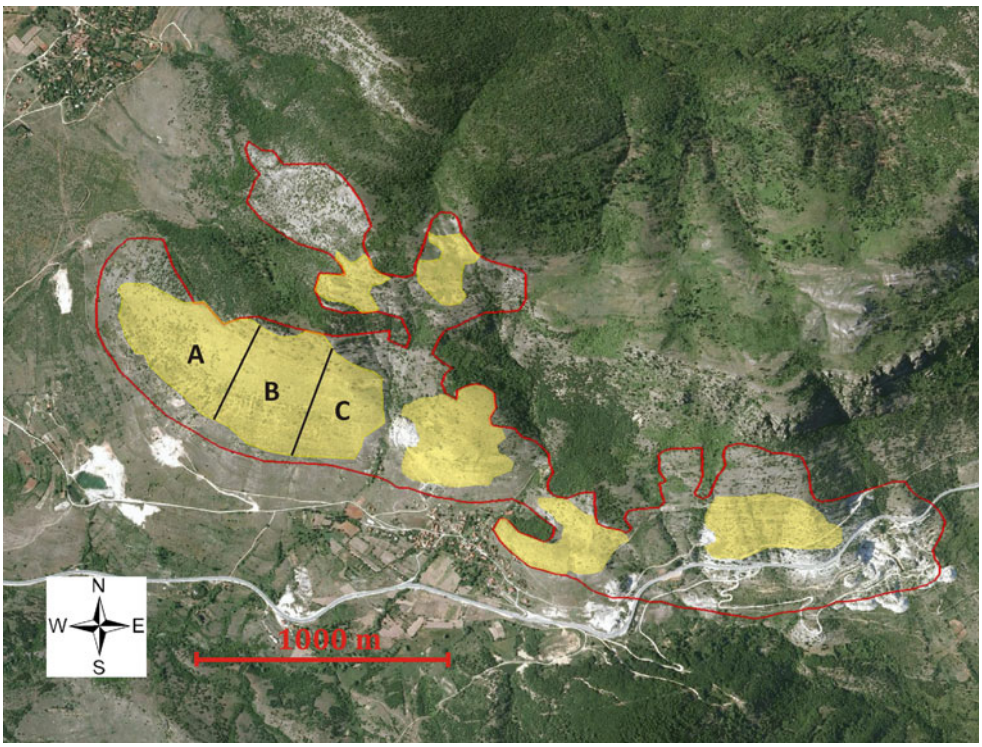

Fig. 2 Distribution of adults of the Macedonian Grayling (*Pseudochazara cingovskii*) at the Pletvar Pass on 7. July 2012 indicated by *yellow* shading. The division of the largest patch as used in MRR study is indicated with A, B and C. (Color figure online)

MRR. Oviposition behaviour was studied during the MRR survey by following females.

Results

In addition to the only known Pletvar Pass population, the Macedonian Grayling was discovered at six new sites (Fig. 1). The general information on each site is summarized in Table 1. Other sites visited (Fig. 1), where adults were not observed, were either too overgrown or the substrate was not marble.

Although the number of adults observed at each site is not directly comparable due to different effort and timing of the visits, it is clear that Mt. Crvenica and Mt. Čave sites host the largest densities of adults and are in that respect comparable to the Pletvar site. The sites north of Pletvar are also less isolated with mean distances between them at about two km, while the Vitolište site is almost 23 km away from the Pletvar pass. During our week of surveys, a total of 68 butterfly species have been found syntopic with the Macedonian Grayling.

At Pletvar Pass, the entire accessible part (some parts are too steep to walk safely) of the putative habitat was surveyed for the presence of adults. Their presence was patchier than expected with no adults seen in more overgrown parts and on west facing slopes (Fig. 2). Single specimens were also seen outside suitable habitat close to the Pletvar village where they were visiting flowers. The observed distribution is based on a single day survey, therefore it should be considered tentative with adults likely to be observed between the patches over a longer period of time. Oviposition was observed only twice at the largest habitat patch during the MRR survey. Eggs were laid either at the base of a half-dried plant or directly on a rock surface in a small crevice, in both cases in close proximity to the potential larval host plant (*Festuca* sp.). Before egg laying the females were walking around the oviposition site probing the surface with their abdomen.

During the MRR study conducted at the largest occupied patch (Fig. 2A–C), we marked a total of 372 individuals. Sex ratio was in favour of females (226/142 + 4 undetermined). Most of the adults captured (260) were fresh individuals without wing damage. A total of 77 specimens were recaptured in 110 recapture events. The daily population size estimated for 8.7.2012 was nearly identical using the Jolly–Seber method (676 ± 199 adults) and the Lincoln–Petersen Index (649 adults).

The mobility of adults was assessed using different letter codes indicating the approximate position of the captured individual (see Fig. 2). The adults proved to be extremely sedentary as 59 % were recaptured at the same part of the slope despite no obvious discontinuities in habitat suitability. Only 15 % of individuals moved from compartment A to C or from compartment C to A (Fig. 2). In

addition, only two marked males were found on the next slope approximately 100 m to the east.

Discussion

Re-evaluation of the threat status

The Macedonian Grayling is a good example of evaluation of extinction risk based on expert judgement, as no detailed studies were done prior to red listing. Its status was inferred from single site endemism (Gross 1973) and the assumption of continuing decline in distribution based on habitat loss due to quarrying (Verovnik, personal observation). Based on lack of information on actual trends it could have also been listed as data deficient. This would probably be a more rational decision, but would push the species much lower down the priority list for conservation actions and funding opportunities (but see Keller and Bollmann 2004).

Our survey was based on prior knowledge of the general habitat requirements of the species and has proven to be extremely successful in identifying additional potential sites, with 60 % of the sites selected harbouring the target species. The specificity of the habitat marked by marble rocky surface also allowed us to accurately map the potential maximum distribution of the species at each site and the calculation of the total AOO of the species. Together with the Pletvar site, the maximum total range of the species is just below the threshold of 10 km^2. However due to IUCN methodological restrictions, where calculation of AOO is based on occupancy at a 2 × 2 km grid square level, the estimated AOO of the Macedonian Grayling is 92 km^2. With all other evaluated parameters remaining unchanged this difference would result in downgrading the extinction risk of the species from CR to endangered.

Unlike in other studied large satyrinae species, *Chazara briseis* (Linnaeus, 1764), which readily disperse among patches up to 7 km apart (Kadlec et al. 2009a) or *Hipparchia semele* where even migrations were recorded (Feltwell 1976; Shapiro 1981; Maes et al. 2006) the Macedonian Grayling proved to be extremely sedentary even within habitat patches. This does not mean long distance movements are not possible, but they would be hard to detect even with lengthy MRR studies. Regardless of potential long distance dispersal, some of the sites are likely to be isolated due to mountainous terrain and the presence of over 10 km of unsuitable habitat between the sites. Thus, the range of the species could be considered severely fragmented in terms of IUCN definition of the term (i.e. criterion B2a—IUCN 2001).

The assumption of continuing decline inferred from habitat loss was also corroborated as five out of the seven sites contained active marble quarries. These have a direct effect on habitat loss through complete destruction as well as indirect (but unquantified) effects of the dust emission which covers large stretches of suitable habitat. This was particularly evident at the Mt. Crvenica site, where more than half of the entire hill has been quarried and the vegetation, especially along the access roads, was covered by a thick cover of dust. Despite that, the density of adults at that site was high, therefore further studies, particularly of larval ecology, would be required to ascertain the effects of the dust emission.

Based on the MRR study conducted at part of the Pletvar site, we consider it likely that the total population size of the species is well over 2,500 individuals, therefore the criteria C and D cannot be used to classify the species as CR (IUCN 2001). The study also expanded the known range of the species from one to seven sites which makes extinction due to stochastic events less plausible. Thus, based on new knowledge, it would be appropriate to downgrade the extinction risk for the Macedonian Grayling to the status of endangered.

Red listing criteria

Despite the methodological restrictions, IUCN Red Lists at different scales are considered one of the most important tools for providing guidelines and set priorities in species conservation (Gärdenfors 2001; Keller and Bollmann 2004; Rodrigues et al. 2006; Vié et al. 2008) as they provide a universally applicable assessment of species extinction risk (Mace et al. 2008). However, the criteria used were developed for taxa for which quantitative data on population size and decline are available, in particularly for mammals or birds. Their use for invertebrates where such data are usually lacking is not so straightforward (Regnier et al. 2009; Cardoso et al. 2011). As was the case for the Macedonian Grayling the major problem in assessment was the almost universal lack of information on population size and trends in invertebrates, which makes only a subset of IUCN criteria suitable for evaluation: in most cases, the changes in AOO or EOO as surrogates of population abundance trends are taken into account (Cardoso et al. 2012; Maes et al. 2012).

Being one of the best studied animal groups, butterflies could be considered an exception among invertebrates. However, some of the criteria are still not applicable due to generally large population sizes in butterflies as a consequence of their high reproduction rate. In the current European Red List of butterflies (Van Swaay et al. 2010), 16 % of threatened species were listed under criteria D indicating small population size or restricted occupancy, while 41 % were listed under criteria B and 43 % under criteria A. The latter two indicate a decline in distribution

and population size respectively. This implies a representative and well-developed butterfly monitoring system throughout Europe, however only a small proportion of Europe is actually monitored (Van Swaay and Van Strien 2008). Thus, the evaluation of extinction risk of European butterflies is still largely based on best expert judgement rather than actual trends and where better data are available, declines are often severely underestimated (Van Swaay et al. 2011). In that respect we must consider the current European Red List of butterflies as highly conservative.

Future prospects

Despite the suggested downgrading of the extinction risk, the Macedonian Grayling still remains one of the most threatened butterfly species in Europe. Active conservation to safeguard its future is therefore essential and the first steps towards this goal have now been undertaken. Firstly, public awareness has been raised locally by the distribution of species leaflets and by placing an information board at the Pletvar site. Secondly, the lack of legislative protection of the species and its habitat has been raised at the national level as well as a potential ban on granting new quarrying concessions at sites occupied by the species. Although quarrying poses the largest single threat to the species, it can in the long run provide a part of the solution if habitats are properly restored once exploitation ceases. Quarries already provide an important secondary habitat for many xerophilous habitat specialists among butterfly species (Beneš et al. 2003; Lenda et al. 2012). This would benefit many other local and potentially threatened species that have been observed syntopic with the Macedonian Grayling.

Further studies of the Macedonian Grayling are needed to address the potential meta-population structure and give more accurate estimates of the population size. Long term monitoring should also be established as an integral part of future conservation efforts. In addition, a study of larval ecology in terms of microhabitat selection and effects of dust deposition is essential to establish effective management of the habitat and regulate the nearby quarrying operations. The two oviposition observations are in line with known oviposition behaviour in other large satyrinae (Steiner and Trusch 2000; Kadlec et al. 2009b). They suggest that larvae may utilize short turf *Festuca* sp. growing on rocky or sandy terrain with sparse vegetation.

In more general terms, the Macedonian Grayling is a good example of an understudied and potentially extremely threatened species for which red listing has provided initial support for field surveys. There are many more such narrow endemic species in Europe and they should be considered priority targets for active conservation (Fontaine et al. 2007) even if there is only limited information on their status. Among butterflies, many small island endemics would require similar initial studies to address their conservation status and provide more robust red listing evaluations. As halting the decline of biodiversity is one of the major nature conservation targets in Europe, such studies should be given international support, as local governments in many countries show little or no interest in species level conservation.

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