Demographic parameters of two sympatric *Maculinea* species in a Romanian site (Lepidoptera: Lycaenidae)

Raluca Vodă, Natalia Timuș, Inge Paulini, Răzvan Popa, Ciprian Mihali, Andrei Crișan & László Rákosy

Summary: Romania is one of the few countries where all five European taxa of the butterfly genus *Maculinea* are known to occur. However, there are very few national studies focused on this group of butterflies, in contrast with the high amount of information available for Central and Western Europe. In this paper, we present the results of the first mark-release-recapture (MRR) study on populations of *Maculinea teleius* and *M. nausithous* that occur sympatrically on a mesohygrophilous meadow near Cluj-Napoca, Romania. By applying the MRR method throughout the entire flight period of both species, we estimated both individual survival and population size. The population size of both species was typical for *Maculinea* (2,480 individuals for *M. nausithous* and 1,198 for *M. teleius*). The estimates of survival were high (on average 0.8 for both species), suggesting relatively long life spans by comparison to results of other studies on the two species. Together with occasional observations of several years, the results of this study suggest that the populations of the studied species are relatively well conserved. This is of particular importance for *M. nausithous* since this species is known from very few localities in Romania and the population studied by us is one of the largest in the country. However, recent changes in habitat management could represent a threat to the long term survival of these Maculinea species and immediate conservation measures have to be taken in order to ensure their persistance.

Key words: distribution, *Maculinea nausithous kijevensis*, *Maculinea teleius*, mark-release-recapture, population size

Introduction

Biodiversity is globally decreasing at a fast rate (May & Tregonning 1998, Thomas *et al.* 2004), with habitat deterioration or destruction being one of the main reasons for biodiversity loss (Warren *et al.* 2001, Stoate *et al.* 2009). The most affected by this process are the highly specialized and sensitive species (Habel *et al.* 2007). The blue butterflies of the genus *Maculinea* have a specialized life cycle, are very sensitive to any change in their habitat and need a particular combination of ecological factors in order to survive (Munguira & Martin 1999, Wynhoff 2001).

They are currently considered highly endangered throughout Europe, mainly because of the changes in agriculture over the past decades and the abandonment of traditional land-use (Van Swaay & Warren 1999, Whynhoff 2001, Thomas & Settele 2004, Schmitt & Rákosy 2007, Stoate *et al.* 2009). *Maculinea* populations have also been reported to be declining in the Carpathian basin and the neighbouring Romania (Bálint 1991, 1993), although, in the latter region, the precarious economical situation largely maintained traditional land-use systems with lesser negative effects on butterfly habitats (Rușdea *et al.* 2005, Schmitt & Rákosy 2007). The decline or even extinction of the *Maculinea* species in some European countries has led to increased conservation efforts and transformed them into a thoroughly studied group of butterflies. Their interesting life cycle involving ant nest parasitism has drawn not only the attention of scientists but also of the public so that these butterflies are currently considered icon species for conservation efforts across Eurasia (Thomas 1995, Thomas & Settele 2004). However, in Romania, apart from limited faunistical data, little is known about any of the *Maculinea* taxa (Rákosy & Vodă 2008, Tartally *et al.* 2008, Rákosy *et al.* 2010). Data related to population size, population dynamics, survival rate etc. were completely lacking until 2009, when we started a research study on populations of *Maculinea teleius* and *Maculinea nausithous* occurring sympatrically on a limited area on the Hills of Cluj and Dej, Romania.

All five European *Maculinea* taxa are present in Romania (Rákosy *et al.* 2003). Their distribution is determined by the availability of suitable

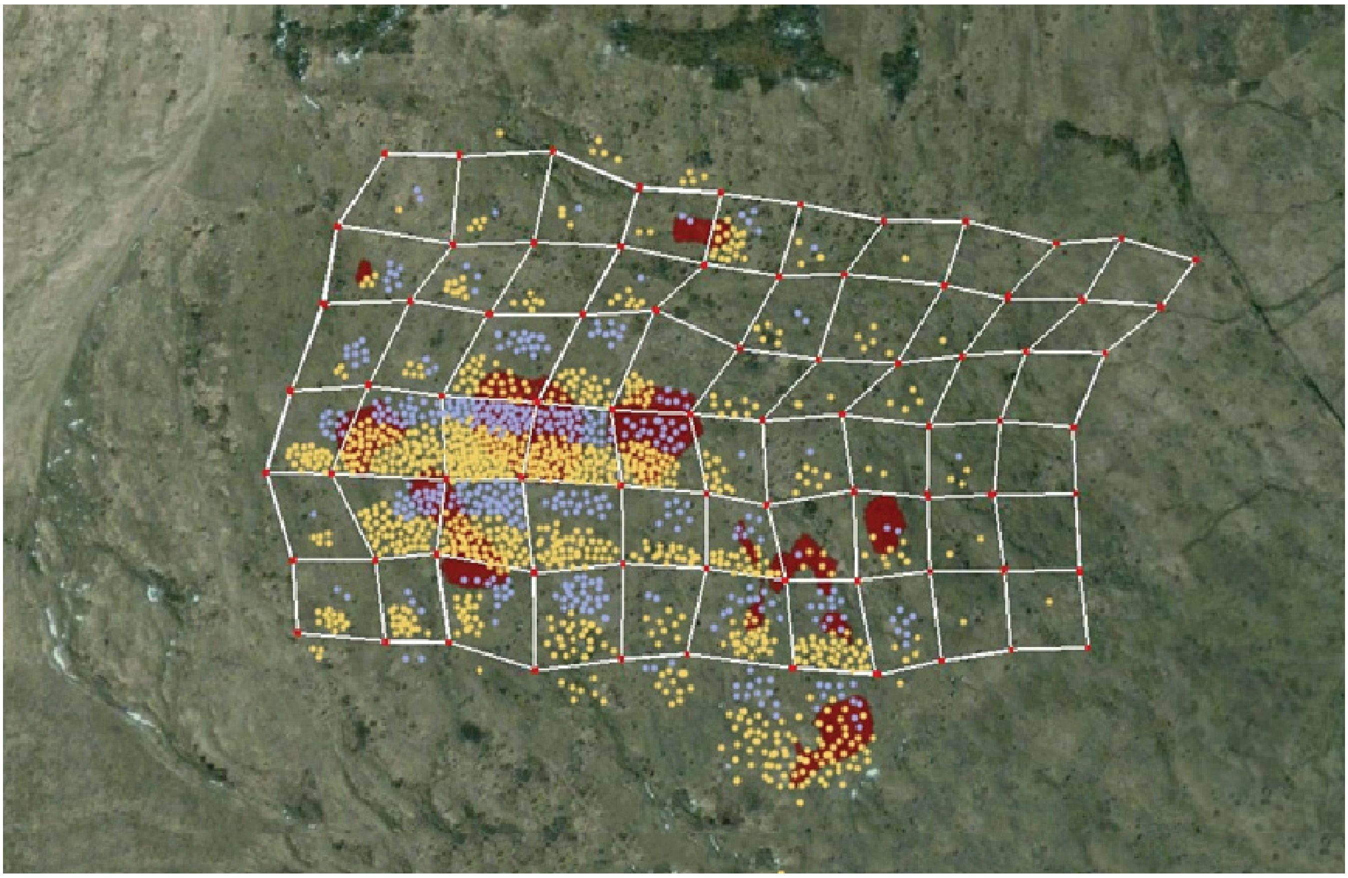

Fig. 1. The distribution of *Maculinea nausithous kijevensis* (yellow dots) and *M. teleius* (blue dots) in the studied area. The red spots represent the patches with a high density of the food plant *Sanguisorba officinalis*. Map: N. Timuş.

habitats, larval host plants and *Myrmica* host ants. Most populations are small and isolated and their habitat is often disturbed by human activities (Rákosy & Vodă 2008). *Maculinea teleius* and *M. nausithous* are species of high conservation concern in Europe, being listed on annexes II and IV of The Habitats Directive 92/43/EEC while in The Red List of Romanian Butterflies they are considered endangered (*M. teleius*) and critically endangered (*M. nausithous*) (Rákosy 2002). Recent studies showed that the populations of *M. nausithous* from Romania belong to a different subspecies compared to the populations from Western and Central Europe (Rákosy *et al.* 2010). This is the subspecies *kijevensis*, first described by Sheljuzhko in 1928 from Ukraine but largely neglected by the scientific community. Moreover, these populations differ from the Western and Central European ones in terms of their ecology and biology, since they occur in different habitats and use a different species of host ant (Tartally *et al.* 2008). Similar specimens have been found in Kazakhstan and steppic habitats from the Western part of Altai Mountains. It is assumed that Romania represents the Western distribution limit of this subspecies (Rákosy *et al.* 2010).

In this paper, we present the results of the first study focused on *Maculinea* populations from Romania. By applying the mark-release-recapture (MRR) method on populations of *M. teleius* and *M. nausithous* ssp. *kijevensis* that occur sympatrically on the Hills of Cluj and Dej, we acquired estimates on population size, survival rate and life span. These data allowed us to objectively assess the conservation status of the species in the targeted area and to propose conservation measures for their safeguarding.

Material and methods

The MRR study was conducted on the meadow Fânaţul Domnesc (Răscruci, Romania: 46º55´N; 23º44´E) at an altitude of 450 m above sea level. The studied area is a mesohygrophilous meadow, where small boggy depressions alternate with dryer patches. *S. officinalis*, the larval host plant of the studied species occurs mainly in nutrient-poor stands on intermittently wet soils belonging to *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*). In the examined area the *Molinion caeruleae* stands grow

Table 1. Parameters of the populations investigated as revealed by the MRR study.

Species	Individuals captured		Seasonal population size (with 95% CI)	Survey rate/day		Lifespan (days)	
	Males	Females		Males	Females	Males	Females
M. teleius	146	122	1,198 (595-2,914)	0.79	0.78	4.31	4.01
M. n. kijevensis	397	262	2,480 (1,634-4,063)	0.83	0.79	5.53	4.20

on north-facing slopes in a vegetation complex with wet, moist and semi-dry herbaceous communities of the classes *Molinio-Arrhenatheretea*, *Festuco-Brometea* and *Trifolio-Geranietea sanguinei* as well as single and grouped shrubs, all together forming an entire hay meadow often delimited from pastures and arable fields by lines of blackthorn and whitethorn shrubs (*Pruno spinosae-Crataegetum monogynae*).

In 2009 we marked *M. nausithous* and *M. teleius* individuals during their entire flight period, starting from the 7th of July until the 31st of August. The 15 ha sample area (300x500m) was divided by wooden stakes into smaller quadrants (50x50m) (Fig. 1). Each stake was assigned a code consisting of a letter and a number allowing us to know where a butterfly has been caught. We marked the GPS coordinates for each stake and also for the patches that had the maximum density of the larval host plant *Sanguisorba officinalis*. The butterflies were caught with insect nets, marked with numbers and released immediately at the place of capture. The marking was made with Staedler Lumocolor Special pens on the underside of the right hind wing. For each individual we recorded the number assigned, the sex and the quadrant in which it was captured. Only two people at once were involved in butterfly capturing, whenever it was possible. This was done mostly depending on weather, during the entire flight period of the two species. Each day was divided into two capture sessions, from 9.00 until 12.00 and from 14.00 until 17.00. We started each sampling day from different points and each quadrant was inspected once.

The data were analyzed using MARK 2.1 software (White & Burnham 1999), according to Cormack-Jolly-Seber type constrained models (Schwarz & Arnason 1996, Schwarz & Seber 1999). We selected the best-supporting models based on the Akaike's Information Criterion (AICc), choosing the ones with the lowest AICc value and with the lowest number of parameters in order to increase the precision of the estimates (Nowicki *et al.* 2005a). For the selected models we obtained the estimates of survival and capture probability. Knowing these parameters we could derive further estimates like daily population size ($\hat{N}_i = n_i / \hat{p}_i$) and recruitment ($\hat{B}_i = \hat{N}_i + 1 - \hat{\varphi}_i \hat{N}_i$), calculated separately for each sex. We also calculated the corrected recruitment, which is the recruitment adjusted for the individuals that could emerge and die between consecutive capture days according to the formula described by Nowicki *et al.* 2005a, 2005b, 2005c: $\hat{N}_i' = \delta_i \hat{B}_i (\hat{\varphi} - 1) / \hat{\varphi}^{\delta i} - 1$. This parameter represents in fact the total size of the population. The average life span was calculated as $\hat{e} = (1 - \hat{\varphi})^{-1} - 0.5$ (Nowicki *et al.* 2005a). Regarding the inter-site movement we recorded the average and the maximum distance an individual could cover.

Results

In 2009, the two species started to fly simultaneously, the first individuals being caught on the 7th of July (a *M. teleius* male) and on the 8th of July (a *M. n. kijevensis* male) (Fig. 2). During the entire flight period, we marked 659 individuals (397 males and 262 females) of *M. n. kijevensis* with 258 of them (192 males and 66 females, representing 45%) being also recaptured. During the sampling period, 161 individuals were recaptured only once, 39 twice and 6 individuals were recaptured at least three times. According to the estimates, the size of the population from Răscruci was 2,480 individuals, 1,329 males and 1,151 females (Table 1). Regarding *M. teleius*, we marked 268 individuals (146 males and 122 females) with 108 of them (70 males and 38 females, representing 40%) being also recaptured. During the sampling period, 57 individuals were recaptured once, 21 twice and only 3 individuals were captured at least three times. According to the estimates the size of the population was 1,198 individuals, 565 males and 633 females (Table 1).

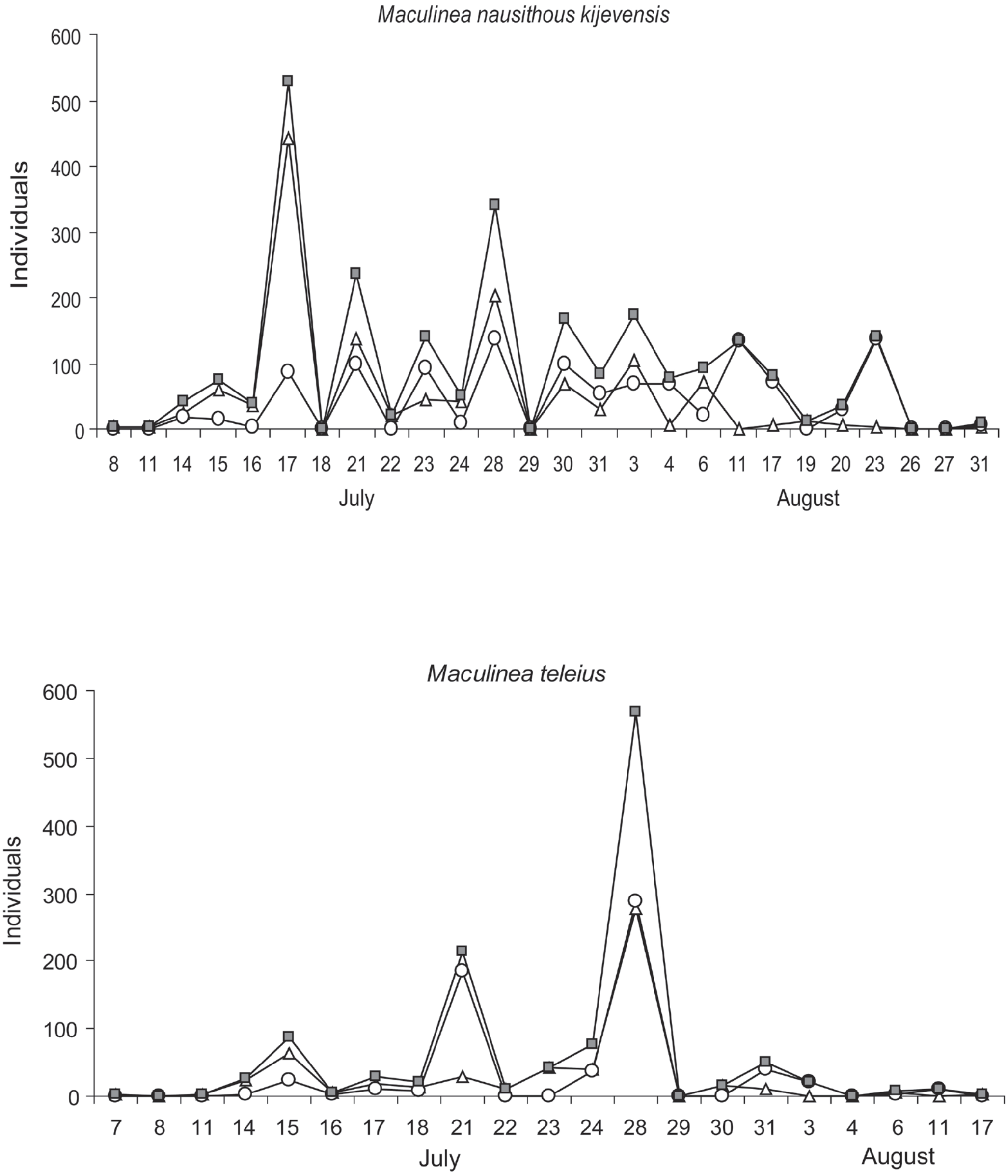

Fig. 2. Within season recruitment dynamics in the populations investigated. Squares represent total recruitment between consecutive capture days. Circles represent recruitment of females and triangles the recruitment of males.

Based on the AICc the model that best fitted the data set was, for *M. nausithous kijevensis*, *Phi*(*s*) *p*(*s*+*t*), meaning that survival (*Phi*) is constant over time but different between sexes and the capture probability (*p*) is different between sexes and varies in time. For *M. teleius* the model that best fitted the data was *Phi*(*s***t*) *p*(*s*+*t*), which means that survival and capture probability is changing over time and is different between the two sexes. The survival rate showed that approximately 20% of the population of each species disappeared (individuals that died or migrated) from one capture day to another (Table 1). The rate of the daily capture probability was very low for both species (in average 0.2) and it was very variable from one capture day to another. Each individual was caught on average 1.39 times, males being recaptured more often (1.48 times) than females (1.25 times). The estimates for survival rate and life span were high compared to other studies on the same species (Nowicki *et al.* 2005a, 2005b). In the case of the populations from Răscruci the males of *M. nausithous kijevensis* lived on avergae 5.53 days, the females 4.20 days and the maximum survival interval for one individual (a male) was of 17 days. In the case of *M. teleius*, the males lived on average 4.31 days, the females 4.01 days and the maximum survival interval for one individual (two males and one female) was of 11 days. Regarding the survival, there were some differences between sexes for both species (Table 1). Males seemed to have survived slightly longer than females, but when testing, the differences between sexes were not significant ($t = 0.9$, $p < 0.05$ for *M. nausithous kijevensis* and $t = 1.05$, $p < 0.05$ for *M. teleius*).

Regarding the larval food plant, we counted on the entire meadow (an area of 40 ha) at least 850 flowering and non-flowering ramets of *S. officinalis*, growing solitary or in small groups of up to a few tens of ramets. We observed an aggregated distribution of the *S. officinalis* ramets on the hay meadow, with 20 % of the ramets occurring on less than 0.2 % of the whole area. In the studied area (15 ha) the individuals of *M. teleius* and *M. n. kijevensis* were clumped in the more humid areas, were the host plant had a high density and the scrubs had a moderate number. In other patches, with a lower number of *S. officinalis*, we captured much fewer individuals which were usually flying rapidly between suitable patches. Around the end of the flying period, we observed that the individuals tended to disperse towards the patches were there were fewer stems of *S. officinalis*. As for the within-site movement the recorded maximum distances observed were of 500 m in the case of *M. n. kijevensis* and 300 m for *M. teleius*.

Discussion

The habitats in which *M. n. kijevensis* and *M. teleius* occur in Romania are, to some extent, different from the typical ones in other European countries, but are very similar to the tall-grass wetland habitats from the South of Siberia. In Transylvania, the two species occur in steppic meadows and tall-herb grasslands, where dryer patches alternate with small boggy depressions (Rákosy *et al.* 2010).

In 2009, the two studied species started to fly simultaneously, although the data from literature and personal observations showed that, *M. teleius* usually starts to fly about one-two weeks earlier than *M. nausithous* (Rákosy 2001, Stankiewicz & Sielezniew 2002, Buszko *et al.* 2005). The simultaneous emergence was probably due to the bad weather at the end of June, with several consecutive rainy days, followed by sunny weather at the beginning of July. Further research is needed to clarify this phenomenon.

Maculinea populations are, in general, medium sized (several hundreds of individuals), although larger ones have also been reported (several thousands of individuals) (Nowicki *et al.* 2005a). According to the estimates, the size of the populations from Răscruci is typical for *Maculinea*: 2,480 for *M. n. kijevensis* and 1,198 for *M. teleius*. Usually, *M. teleius* is more rare and has a greater decline compared to *M. nausithous* (Wynhoff 1998a,b; 2001). Our results confirm a similar situation for the studied area regarding the size of the populations, although in Romania *M. teleius* is more widespread than *M. nausithous* (Rákosy & Vodă 2008).

Regarding the daily capture probability (p), this value is rather low and is very variable from one capture day to the other. Generally, the capture probability may be influenced by wind speed and cloud coverage, since the higher they are the value of p decreases significantly (Árnyas *et al.* 2005). In this case, we can also explain the small value of p by the low number of people involved in the mark-recapture activity compared to the size of the investigated area. Moreover, the probability of capturing males was higher than of females. Probably, males are easier to capture because they have the tendency to fly more often and higher, looking for females to mate with. The females are less mobile, as they tend to fly lower, searching for suitable plants for oviposition.

The estimates of survival are rather high, suggesting relatively (to other studies on the species) long life spans (Nowicki *et al.* 2005a, 2005b). This may be partially due to favourable climatic conditions during July and August 2009, when we performed the field work.

Nowicki *et al.* (2007) showed that one of the limitative factors of *M. teleius* and *M. nausithous* populations is the size and shape of their habitat. The larger parcels with a high internal fragmentation usually have the highest densities of the two species. The main reason for this stands in the differences regarding the relative abundance of *Myrmica* species, a vital resource for the *Maculinea* butterflies (Nowicki *et al.* 2007). In the patches were *S. officinalis* grows the pressure of the butterfly parasitism is possibly much higher so that the ants find refuges in the marginal patches. Therefore, it is to be expected that the density of the ants will be smaller inside the habitat and higher on the margins, so higher in small and fragmented areas (Nowicki *et al.* 2007). This hypothesis has not yet been confirmed at Răscruci.

According to some recent observations, the highest density of *Myrmica scabrinodis* was found only in the more humid patches of the habitat (up to nine colonies of *My. scabrinodis* in a circle relevee with two metres radius; Markó B., *pers. obs.*). These humid patches are in the central part of the studied area, where also the host plant density is the highest.

The species of this genus can survive in isolated populations, in terrestrial islands or in typical metapopulational systems, although they are characterized by low mobility (Nowicki *et al.* 2005d, 2005e). Exchange of individuals between local populations rarely exceeds 20%. Although there have been recorded flight distances between two and six kilometers, these movements are very rare, the dispersion between different sites being limited to less than 500 m for all the species of the genera (Nowicki *et al.* 2005d). In the meadow from Răscruci the maximum distance recorded inside the site, was 500 m for *M. nausithous* and 300 m for *M. teleius*. In future studies we intend to extend our research outside this area and observe if marked individuals from the studied population can also fly in other proper habitats and thus allow genetic flow between populations. Although we have not started the studies outside Fânațul Domnesc, in the North-Western side of the Hills of Cluj and Dej there are several other *M. n. kijevensis* and *M. teleius* populations and we suppose that all of them are integrated in a metapopulational system.

Protection and conservation

Habitats in which we find *M. teleius* and *M. n. kijevensis* are a part of the Transylvanian cultural landscape, which is a result of the traditional activities that were applied during the last millennium. Both species are strongly dependent on *Molinietum caeruleae* habitats with high densities of *S. officinalis*. The *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils are an endangered vegetation type throughout Europe, listed in Annex I of the EU Habitats Directive. The main reason for their vulnerability is the dependency of a constant traditional management with no or little use of fertilizers and low mowing rates, which are examples of extensive land use types that have been replaced by intensive management in many parts of Europe (Poschlod *et al.* 2005). Regular and early mowing leads to very species-rich habitat, less than one-meter high stands with a dense lower and a sparse upper herb layer (Burkart *et al.* 2004). If the meadows are mown later in the year or lay fallow, single plant species like *Molinia caerulea* and *Iris sibirica* become dominant, forming species-poor, tall growing stands without many of the order and class character species, such as the host plant *S. officinalis*. In the study area, both of these types of *Molinietum caeruleae* meadows as well as transition stages can be observed. Land use change can be observed, too, namely abandonment of hay crop and increasing grazing pressure. The tendencies in Romanian agriculture policy towards adaptations to free market economy are likely to cause land use changes beyond the observed extent and demand urgent protection measures for the *Molinion caeruleae* and other oligotrophic habitat types, by establishing well-managed protected areas and also by providing financial support for extensive farming and adapted land use management.

The main reason for the decline or the extinction of some populations of *M. teleius* and *M. n. kijevensis* is the cessation of traditional agricultural practices (grazing and manual mowing) and abandonment of land. Instead of manual mowing (once a year) and extensive grazing by sheep and/or cattle, we witness an increase of grazing by sheep throughout the year, including in winter, or the abandonment of land. If, on one hand, the lack of grazing gradually leads to the alteration of the structures that are specific to the habitat preferred by *Maculinea* by favoring bush development, on the other hand intensive grazing has an immediate negative effect both on the structure of the habitat and on population effectives.

Other factors that have a negative impact on the populations of *Maculinea* and their habitat are: construction plans, drainage of humid areas and the alteration of proper, benefic habitats for *Maculinea* by planting *Robinia pseudoacacia*, *Pinus sylvestris* or *Pinus nigra*. Usually, in spring and autumn, farmers are setting on fire the dry vegetation but the effects of this impact are yet unknown.

Given the estimated populations sizes, both studied species are not endangered at present. This is of special importance for *M. nausithous* which is very rare and local in Romania, with recently confirmed occurence in only four localities (Rákosy & Vodă 2008). However, recent changes in land management could have a negative impact in medium and long term. It is therefore essential to maintain a traditional management, namely manual mowing once a year, in the second part of September, and controlled grazing with a small number of sheep.

Acknowledgements

We would like to express our gratitude to P. Nowicki for his help in the population analysis and for advice regarding field work methodology. We thank Z. Varga for comments on the manuscript and for participating in many of the field trips. We are also grateful to G. Nève for comments on the manuscript. M. Beldean, S. Badarau and C. Malos provided useful information about the vegetation in the studied area. Financial support for this research was provided by ID-552 IDEI PNII grants (Bucharest, Romania), German Federal Foundation for Environment (Deutsche Bundesstiftung Umwelt DBU, AZ 27559) and by POSDRU 6/1.5/S/3 to A. Crişan.

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Raluca Vodă
Dept. of Taxonomy and Ecology,
Babeş-Bolyai University,
Str. Clinicilor 5-7, 400006
Cluj-Napoca, Romania
raluvoda@yahoo.com

Inge Paulini
Dept. of Geobotany & Nature Conservation,
University of Bonn,
Karlrobert-Kreiten-Str. 13,
53115 Bonn

Natalia Timuş, Răzvan Popa, Andrei Crişan, Ciprian Mihali, László Rákosy
Dept. of Taxonomy and Ecology,
Babeş-Bolyai University,
Str. Clinicilor 5-7, 400006
Cluj-Napoca, Romania

Ciprian Mihali
Electron Microscopy Center,
Babeş-Bolyai University,
Str. Clinicilor 5-7, 400006,
Cluj-Napoca, Romania

Received: 9.11.2010
Accepted: 13.12.2010
Printed: 20.12.2010