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# Summary of the PhD Thesis


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## Taxonomy, biology and ecology of myrmecophilous butterfly *Maculinea teleius* (Lepidoptera, Lycaenidae) from Cluj and Dej Hills Area (Cluj County)


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# Content



Key words: *Maculinea*, *Myrmica*, Laboulbeniales, *Ichneumonidae*, myrmecophily, social parasites, parasitoids, morphology, ecology, taxonomy, biological communities, mark – release – recapture (MRR), population ecology, local specialisation, polymorphic larval growth, metapopulation system, source – sink dynamics, traditional land-use, conservation, Transylvania, România.

# Introduction

The butterflies of genus *Maculinea* Van Eecke 1915 (Lepidoptera, Lycaenidae) are extremely specialised myrmecophilous species with a complex life-cycle, that involves diferent species of plants (Asteraceae şi Rosaceae) and species of ant from *Myrmica* Latreille 1804 genus (Elmes & Thomas 1992, Fiedler 1998).

The Large Blues are examples of species adapted to traditional cultural landscapes that are threatened at European level (Elmes & Thomas 1992, Grill et al. 2008, van Swaay et al. 2012). In Romania the main factors for the decline or the extinction of some populations of *Maculinea* species are the habitat fragmentation, long-term abandonment of traditional land-use (hand-mowing, extensive grazing etc.), the inappropriate period of mowing (in the flight season of *Maculinea*), intensification of grazing (especially with sheep), drainage works, the conversion of grasslands to arable land and local urban development plans (Vodă et al. 2010, Timuș et al. 2011, Rákosy 2013, Timuș et al. 2013a).

*Maculinea* butterflies are considered umbrella species and their conservation benefits many other threatened species (Thomas et al. 2005, Skórka et al. 2007, Anton et al. 2007). Also they are regarded as the ‘flagships’ of biodiversity conservation in Europe (Thomas and Settele 2004, Kühn et al. 2005), epitomising the way that the management of their protection goes hand in hand with the preservation and conservation of human culture (Grill et al. 2008).

Up until 2009 in Romania, apart from limited faunistical data, little was known about any of the *Maculinea* taxa. The Large Blues butterflies have started being thoroughly studied after sites with *Maculinea* taxa from ‘Dealurile Clujului Est’ area were dicovered by Prof. Dr. László Rákosy. The habitats in the Natura 2000 site ‘Dealurile Clujului Est’ are probably unique in Europe, because in some northern exposed meso-higrophilous meadows five of the European *Maculinea* taxa cohabit syntopically: *Maculinea arion* Linnaeus 1758, *M. alcon ”pneumonanthe”* Denis & Schiffermüller 1775, *M. alcon “cruciata”* (also on southern exposed sites), *M. teleius* Bergsträsser 1779 and *M. nausithous kijevensis* Sheljuzhko 1928 (Rákosy 2013, Timuș et al. 2013a). This was the reason why in 2009 a series of biological and ecological studies, such as mark-release-recapture studies, *Myrmica* nests investigation, laboratory experiments etc., were initiated.

## Study area

Our studies were carried out in Natura 2000 site Dealurile Clujlui Est. Study sites:

1. meso-higrophilous abandoned meadow of cca 41 ha named Fânațul Domnesc (Chapters 1, 2, 3, 4, 5 and 6)
2. meso-higrophilous hay meadow of cca 92 ha named Fânațul Sătesc (Chapters 4, 6)
3. meso-higrophilous hay meadow of cca 81 ha named Secheliște (Chapter 6)

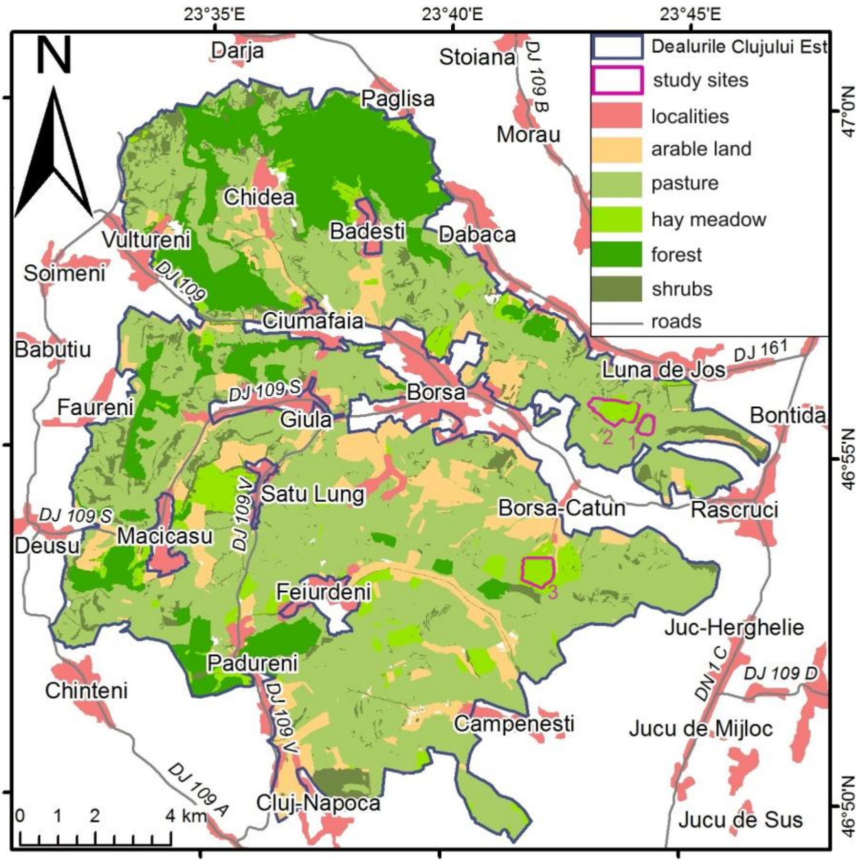


Fig.1. Map of „Dealurile Cluj Est", Natura 2000 site, with the main categories of land-use in 2012 (modified after the map created in the project „Elaborarea planului de management integrat pentru situl de importanță comunitară ROSCI0295 - Dealurile Clujului Est", Romanian Lepidopterological Society)

# **Chapter 1.** General aspects of ecology and taxonomy in *Maculinea teleius*

## *Maculinea teleius* life cycle

In Romania the Scarce Large Blue (*Maculinea teleius*) butterfly is on the wing from July till the end of August. Females oviposit [Fig.1.1 (1)] on the young inflorescences of *Sanguisorba officinalis*, the only host plant used by these butterflies (Malicky 1968, Thomas 1984). During the first three instars (3-4 weeks) caterpillars will stay on the flowerheads and feed monophagously on the developing seeds of the host plant (Thomas 1984).

Immediatly after the last moulting [L3 exuvia Fig.1.1(2)], fourth instar caterpillars of *M. teleius* descend on the ground with the help of a silk filament (Fig.1.1 (3)] secreted from specialised glands. The first interaction with host ants evolves into an adoption ritual that varies depending on *Maculinea* and *Myrmica* species. In the case of *M. teleius* adoption process can last from tens of minutes to several hours, in which time the caterpillars are palpated by the ants with their antennas. In response to antennation, caterpillars offers droplets secreted from dorsal nectary organ (DNO) (Malicky 1968). The ants eagerly imbibe each droplet [Fig.1.1(4)], then will carry the caterpillars to the their nest. [Fig.1.1(5)] (Frohawk 1924, Thomas 1984).

While living in *Myrmica* nests, *Maculinea* caterpillars feed in two different ways: caterpillars of *M. "rebeli"* (="*cruciata*" ecotype) and *M. alcon* are fed directly by nurse ants via trophallaxis and are termed "cuckoo" species (Elmes et al. 1991), whereas caterpillars of *M. teleius*, *M. nausithous* and *M. arion* prey on ants brood and are called "predatory" species (Thomas & Wardlaw 1992).

The caterpillars hibernate in host ant nests (Thomas & Wardlaw 1992). In spring (after 10/23 months, Witek et al. 2006) *M. teleius* caterpillars grow rapidly [Fig.1.1(6)] (Witek et al. 2011) till pupation [Fig.1.1(7)], wich takes place in the upper chambers of the ants' nests called *solarium* [Fig.1.1(8)]. The adults eclose after 2 weeks [Fig.1.1(9)] (Thomas 1984, Witek et al. 2006).

## Adaptations to myrmecophily of *Maculinea* species

The fourth instar caterpillars possess a range of morphological, physiological, chemical and behavioural adaptations that enable them to enter and exploit *Myrmica* host ant colonies. Some of them are described below alongside with our own observations. *Maculinea* caterpillars have a thick and tough cuticle. As has been demonstrated by Malicky (1970), the cuticle of lycaenid caterpillars is 5-20 times thicker than that of other lepidopteran caterpillars of comparable size.

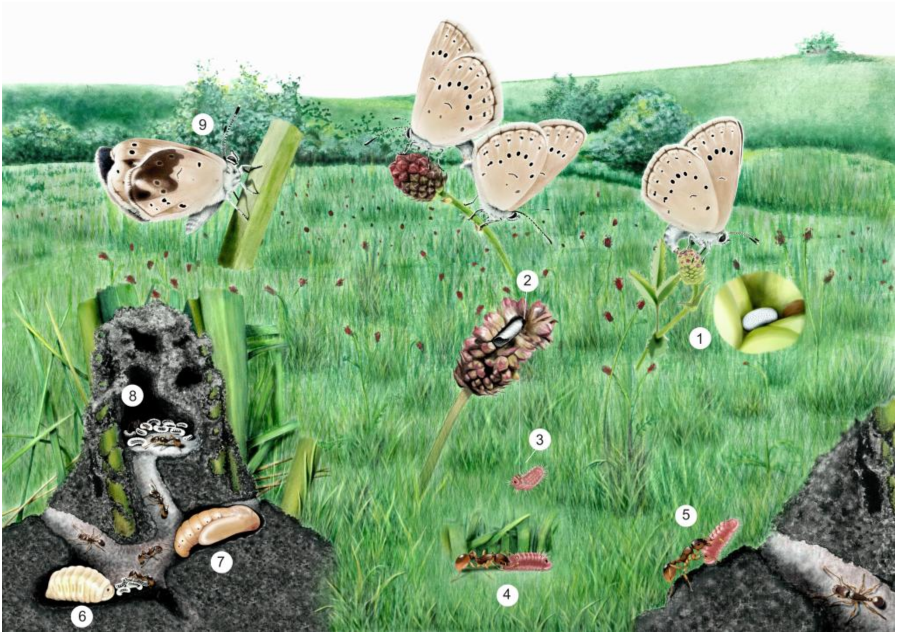


Fig.1.1. *Maculinea teleius* life cycle.

The *Maculinea* caterpillars can also retract their head under their **prothoracic shield** (Fig.1.2.a) – a cuticular fold of T1 (thoracic segment 1) (Fiedler 1991, Śliwińska et al. 2006). Thus, the most vulnerable organs (nervous system) are well protected against possible ant-attacks (Fiedler 1991) (Fig.1.2.b). When feeding on ant larvae, the *M. teleius* caterpillars pull the prothoracic shield over the head, thereby hiding their pray.

The predatory caterpillars of *M. teleius* (LIV) have on dorsal surface **long and thick setae** disposed regularly in pairs with 3/2 setae on thoracic segments and a single pair of setae on abdominale segments (AII – AVII) (Fig.1.2a). *M. nausithous kijevensis* caterpillars always have on the dorsal surface 4 long and thick setae disposed in pairs on thoracic segments T2 and T3 (pers. obs.) (Fig1.2b). The presence of the two pairs of long setae could be a specific morphological trait of *M. nausithous kijevensis* caterpillars, differentiating it from *M. nausithous* species. Studies are needed for confirmation.

During the adoption process, *M. teleius* caterpillar contracts the **first abdominal segment** (AI) (Fiedler 1990), which is smaller and without dorsal setae (Fig.1.2.b). This feature enables the ant to use its mandibles in order to pick up the *M. teleius* caterpillar and carry it into the nest.

The *Maculinea* caterpillar emanates **sounds** from muscular contractions in the abdomen or from sclerotized structures (Fig.1.2.d) between segments 4 and 7 (Barbero et al. 2009). Sounds produced by pupae and larvae of the butterfly *Maculinea* mimic those of queen ants more closely than those of workers, enabling them to achieve high status within ant societies (Barbero et al. 2009).

Over the dorsal surface of *Maculinea* caterpillar are scattered small epidermal glands called Pore Cupola Organs (**PCOs**) (Fi.1.2.f). PCOs secrete substances that generally may serve to pacify aggressive ants (Malicky 1970, Fiedler 1991).

The dorsal nectary organ (**DNO**) is located on the seventh abdominal segment (Fig.1.2.e,f) and is a specialised exocrine gland that produces nutritious secretions (Fiedler 1991). The DNO secretes droplets when stimulated by ants via antennation (Malicky 1970).

The DNO of *M. teleius* and *M. n. kijevensis* caterpillars (LIV) is surrounded by a field of **dendritic setae** (pers obs.) (Fig. 1.2.f). It is highly likely that these are specialized setae with mecanoreceptive properties as was described for *Polyomatus icarus* caterpillars which dendritic setae are able to preserve the exact time pattern of tactile stimulation of a specific species of ants (Tautz & Fiedler 1992). All our attempts to elicit the DNO secretion of *M. teleius* caterpillars (tactile stimulation with human hairs, brushes, amputated ant heads) were unsuccessful. Only live ants antennation during adoption procces could obtain the DNO secretion. Probably the mechanosensory hairs of *M. teleius* and *M. n. kijevensis* caterpillars can recognise the antennation of *Myrmica* host ants.

The PCOs and mechanosensory hairs are more dense in *M. nausithous kijevensis* caterpillars then in *M. teleius*.

## Taxonomy aspects

The genus *Maculinea* Van Eecke 1915, Lycaenidae family, Polyommatinae subfamily, comprises species restricted to the Palaearctic region (Sibatani et al. 1994, Wynhoff 1998).

Recent publications based on both molecular and morphological data (Als et al. 2004, Pech et al. 2004, Fric et al. 2007), have shown that species of *Maculinea* and Oriental genus *Phengaris* form a monophyletic group and according to Fric et al. (2007) *Maculinea* Van Eecke, 1915 should be considered a junior subjective synonym of *Phengaris* Doherty, 1891. Due to the widespread usage of the name *Maculinea* (references in Barbero et al. 2012) some of the authors have asked the International Commission on Zoological Nomenclature to conserve the name *Maculinea* against *Phengaris*. It was due to the folowing reasons that I used the name *Maculinea* in the doctoral thesis: the decision by the ICZN is still pending, in Romania a new package of the national agri-environment scheme was introduced in 2012: Agri-environment Package 6 "Grasslands important for butterflies esp. ***Maculinea*** spp".

Cryptic speciation has been hypothesised for ***Maculina teleius*** based on divergent mtDNA sequences (Als et al. 2004, Fric et al. 2007, Ugelvig et al. 2011). According to recent sudies (Ritter et al. 2013) based on mtDNA barcoding, nuclear microsatellite analyses and *Wolbachia* screening, hypothesis of cryptic speciation within *Maculinea* (*Phengaris*) *teleius* is rejected. The major splits in the mtDNA phylogeny can be explained by *Wolbachia* infections. Furthermore, the geographic isolation during Pleistocene glaciations, which likely took place in Central or Eastern Asia, contributed to differentiation of mitochondrial and nuclear genomes (Ritter et al. 2013). The phylogeographic hypothesis proposed by Ritter et al. (2013) is also corroborated by the fact that all described subspecies in *M. teleius* are restricted to the Eastern Palaearctic (*M. t. sinalcon* Murayama 1992, *M. t. obscurata* Staudinger 1892, *M. t. euphemia* Staudinger 1887) and mainly to Japan (*M. t. hosonoi* Takahasi 1973, *M. t. kazamoto* Druce 1875, *M. t. ogumae* Matsumura 1910 and *M. t. daisensis* Matsumura 1926) (Sibatani et al. 1994).

## Other taxa recorded in *Maculinea-Myrmica* system

In the study area (Dealurile Clujului Est) the *Myrmica scabrinodis* Nylander 1846 ant species is the main host for the *Maculinea* taxa*,* and for the syrphid ***Microdon myrmicae*** Schönrogge et al. 2002 (Fig. 1.3.a,b,c). Also these ants are parasitised by ectoparasitic fungus ***Rickia wasmannii*** Cavara 1899 (Tartally et al. 2007) (Fig.1.3.d,e).

In Romania there are limited available data relating to *Maculinea* parasitoids species. Pupae belonging to the two *Maculinea alcon* ecotypes parasitised by *Ichneumon eumerus* were found at Șardu and Răscruci (Tartally 2008). As a result of investigations carried out on *Myrmica* nests in 2010 and 2012 Luna de Jos, Cluj county, Transylvania, Romania), *M. alcon* („pneumonanthe" ecotype) pupae were found in three *Myrmica scabrinodis* colonies. In total 13 infested pupae (Fig.1.3.g) were collected and kept under laboratory conditions, from which 3 males and 5 females *Ichneumon balteatus* Wesmael 1845 (Fig.1.3.h) emerged after 1-2 weeks. Prior to our recent discovery, *Ichneumon balteatus* was only known to utilise two host species: *Melitaea cinxia* (L., 1758) (Nymphalidae) and *Calliteara pudibunda* L. (Lymantriidae) (Constantineanu 1959, Paul & Hanski 2004), and *Ichneumon eumerus* was the only recorded parasitoid of *M. alcon* butterflies (Shaw et al. 2009). Therefore the association *Maculinea alcon* and *Ichneumon balteatus* is new to science (Timuș et al. 2013b).

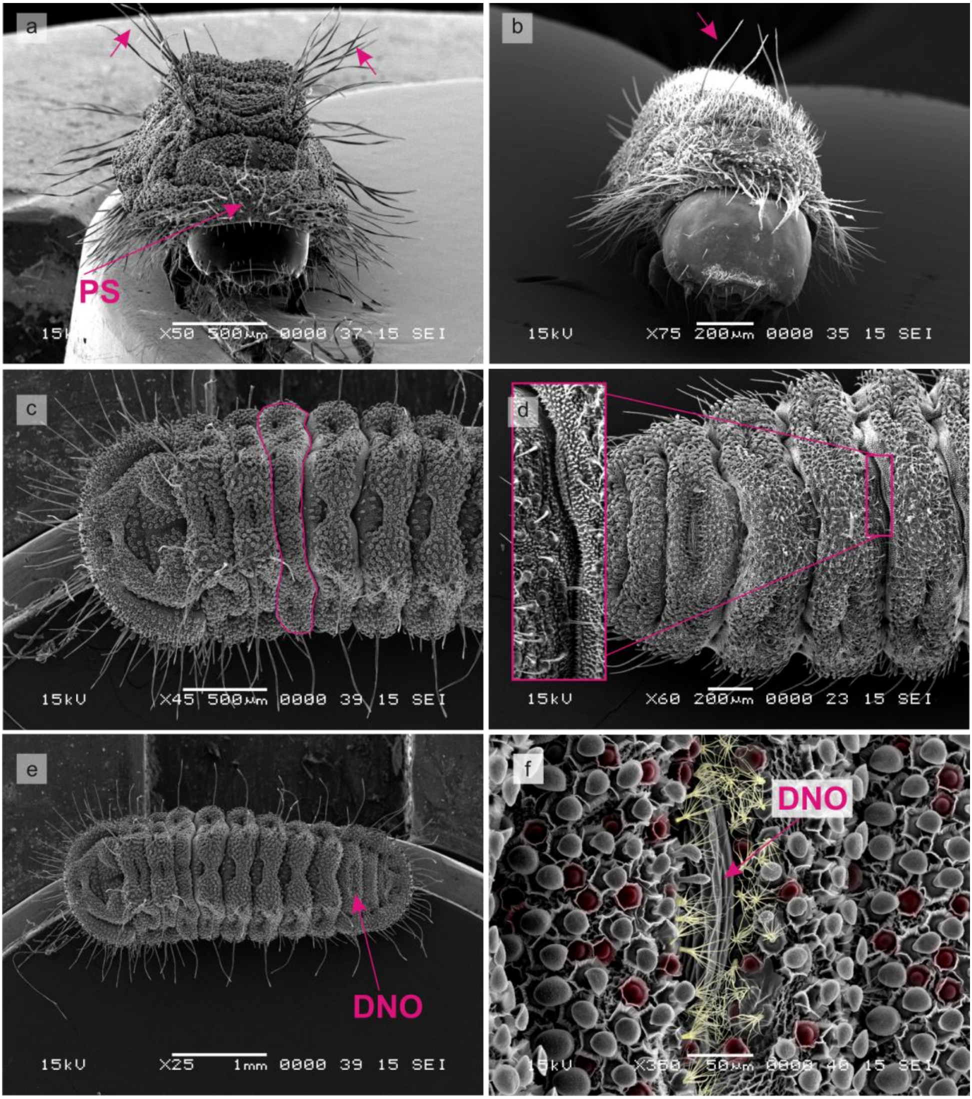


Fig. 1.2. SEM photo Ciprian Mihali

a) Dorsal setae in *Maculinea teleius* caterpillar (LIV), PS - prothoracic shield. b) Dorsal setae in *M. nausithous kijevensis* caterpillar (LIV). c) The first abdominal segment (AI) of *Maculinea teleius* caterpillar. d) Detail - sclerotized structures between abdominal segments, possible stridulatory organ in *M. nausithous kijevensis* caterpillars. e) Dorsal view of *Maculinea teleius caterpillar*, DNO – dorsal nectary organ. f) Mechanosensory hairs (yellow), Pore Cupola Organs (red), DNO – dorsal nectary organ.

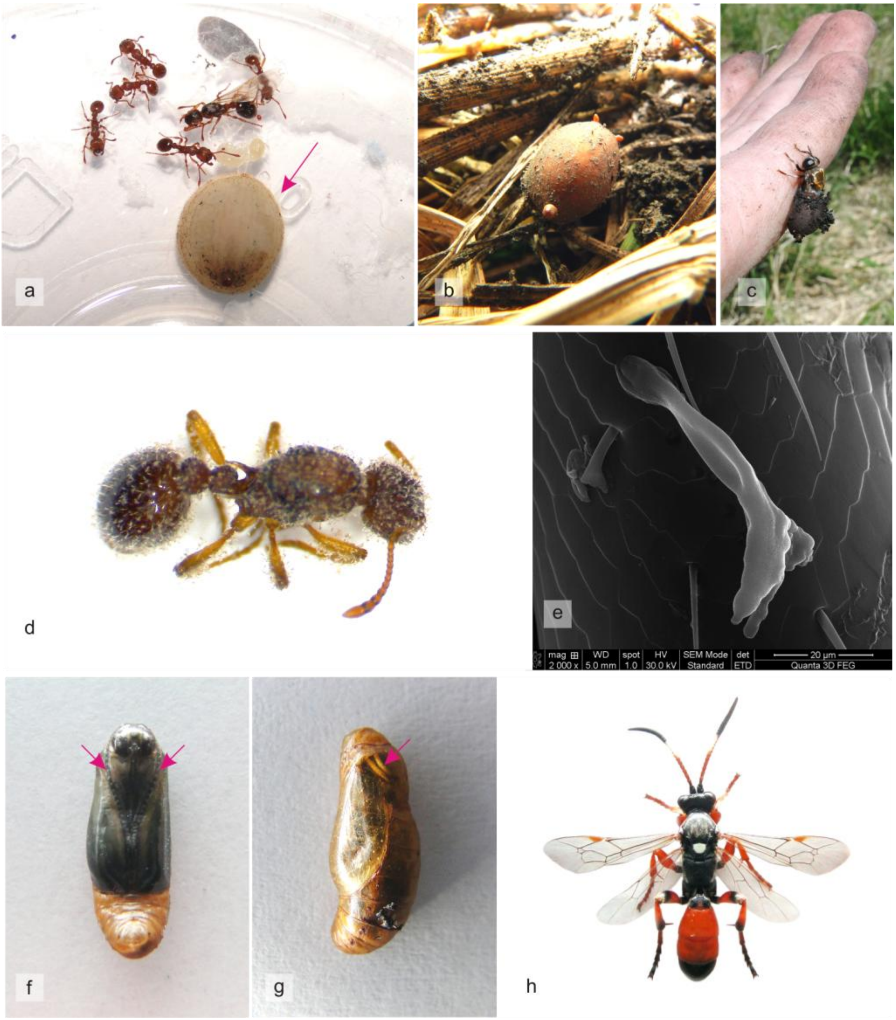


Fig. 1.3. Photo: Natalia Timuş, SEM photo: Lucian Barbu Tudoran

a) *Microdon myrmicae* larva (LIII) and *Myrmica scabrinodis* ants. b) *Microdon myrmicae* pupa. c) The eclosion of *Microdon myrmicae* adult, 2012 May, Fânaţul Domnesc, (Luna de Jos, jud. Cluj). d) *Myrmica scabrinodis* queen infested with *Rickia wasmannii*. e) *Rickia wasmannii* thallus on *Myrmica scabrinodis*. f) Ventral view of *Maculinea alcon “pneumonanthe”* pupa, arrows indicate the butterfly antennae. g) *M. alcon “pneumonanthe”* pupa (lateral view) infested with *Ichneumon balteatus*, the arrow indicate the parasitoid antennae. h) *Ichneumon balteatus* male.

# **Chaper 2.** The adoption process of *Maculinea* caterpillars by *Myrmica scabrinodis* ants infested with ectoparasitic fungus *Rickia wasmannii versus* non-infested

**Timuș N.,** Csata E., Witek M., Babik H, Czekes Z., Erős K., Rákosy L., Markó B.: Parasitic fungi as key to the ant social system. In prep.

## Aim of the study

The presence of a parasite in a system (*Rickia wasmannii*) could decisively influence the infiltration success of another parasite (*Maculinea spp*) either negatively or positively. During our study we investigated in laboratory conditions the differences between *R. wasmannii* infested and non-infested *Myrmica scabrinodis* colonies with regard to the adoption process (duration and complexity) of caterpillars of four different *Maculinea* taxa: *M. alcon "cruciata"*, *M. a. "pneumonanthe"*, *M. nausithous kijevensis* and *M. teleius* (Fig.2.2. a,b,c,d).

## Materials & methods

The infested and non-infested *Myrmica scabrinodis* colonies were collected (12.05-21.07.2012) from Fânațul Domnesc, where all four *Maculinea* taxa cohabit syntopically (Timuș et al. 2013a) and parasitize *Myrmica scabrinodis* (Tartally et al. 2008, pers obs.). *Maculinea* caterpillars of the fourth larval stage were obtained by collecting host plants with larvae of the different species from the 30$^{th}$ of June until the 15$^{th}$ of August (2012).

The adoption experiment was carried out from July to August 2012. In a *formicarium* (queenless nests containing 50 workers Fig.2.2. e,f) a single *Maculinea* caterpillar of the 4$^{th}$ larval instar was introduced. The time elapsed from the placing of the caterpillar until its discovery by ants and its transportation in the shelter (Fig.2.2.g), respectively, was recorded on a minute per minute basis for 120 minutes. In addition, the behavioural responses of ants towards the caterpillar were also noted [antennation, ant harvesting the DNO secretions droplets (Fig.2.2.h), transportation of a caterpillar in to the nest etc.]. Altogether 132 caterpillars of the four *Maculinea* forms were included in the experiment: 65 infested colonies *versus* 67 non-infested colonies.

The effect of infestation and of the effect of *Maculinea* species on the adoption rate of caterpillars was analyzed with a Cox regression approach (proportional hazard, efron method for handling ties). The sum of interactions occurring between ant workers and caterpillar was analyzed with Generalized Linear Mixed Model (GLMM) approach, Poisson error.

## Results & Discussion

Infestation of *Myrmica scabrinodis* ants with the fungus *Rickia wasmannii* influences differentially the adoption success and the duration of the adoption process of *Maculinea* species. *M. alcon "pneumonanthe"* (significant) and *M. teleius* (marginally significant) caterpillars were adopted sooner and in higher percentage by infested ants, whereas *M. nausithous kijevensis* (marginally significant) by non-infested ants. In the case of *M. alcon "cruciata"* the differences between infested and non-infested were not significant. The different effects of fungal infestation of *Myrmica scabrinodis* ants could be the result of local specialisation of *Maculinea* species for infested or non-infested ants located in the vicinity of specific host plants.

In this study we discovered that *Myrmica scabrinodis* ants, irrespective of the infestation, perform a specific adoption rituals towards *Maculinea* species. The most simple and the shortest adoption process (seconds-5min) was in *M. alcon "pneumonanthe"* (Fig.2.1). The longest (50 min) and complex (high and divers *No* of behavioral interactions) adoption ritual was with *M. teleius* caterpillars. In *M. alcon "cruciata"* the adoption process was longer (30 min) (Fig.2.1) and more complex than in *M. alcon "pneumonanthe"*. *M. nausithous kijevensis* caterpillars were significantly more inspected (No of antennation) but the duration of adoption process was shorter (15 min) than *M. alcon "cruciata"* and *M. teleius.*

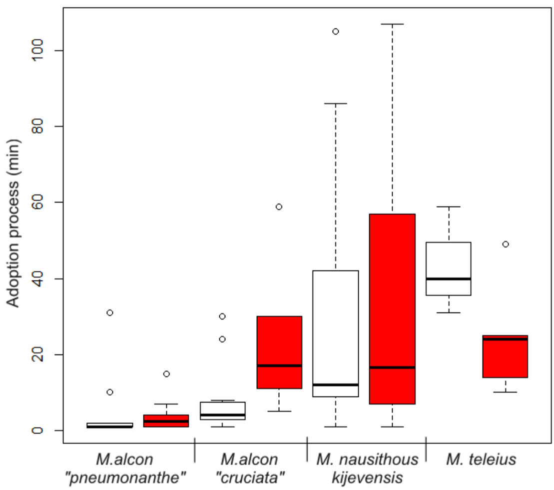


Fig. 2.1. The duration af adoption process of *Maculinea* species with *Myrmica scabrinodis* infested ants (red) and not infested (white).

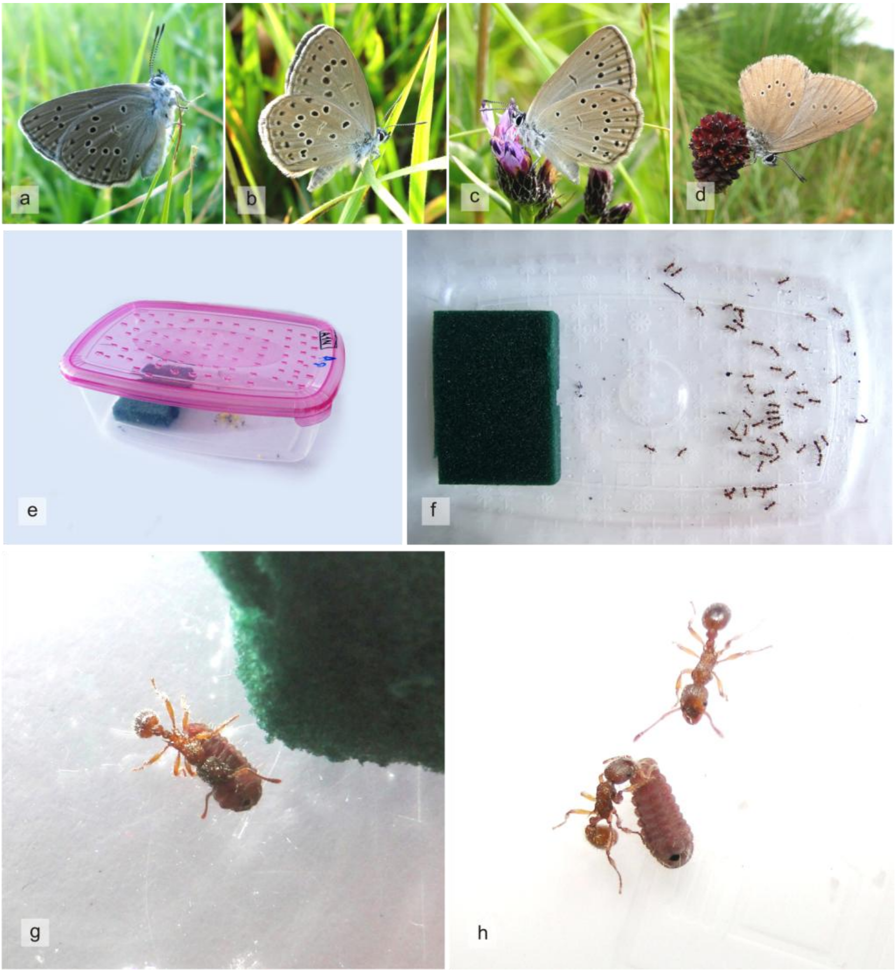


Fig.2.2. Photo: Natalia Timuş

a) *Maculinea alcon "cruciata".* b) *Maculinea alcon "pneumonanthe*". c) *Maculinea teleius*. d) *Maculinea nausithous kijevensis*. e) Formicarium f) *Myrmica scabrinodis* colony wth 50 worker ants. g) *Rickia wasmannii* infested ant of *Myrmica scabrinodis* transports *M. teleius* caterpillar. h) *Myrmica scabrinodis* ant "imbibe" the droplet secreted by *M. teleius* dorsal nectary organ.

# **Chapterl 3.** Survival and development of *Maculinea* caterpillars in *Myrmica scabrinodis* colonies infested with *Rickia wasmannii* versus non-infested

Some results of this study in: Markó B., Csata E., Timuș N., Hughes M., Tartally A., Csősz S., Rózsa L.: A unique multispecies parasitic system worth protecting in ants. In prep.

## Aim of the study

We analysed the survival (days) and growth (mg) of caterpillars of four different *Maculinea* taxa (*M. alcon "cruciata", M. a. "pneumonanthe" M. teleius*, *M. nausithous kijevensis*) in *Myrmica scabrinodis* host-ant colonies infested with ectoparasitic fungus *Rickia wasmannii* versus not-infested colonies. The main objectives was to identify: the effects of fungal infestation of *Myrmica scabrinodis* ants on survival and growth of *Maculinea* caterpillars in the same laboratory conditions; integration level of *Maculinea* species in *Myrmica scabrinodis* colonies; differences between *Maculinea* "cucko" species (*M. alcon "cruciata", M. a. "pneumonanthe"*) and "predatory" species (*M. teleius*, *M. nausithous kijevensis*).

## Materials & methods

Infested and non-infested *Myrmica scabrinodis* colonies and specific host plants as source of *Maculinea* caterpillars were collected from Fânaţul Domnesc. Altogether 158 caterpillars of the four *Maculinea* taxa were included in the experiment: 79 in colonies with infested ants versus 79 in colonies with non-infested ants. Survival and growth (body mass changes) of *Maculinea* caterpillars were assessed at 10-day intervals. The experiment was carried out from 1 July 2012 to 26 July 2013. The effect of infestation on survival of *Maculinea* caterpillars was analysed wiht Generalized Linear Mixed Model approach. Statistical analysis Mann-Whitney, Wilcoxon (comparison of general growth pattern of *Maculinea* caterpillars in infested and non-infested ant colonies), Kruskal-Wallis (comparison of general growth pattern between *Maculinea* species) were realised with the program PAST (Hammer et al. 2001). Bonferroni correction was used for each post hoc comparison.

## Results & Discussion

Survival rate and median survival time were higher in the "cuckoo" *Maculinea* species and they were very reduced in "predatory" species (Fig.3.1.). *M. teleius* caterpillars survived until 20 weeks and *M. n kijevensis* until 14 weeks. The "cuckoo" caterpillars of the *M. alcon* ecotypes survived until 11 months inside ant nests. The fungal infestation of ants influences differentially the survival of *Maculinea* species (Fig.3.1.). The results showed a reduced survival rate (Fig.3.2.) and median survival time of *M. n. kijevensis* caterpillars in infested colonies. The infestation of ants have a positve effect on *M. teleius* demonstrated by higher values of survival rate (Fig.3.2.) and body mass (is the only case were the infestation have an effect on growth of caterpillars). In *M. a. "cruciata"* survival rate and median survival time, although marginally significant, were higher in infested colonies. For *M. a. "pneumonanthe"* the difference between infested and non-infested ants, was insignificant.

All studied *Maculinea* taxa (exception *M. a. "cruciata"* ) grew rapidly in the first 10 days (Fig.3.2., Fig.3.3.d), achieving a specific post-adoption weight. *M. a. "cruciata"* caterpillars grew constantly till pupation (Fig.3.2). The specific post-adoption weight of *M. a. "pneumonanthe"* remained stable from August till March-April, when it was possible to distinguish two groups of caterpillars, faster and slower growing caterpillars, which may indicate the presence of polymorphic larvae. Differences in general growth pattern between *M. alcon* ecotypes it is likely to be a mechanism for synchronizing their flight periods with the availability (phenology) of specific host plants (Sielezniew & Stankiewicz 2007).

Irrespective of the infestation the *Maculinea* caterpillars had a specific level of integration in ant colonies. *M. a. "pneumonanthe"* and *M. a. "cruciata"* were extremely well integrated in host ant colonies (Fig.3.3.a), *M. n. kijevensis* are well integrated (Fig.3.3.b), and *M. teleius* caterpillars were tolerated or ignored by host ants. *M. n. kijevensis* showed "cuckoo" and "predatory" behaviour, and most important aspect was the re-adoption of caterpillars after reintroduction in their host ant colonies. Only "cuckoo" *M. a. "pneumonanthe"* and *M. a. "cruciata"* caterpillars became infested by *R. wasmanii* after one month spent in infested colonies (Fig.3.3.e,f). The infestation of *Maculinea* caterpillars had no detectable effect. Only a single caterpillar of *M. alcon "pneumonathe"* pupated wich was infested with *Rickia wasmannii*. Also, I found infested *M. alcon "pneumonathe"* caterpillars in the field (June 2013, Fânaţul Domnesc).

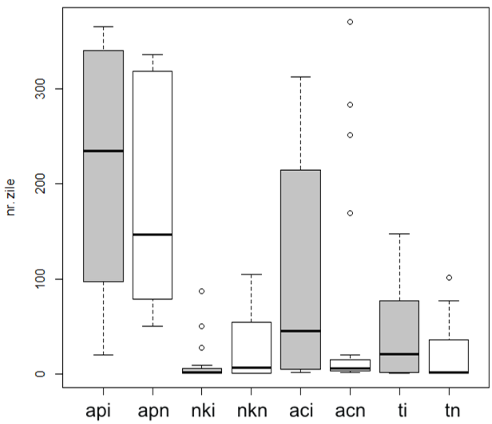


Fig. 3.1. The median survival time of *M. a.* "*pneumonanthe*" in infested colonies (api) vs non-infested (apn), *M. a.* "*cruciata*" (aci vs acn), *M. n. kijevensis* (nki vs nkn), *M. teleius* (ti vs tn).

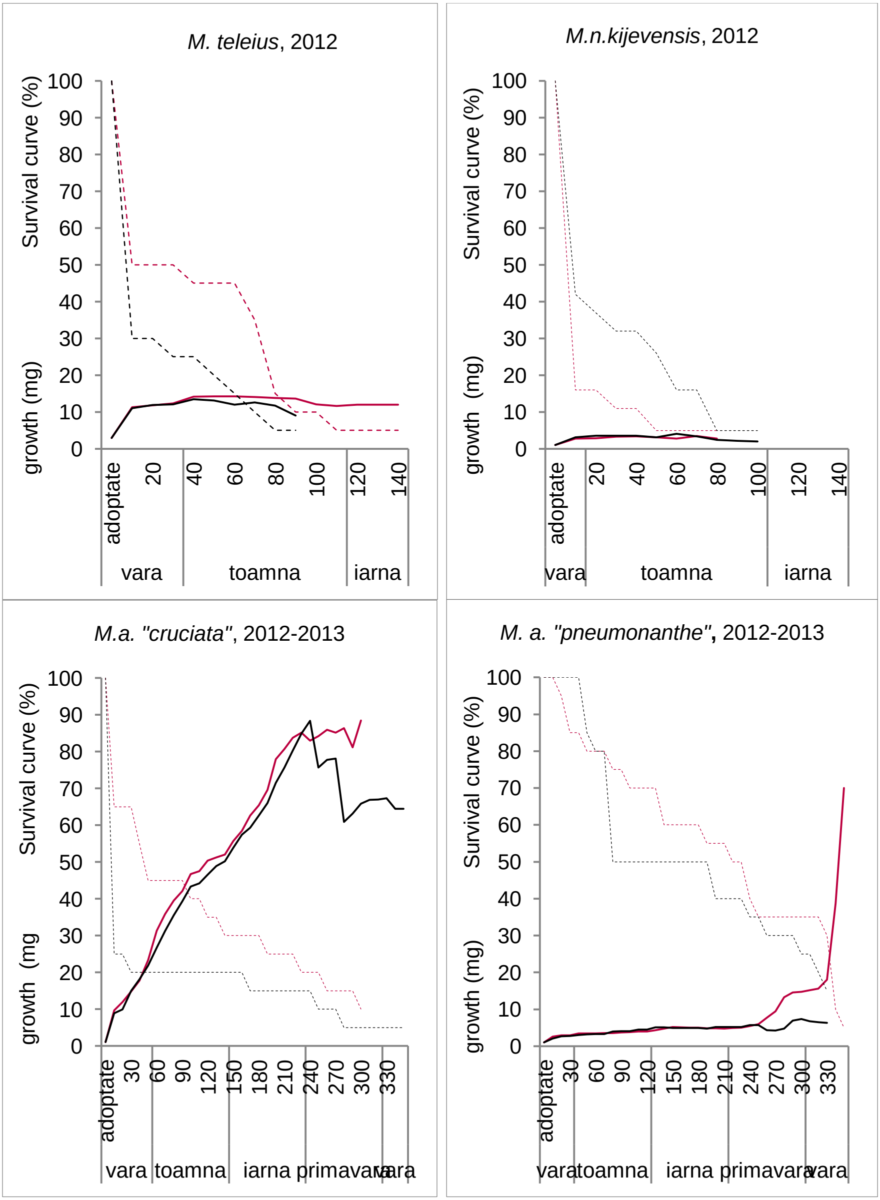


Fig. 3.2. Survival curve (interrupted line) and growth curve (continuous line) of *Maculinea* caterpillars in infested *Myrmica scabrinodis* colonies (red) and non-infested colonies (black).


Fig.3.3. Photo: Natalia Timuş

a) *Myrmica scabrinodis* ant transorts *M. alcon "pneumonanthe"* caterpillar. b) Adopted caterpillar of *M. n. kijevensis* (arrow) among *Myrmica scabrinodis* ant brood, beneath floral foam (artificial nest). c) Pre-adoption fourth instar caterpillar of *M. teleius* (2,9 mg). d) Post-adoption fourth instar caterpillar of *M. teleius* (11,7 mg) after 10 days spent in host ant colony e) *M. alcon "pneumonanthe"* caterpillar infested with *Rickia wasmanii*. f) *M. alcon "cruciata"* caterpillar infested with *Rickia wasmanii*.

# **Chapter 4.** Demographic parameters of *Maculinea teleius* and *M. nausithous kijevensis* from "Dealurile Clujului Est"

Parts are published as: Vodă et al. 2010, Timuş et al. 2011

## Aim of the study

We applied the mark-release-recapture (MRR) method to study the adult populations of the *M. teleius* and *M. nausithous kijevensis* species with the aim of gathering knowledge of importance for their conservation. In the study area both species have similar ecological requirements: *Sanguisorba officinalis* as host plant and *Myrmica scabrinodis* as host ants.

## Materials & methods

The population of *Maculinea teleius* (Fig.4.2a) from Fânațul Domnesc was analysed with mark-release-recapture (MRR) method in 2009, 2010 and 2011. The MRR method was applied in 2011 on syntopic populations of *Maculinea teleius* and *M. nausithous kijevensis* from Fânaţul Domnesc (Fig.4.2.a,c) and Fânaţul Sătesc (Fig.4.2.b,d). Data were analysed separately for each species with the Cormack-Jolly-Seber type constrained models (Schwarz & Arnason 1996) using the program MARK 6.0 package (Cooch & White 2010).

## Results & Discussion

The adults of *M. teleius* and *M. n. kijevensis* flew for approximately 7 weeks, between the 7$^{th}$ July – 25$^{th}$ August. The average life span of both species was 3-5 days (Table 4.1). The sex-ratio dynamics within season of *M. teleius* and *M. n. kijevensis* always showed a pattern of proterandry, a common fenomena in *Maculinea* butterflies (Nowicki et al. 2005a).

According to the estimates, the size of the population of *M. teleius* from Fânațul Domnesc (2009-2011) was 1000 – 1800 individuals and 1200 individuals for *M. n. kijevensis* in 2011. In the year 2011 in Fânaţul Sătesc a population of 2800 individuals was estimated for *M. teleius* and 1700 individuals for *M. n. kijevensis* (Table 4.1, Fig.4.2. a,f). The *M. teleius* experienced small demographic fluctuations through the years.

For both species within-season daily population size dynamics and the recruitment of both males and females consistently followed a bimodal pattern (Fig.4.1.), as was showed in other studies (Nowicki et al. 2005b).

Only 3 individuals of *M. teleius* and 2 individuals of *M. n. kijevensis* changed sites. The results indicate that there are necessary measures (corridors and stepping stones) to enhance metapopulation viability.

**Tabelul 4.1. Basic parameters of populations of *Maculinea teleius* and *M. nausithous kijevensis (M.n.k.)* as revealed by MRR studies in Fânațul Domnesc (FD) and Fânațul Sătesc (FS).**

| | Study area & year | N.M. | N.E. | M : F (%) | φ | | p | Life span | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | M | F | M + F | M | F |
| ***Maculinea teleius*** | FD 2009 | 268 | 1231 | 48 : 52 | 0,71 | 0,71 | 0,23 | 4,34 | 4,34 |
| | FD 2010 | 279 | 981 | 46 : 54 | 0,81 | 0,81 | 0,42 | 4,66 | 4,66 |
| | FD 2011 | 342 | 1774 | 55 : 45 | 0,81 | 0,75 | 0,20 | 4,73 | 3,54 |
| | FS 2011 | 332 | 2808 | 59 : 41 | 0,73 | 0,73 | 0,15 | 4,72 | 4,72 |
| ***M.n.k.*** | FD 2011 | 235 | 1204 | 63 : 37 | 0,85 | 0,78 | 0,15 | 3,33 | 2,96 |
| | FS 2011 | 247 | 1676 | 60 : 40 | 0,81 | 0,59 | 0,20 | 4,85 | 1,93 |

N.M. – no of marked individuals, N.E. – no of estimated individuals, *p* -average daily capture probability; *φ*-average daily survival rate; M - males, F -females, M : F- sex ratio (%).

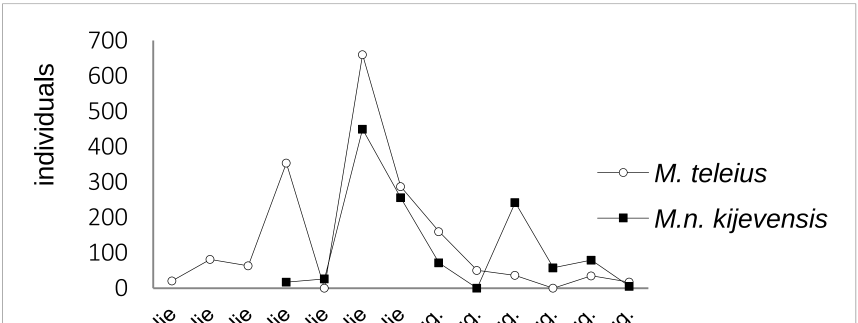


Fig. 4.1. Within – season recruitment in the populations investigated: *M. teleius* and *M. n. kijevensis*, Fânațul Domnesc, 2011.

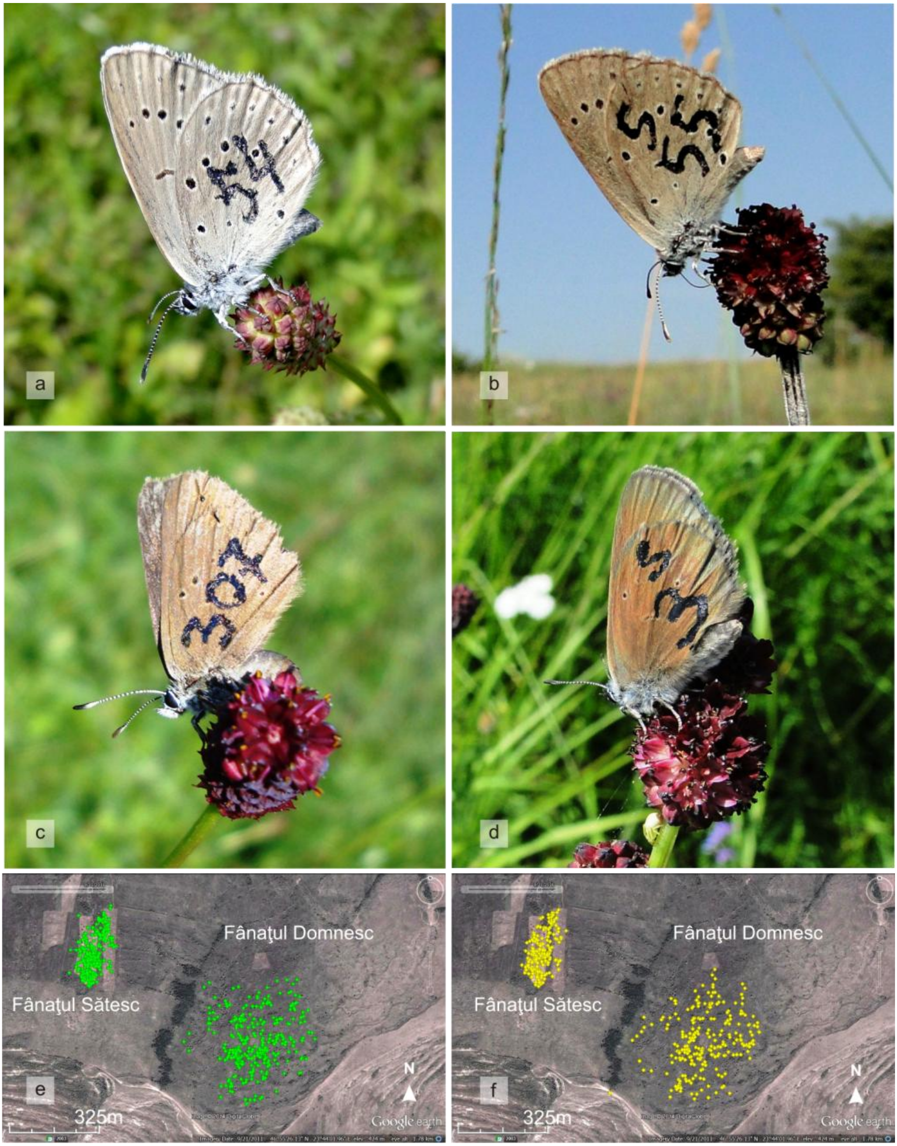


Fig. 4.2. Photo: Natalia Timuş

Marked *Maculinea teleius* individuals in Fânaţul Domnesc a) and Fânaţul Sătesc b)
Marked *M. nausithous kijevensis* individuals in Fânaţul Domnesc c) and Fânaţul Sătesc d)

e) GPS coordinates of captured *M. teleius* butterflies in Fânaţul Domnesc and Fânaţul Sătesc. f) GPS coordinates of captured *M.n.kijevensis* butterflies in Fânaţul Domnesc and Fânaţul Sătesc (Luna de Jos, jud. Cluj).

# **Chapter 5.** Mobility and spatial distribution within habitat of *Maculinea teleius* and *Maculinea nausithous kijevensis* butterflies

Results of this study in: Timuș N., Nowicki P., Rákosy L.: Within-population source-sink dynamics in *Maculinea* butterflies. In prep.
Timuș N., Czekes Z., Craioveanu C., Nowicki P., Rákosy L.: Movement patterns of two syntopic *Maculinea* species. In prep.

## Aim of the study

Recent studies (e.g. Kőrösi et al. 2012, Wynhoff et al. 2011, Nowicki et al. 2013, Skórka et al. 2013a,b) revealed that *M. teleius* and *M. nausithous* species, even occuring syntopically, have a regional and species-specific pattern of mobility and spatial distribution, shaped by ecological requirements, arrangement of resources within habitat, behaviour of resource exploitation (host plants and host ants), physical structure of available habitats. The aim of our study was to indentify the movement and spatial distribution patterns within habitat of syntopic species *M. teleius* and *M. nausithous kijevensis* from Fânațul Domnesc. In the study site both species have the same ecological requirements: *Sanguisorba officinalis* utilised as host plant and *Myrmica scabrinodis* as host ants species.

## Materials & methods

We used the mark-release-recapture (MRR) data from 2009, 2010 and 2011 and GPS coordinates. The mobility of butterflies was analysed with Generalized Linear Mixed Model and Mann-Whitney U-test. The transition probability of butterflies between high-quality habitat (source) and low-quality habitat (sink) was calculated with *multi – state* models for live recaptures.

## Results & Discussion

Our results showed that the mobility and spatial distribution of *M. teleius* and *M. nauisthous kijevensis* from Fânațul Domnesc have a specific pattern with no significant inter-specific differences. The butterflies are characterised by home-range behaviour and relatively low within-habitat mobility, the mean seasonal flight distance was 100 – 200 m. The females of both species were more mobile than males. The mobility of butterflies increased with season progression (Fig.5.1), especially females tend to cover longer distances at the end of the flight period (Fig.5.2, Fig.5.3), behaviour caused by oviposition preferences (Fig.5.5. a, b) and host plants availability. The populations of *M. teleius* and *M. nauisthous kijevensis* from Fânațul Domnesc are characterised by *source-pseudosink* dynamics within habitat: source–high-quality habitat, consistent net exporter of organisms and pseudosink - low-quality habitat, net importer, but without immigration they can sustain populations and sometimes can even become netexporters (Watkinson &

Sutherland 1995, Boughton 1999). The transition probability of butterflies from source to pseudosink increases rapidly after the peak of flight period (Fig.5.4, Fig.5.5 e,f)). The tendency to leave the source was more pronounced in females (Fig.5.4) than males, in order to avoid inter an intra-specific competition by finding host plants with no eggs and in a suitable phenological stage.

Source-pseudosink dynamics of studied butterflies could be a regulating factor of populations that mentain a diversity of syntopic *Maculinea* species in Fânațul Domnesc, through absorbtion of the individuals excess by pseudosink, thereby reducing the parasite pressure on *Myrmica* ants from source.

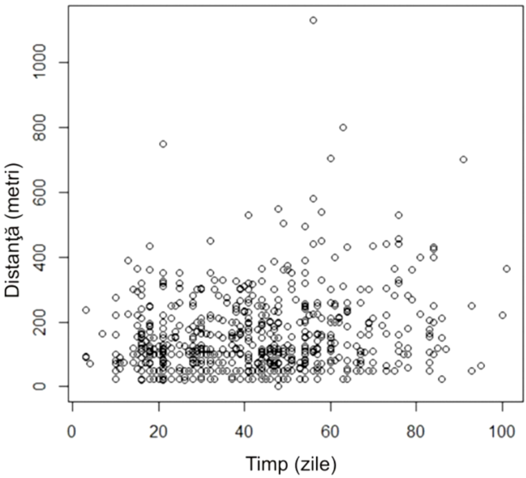


Fig. 5.1. The increasing of flight distances of *M. teleius* and *M. n. kijevensis* with season progression (time in days between the start of the season and the middle of the period between captures), MRR data from 2009-2010, GLMM.

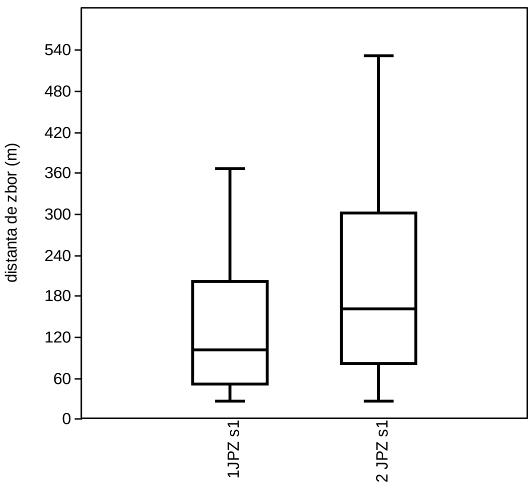


Fig. 5.2. The mean flight distance of *M. teleius* females in the first half (1JPZ s1) and the second half (2JPZ s1) of flight season, MRR data from 2009- 2010

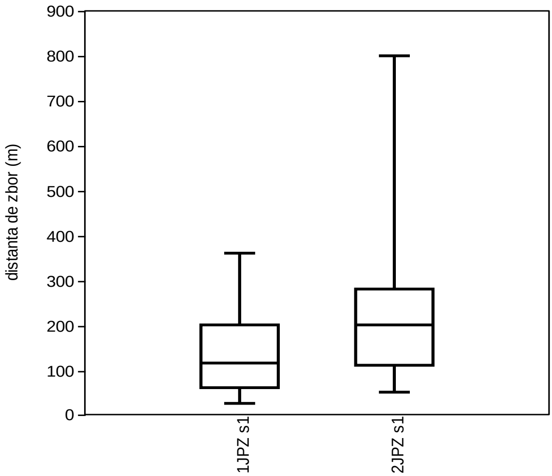


Fig. 5.3. The mean flight distance of *M. n. kijevensis* females in the first half (1JPZ s1) and the second half (2JPZ s1) of flight season, MRR data from 2009- 2010

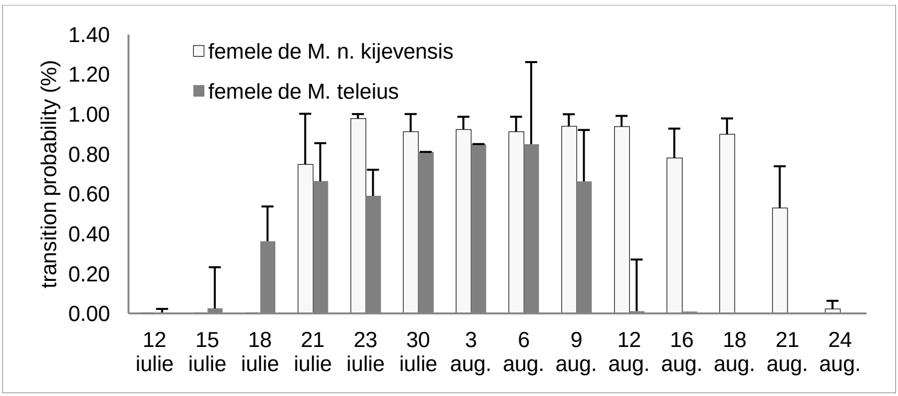


Fig. 5.4. – Probability (%) of dispersion from source to pseudosink of *M. teleius* and *M. n. kijevensis* females, 2010 (Psi A to B, multi-state model).

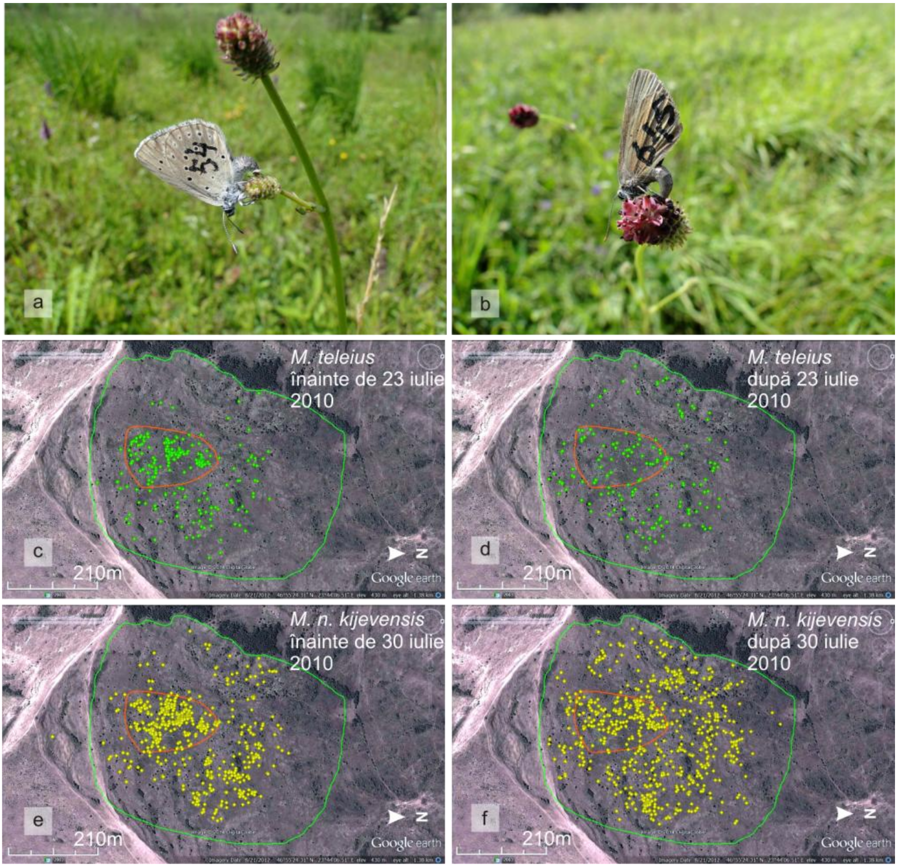


Fig.5.5. Photo: Natalia Timuş

a) Female of *M. teleius* (marked with number 54) ovipositing on young inflorescence of *Sanguisorba officinalis*. b) Female of *M. n. kijevensis* (marked with number 645) ovipositing on mature inflorescence of *S. officinalis*.

The maps shows the source (orange line) and pseudosink areas (green line) in Fânaţul Domnesc.

Captured *Maculinea teleius* individuals in the first c) and second d) half of the fight season. Captured Maculinea nausithous kijevensis individuals in the first e) and second f) half of the fight season.

## **Chapterl 6.** The land-use impacts on *M. teleius* populations from Natura 2000 site “Dealurile Clujului Est”

Parts are published as: Timuş et al. 2011

### Aim of the study

The aim was to identify in which type of land-use (abandoned, mown, grazed) *Maculinea teleius* populations are the most prosperous . Another objective was to find out if transect method could be useful to assess the land-use impacts on *M. teleius* populations. According to our results obtained from MRR method, transect counts, and information related to land-use history, we indicated the most threatening factors for *M. teleius* populations in the study area, and described specific conservation measures in order to insure the long term survival of these butterflies.

### Materials & methods

This study was carried out in three meso-higrophilous meadows: Fânațul Domnesc (41ha)- partially abandoned and intensively grazed by sheep (Fig.6.4.c), Fânațul Sătesc (93ha) - mown in a mosaic-like manner and partially grazed by sheep (Fig.6.4.b), Secheliște (89ha) – mown in a mosaic-like manner, the grazing is forbbiden (Fig.6.4.a). In each meadows we delimited the habitats with *Maculinea teleius*: 20 ha in Fânațul Domnesc (FD), 5 ha Fânațul Sătesc (FS), 15 ha Secheliște A (SA) and 3,8 ha Secheliște B (SB). We recorded *M. teleius* individuals with transect method in FD, FS, SA and SB, and a mark-release-recapture (MRR) method was applied in FD and FS. The MRR data were analysed using the program MARK 6.0 package (Cooch & White 2010). Transects data and MRR data were compared through an analysis of variance (one-way ANOVA).

### Results & Discussion

Using the transect method the lowest values were recorded in Fânațul Domnesc (53 individuals) and Fânațul Sătesc (62 individuals) (Fig.6.1., Fig.6.2.). Higher values were obtained in Secheliște A (108 individuals) and Secheliște B (140 individuls) (Fig.6.1., Fig.6.2.). Transect count results are highly correlated with estimates obtained with MRR (Fig.6.2.). The estimated populations of *M. teleius* were: 5276 individuals in Secheliște B, 2808 de indivizi în Fânațul Sătesc, 1937 de indivizi în Secheliște A, 1774 de indivizi în Fânațul Domnesc (Fig.6.3.).

*M. teleius* thrives in meadows (Dealurile Clujului Est) were mowing was applied tardily, asynchronously and in a mosaic-like way. The grazing on these medows was forbbiden. We recommend avoiding the mowing and grazing of meadows on which *M. teleius* colonies were identified between 1.06 – 10.09. A compromise solution, which could be convenient for farmers and hay production and also maintaining *M. teleius* population at a balanced level, is that of mowing being applied after 25.08. Transects method may be a useful tool in tracking population trends over time and their response to changing land use.

The main reason for the decline or the extinction of some populations of *M. teleius* in the investigated area is the conversion of grasslands to arable land (2013, 2014) and the mowing of meadows in the flight season of these butterflies (2012) (Fig.6.4.d). Factors that have a negative impact on the populations of *Maculinea* and their habitat are: the cessation of traditional agricultural practices (grazing and hand-mowing) and abandonment of land, construction plans, drainage of humid areas (Fig.6.4.f) and the alteration of proper, benefic habitats for Large Blues by planting *Robinia pseudoacacia*, *Pinus sylvestris* or *Pinus nigra*. Usually, in spring and autumn, farmers are setting on fire the dry vegetation (Fig.6.4.e) but the effects of this impact are yet unknown.

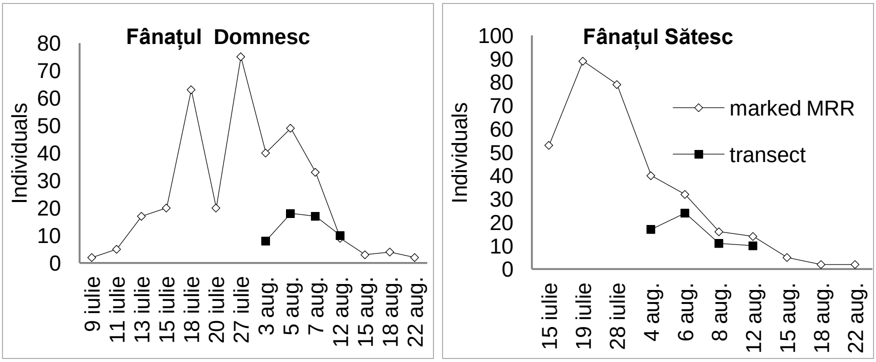


Fig. 6.1. Number of *M. teleius* individuals recorded with transect method and number of individuals marked with MRR method, 2011.

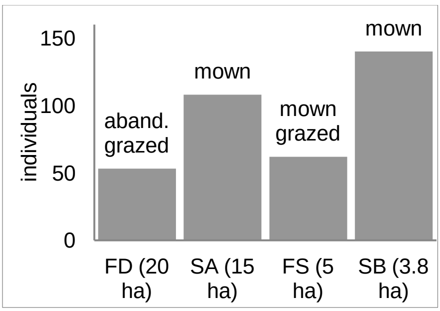


Fig. 6. 2. Number of recorded *M. teleius* individuals with trensect counts in Fânațul Domnesc (FD), Fânațul Satesc (FS), Secheliște A (SA), Secheliște B (SB), 2011.

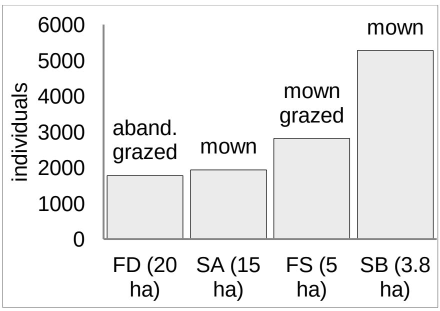


Fig. 6.3. The estimated population size (MARK) of *M. teleius* in Fânațul Domnesc (FD), Fânațul Satesc (FS), Secheliște A (SA), Secheliște B (SB), 2011.

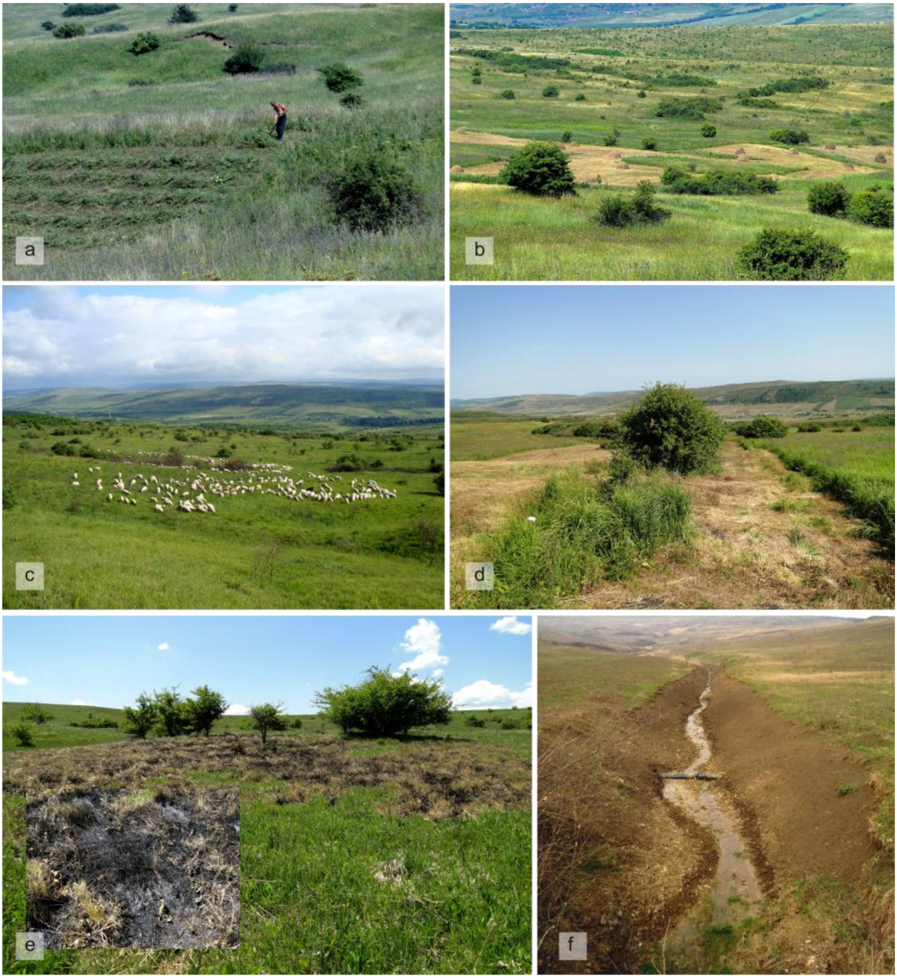


Fig.6.4. Foto: Natalia Timuş

a) Sechelişte – mown in a mosaic-like manner, the grazing is forbbiden. b) Fânaţul Sătesc - mown in a mosaic-like manner and partially grazed by sheep. c) Fânaţul Domnesc- abandoned and partially and intensively grazed by sheep. d) Site with syntopic *Maculinea* species from Fânaţul Sătesc – mown in July 2012.
e) Burnt grass, *Molinia caerulea* patches in Fânaţul Domnesc (May 2011). f) Drainage canal (2009) on site (southern exposed) opposed to the *Maculinea* site Fânaţul Domnesc (northern exposed).

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**Timuş N**., Nowicki P., Rákosy L.: Within-population source-sink dynamics in *Maculinea* butterflies

**Timuş N**., Czekes Z., Craioveanu C., Nowicki P., Rákosy L.: Movement patterns of two syntopic *Maculinea* species.

*Prizes:*

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