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Facultative myrmecophily of some Lycaenid butterflies
(Lepidoptera) in Transylvania

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1. FACULTATIVE MYRMECOPHILY

Facultative myrmecophilic relationships are looser, than obligatory relationships, thus they are not characterized by host species specificity, and the larvae do not reside in the host ant nest, however the mortality of larvae is decreased thanks to the presence of ants. The survival rate of the facultative myrmecophilous *Polyommatus icarus* has raised from 55% to 94%, when being attended by the *Formica podzolika*, *F. neogaagtes* and *Tapinoma sessile* species, compared to the larvae not attendance by the ants (Axén 2000). Most Lycaenidae butterflies species are engaged in facultative mutualistic relationship with different ant species (Fiedler 1991, Eastwood and Fraser 1999, Axén 2000), such as e.g. *Tetramorium caespitum*, *Formica pratensis*, *Camponotus aethiops*, *Lasius alienus* in Europe (Fiedler 1991).

The ants protect the caterpillars from various predators and parasites (Axén et al. 1996, Fiedler 1998, Axén 2000), while the larvae reward the ant attendance with droplets rich in amino acids and sugars (Fiedler and Maschwitz 1989b, Ballmer and Pratt 1991, Fiedler 1991, Agrawal and Fordyce 2000, Wardlaw et al. 2000, Axén 2000, Weeks 2003, Fiedler 2006, Tartally and Varga 2008), which are harvested by the ants (Fiedler 1991, Weeks 2003). The dorsal nectary organ is to be found on the 7th abdominal segment and on the 8th segment is the tentacular organ. The former produces volatile substance, while the latter secretes honeydew consumed as nutrient by the ants (Axén et al. 1996, Hojo et al. 2014), which begins to function only from the 3rd-4th larval stage and this is why in a few cases the ants attack the larvae in their 2nd larval stage (Robbins 1991). Therefore, in the case of the butterfly *Neomyrma nivea periculosa*, the larvae of which is in facultative mutualistic relationship with *Tapinoma*, *Crematogaster* (Igarashi and Fukunda 2000) and *Lasius* species (Saarinen 2004), the larva allocates a lot more energy to emit

signaling behavior (larvae did not feeding, when ants are presents) in the 1st and the 2nd larval stage (Saarinen 2004) in order to avoid the aggressiveness of ants. The secretion of honeydew is very costly (Heil and McKey 2003, Pierce et al. 2002, Hojo et al. 2014), consequently these larvae will have a longer development time (Robbins 1991), will be smaller in size, and their fitness is lowered (Pierce et al. 1987, Robbins 1991, Kaminski et al. 2010, Hojo et al. 2014), but their survival rates are higher. The larvae of *Arawacus lincoides* larva has a longer development time thanks to costs related to ant attendance, but the survival rate of the larvae is higher (Robbins 1991). The same is valid for the larvae of *Apodemia paucipuncta* visited by ants belonging to the genus *Camponotus* (DeVries et al. 2004). At the same time, the parasitism rate of larvae is lower thanks to the protection of the ants (Pierce et al. 2002).

In the event of competition among facultative myrmecophilic caterpillars rivals may increase the energy invested in the secretion of honeydew (Hojo et al. 2014), as the invested energy will be returned by the positive impact of protection. A great number of factors can affect the costs and the benefits of the ant-caterpillar relationship (Axén et al. 1996). In the case of a large colony, the need for the honeydew (produced by the caterpillar) is determined by the various food reserves e.g. the abundance of aphids and the amount of extrafloral nectaries (nectar-producing plant organs outside of the inflorescence on leaf stalk or on leaves) (Axén et al. 1996), by the effect of which, the abundance of ants may increase on the food plant (Kaminski et al. 2010).

Although facultative relationships are considered looser and less specific, that does not mean, that at least a limited host-preference cannot be found. E.g. caterpillars secrete twice as much honeydew as a reaction to the frequent and extended tending by the small-sized *Tapinome sessile* species, than in the case of other ant species (Axén 2000). *Camponotus* species are also known as important partners of the *Lycaenidae* (Fiedler 2006, Bächtold et al. 2014). Ant abundance has also an effect on the amount of honeydew secreted by the larvae. The larvae of *Polyommatus icarus* facultative myrmecophilous butterflies increase the amount of honeydew produced in accordance with the ant abundance. However, when the ant abundance exceeds a certain level, the amount of the honeydew secreted by the larvae will decrease sharply (Axén, et al. 1996). As a consequence of the continuous visits from the ant, the larva has no possibility to feed (Kaminski and Rodrigues 2011). Kaminski and Rodrigues (2011) found that the cost of the

protection has species specificity, because when the facultative myrmecophilous *Parrhasius polibetes* (Lycaenidae) larva was visited by *Camponatus crassus* the weight of the pupa was lower, than in the case of the control larva. However, when the same larva was visited by the *C. melanoticus* ant species they have not recorded significant changes regarding the weight of the pupa, but the development period of the larvae was longer. Hojo et al. (2014) found, that the cuticular carbohydrate (CHC) profile of larvae of the facultative myrmecophilous species *Narathura japonica* is learned more easily by the ant *Pristomyrmex punctatus*, than that of non-myrmecophilic butterflies species. In the case of the facultative ant-larvae relationship, the larvae has to consider the invested cost and recoverable benefit rate, due to this pressure is put on the different Lycaenidae species (Kaminski et al. 2010).

2. THE AIMS OF THE STUDY

More than 75% of the Lycaenidae species are in different relationships with the ants. These relationships are characterized mainly by mutualism, which may be obligate or facultative relationship. The most common type of relationship between butterflies and ants is mutualism, when the ant protects the larva from predators and parasites in exchange for the food rich in sugars (honeydew) (Wilson and Hölldobler 1990, Ballmer and Pratt 1991, Fiedler 1991 Fiedler and Saam 1995, Fiedler 1998, Axén 2000, Weeks 2003).

Due to the mosaic character of Transylvanian Basine, many Lycaenidae species are provided with adequate habitat and they are in mutualistic relationship with certain ant species (e.g. *Maculinea* spp., *Pseudophilotes bavius hungarica*, *Kretania sephirus*, *Lycaena dispar* etc.). The population of these butterfly species are usually small and isolated, consequently they are often vulnerable. The aim of nature conservation is to preserve the populations of the species and subspecies whose distribution area is reduced. The isolation and the small population size have negative effect on the population fitness (Hill et al. 1996, Kéri et al. 2001), and is less resistant against the herbivores and the pathogens attacks (Ericson et al. 1999).

Kretania sephirus is a protected Lycaenid butterfly of semi-open grasslands, especially *Astragalo-Festucetum rupicola* associations. The investigation of the populations from the Carpathian basin is relevant from various perspectives. It is one of the important zoogeographic elements of the Carpathian basin, reaching in range the northwestern border of the Pannon region. There are several studies regarding the distribution of the imago (Fiedler and Bálint 1994), but there are only few studies dealing with the developmental forms (Bálint and Kertész 1990, Vojnits and Ács 1995) (caterpillar, adult) and their lifestyle, however we don't have knowledge about the egg laying strategy of females and the food plant preference of females and caterpillars.

Kretania sephirus is in a facultative mutualistic relationship with certain ant species as e.g. *Tetramorium* cf. *caespitum*, *Formica pratensis*, *Camponotus aethiops*, *Lasius alienus*, since according to the observations made by Fiedler and Bálint (1994), these ant species were seen on the foodplant or near the larvae. However, all these data are resulting from unsystematic field observations, no systematic data collection was designed regarding the true nature of the myrmecophily of this butterfly. The relationship of the larvae with the ants remained understudied (Fiedler and Bálint 1994). At present, in terms of the relationship with the ants, there is very little information available; numerous questions are still waiting to be answered.

Pseudophilotes bavius hungarica the Transylvanian Blue is an endemic and also a protected butterfly subspecies of steppe-like habitats. Such steppe species with relic character existing today in the Transylvanian Lowland invaded the Carpathian Plateau before the last glaciation. These populations became isolated and, in many cases, new species or subspecies have developed. The relic-endemic *P. b. hungarica* has a similar history. According to Crişan et al. (2011), the butterfly is also a facultatively myrmecophilous species (e.g. engaged in such relationship with the ant *Camponotus atricolor*). We know, though, very little about the specificity of this relationship, whether it is facultative or obligatory, and how the butterfly chooses the host plant. Similarly as in the previous case, in this case it is as well valid that all these data are resulting from unsystematic field observations, no systematic data collection was designed regarding the true nature of the myrmecophily of this butterfly.

Therefore the aim of the current study is to answer the following questions:

- (1) Which factors affect the oviposition strategy of *Kretania sephirus*?
- (2) Which are those host plant characteristics that could predict the presence of the butterfly larvae and ants on host plants in the case of both species?
- (3) How specific is the myrmecophily of the caterpillar, that is are there specific ant species that tend the caterpillars, which could also differ in their behaviour towards the caterpillar compared to other non-tending ants, in the case of both species?
- (4) Is there any food preference in the ants (e.g. protein vs carbohydrate sources), that could be a major driving force in the formation of their relationship with the caterpillar of the *Kretania sephirus* habitat?
- (5) What is the ant community structure of the *Kretania sephirus* habitats?

The answers to the above formulated questions promote an in-depth knowledge, which may be of use for the elaboration of appropriate conservation plans for both species in addition to offering important insights into the nature of facultative relationships between Lycaenid caterpillars and ants.

3. STUDY SPECIES

3.1. *KRETANIA SEPHIRUS*

Kretania sephirus (Frivaldszky 1838) the Sephir Blue is a univoltin Palaearctic species; the adults begin to fly at the same time from the end of May till the beginning of June, in the same period when the food plant flowers. They stick to their area, they do not leave the habitat of the host plant. Based on observations, the male Sephir Blue butterflies fly over a relatively small area, but they move a lot within that area (Bálint 1991). In the flight period the females are laying eggs. In about 10 days the eggs hatch and the larvae emerge, which after a feeding period become inactive, which lasts from the beginning of July until the middle of April next year. After

this, the butterfly pupates and this stage lasts for 10 days at the end of which the adult butterfly emerges.

Previous studies showed that *Kretania sephirus* is in mutualistic relationship with certain ant species (*Tetramorium* cf. *caespitum*, *Formica pratensis*, *Camponotus aethiops*, *Lasius alienus*) (Fiedler and Bálint 1994). According to the observations of Fiedler and Bálint (1994), the females lay eggs on those host plants on which certain ant species have a high number of individuals. In some situations the caterpillars may overwinter at the entrances of the ant nest (Fiedler and Bálint 1994). However, all these data are resulting from unsystematic field observations.

3.2. PSEUDOPHILOTES BAVIUS HUNGARICA

Pseudophilotes bavius hungarica (Diószeghy 1913) (Fig. 5) the Transylvanian Blue is an endemic, protected butterfly subspecies inhabiting fores-steppe patches of the Transylvanian Basin, which evolved and survived on patches with steppe-like vegetation (Crişan et al. 2011). Its host plant is *Salvia nutans*, a post-glacial relic in the Carpathian Basin (Németh 1990, Simon 1992). On the Western edge of the area, in the Transylvanian Basin, in Vojvodina in Serbia and in the Hungarian Plain, it has a patchy distribution (Jakab and Bota 2002). László Diószeghy painter and lepidopterologist described the species in 1913, collecting his specimens from Viţa (currently in Bistriţa-Năsăud County). *Pseudophilotes bavius hungarica* flies in the first or second half of May depending on weather conditions, and the female lay eggs on *Salvia nutans* in its early stage of inflorescence. In 7-12 days the whitish coloured larvae hatch (Crişan et al. 2011), and then after the first ecdysis they change colour and purple stripes appear on them similar to the calyces of the host plant (Fig. 6.). In the second half of June, after the last larval stage, they crawl down to the litter and pupate Crişan et al. (2011).

The caterpillar is engaged in facultative myrmecophily (Crişan et al 2011). Crişan et al. (2011) reported that the larvae are often visited by the ant *Camponotus atricolor* (Nylander, 1849) in Transylvania, while according to Jutzeler et al. (1997) the ant *Crematogaster sordidula*

(Nylander, 1849) also visit the *Pseudophilotes bavius hungarica* larvae, but *C. sordidula* not is present in Suatu, only in Dobrogea. However, all these data are resulting from unsystematic field observations.

4. RESULTS

4.1. Oviposition of *Kretania sephirus* on a different host plant

To predict the population size and the foodplant preference of the butterflies, many researchers studied their egg laying strategy, mostly focusing on the species belonging to familia *Lycaenidae* (Árnyas et al. 2009, Czekes et al. 2014). We have no information about the oviposition strategy of *Kretania sephirus*, so the aim of our research was to answer the following questions:

1. How does the quality of the host plant influence the oviposition behaviour of the *Kretania sephirus* butterfly?
2. Has the density of the food plant any effect on the egg laying strategy of the butterfly?
3. Are there any differences between populations inhabiting areas with different host plants?

We studied the distribution of eggs in habitats with different foodplant species, as *A. monspessulanus* at Căianu-Vamă and Bărai, *A. dasyanthus* at Bărai and Vișea and *A. exscapus* at Cojocna villages, in June 2013 and 2014, respectively. In every site we selected 60 food plants randomly, with at least 2 m distance between neighboring individuals.

When analyzing the four populations together with the help of GLMM (Poisson error term) the length of leaves of the foodplants (GLMM $z = 4.16$, $p < 0.001$, $n = 240$) (Fig. 15a) the number of its leaves ($z = 12.921$, $p < 0.001$, $n = 240$) (Fig. 15b), the distance of the first ($z = 4.34$, $p < 0.001$, $n = 240$) (Fig. 16a) and second ($z = 2.57$, $p = 0.01$, $n = 240$) (Fig. 16b) neighboring host plant ($z = 4.34$, $p < 0.001$, $n = 240$) (Fig. 16a), the length of their leaves ($z = 2.319$, $p = 0.02$, $n = 240$ and $z = -3.18$, $p = 0.001$, $n = 240$) (Fig. 16c, d) and presence of eggs on

them ($z = 2.5$, $p = 0.013$, $n = 240$ and $z = 6.44$, $p < 0.001$, $n = 240$), the vegetation height ($z = -3.55$, $p < 0.001$, $n = 240$) (Fig. 15c) and the vegetation cover ($z = -4.31$, $p < 0.001$, $n = 240$) (Fig. 15d) had had significant effect on the eggs abundance (Fig. 1., 2.).

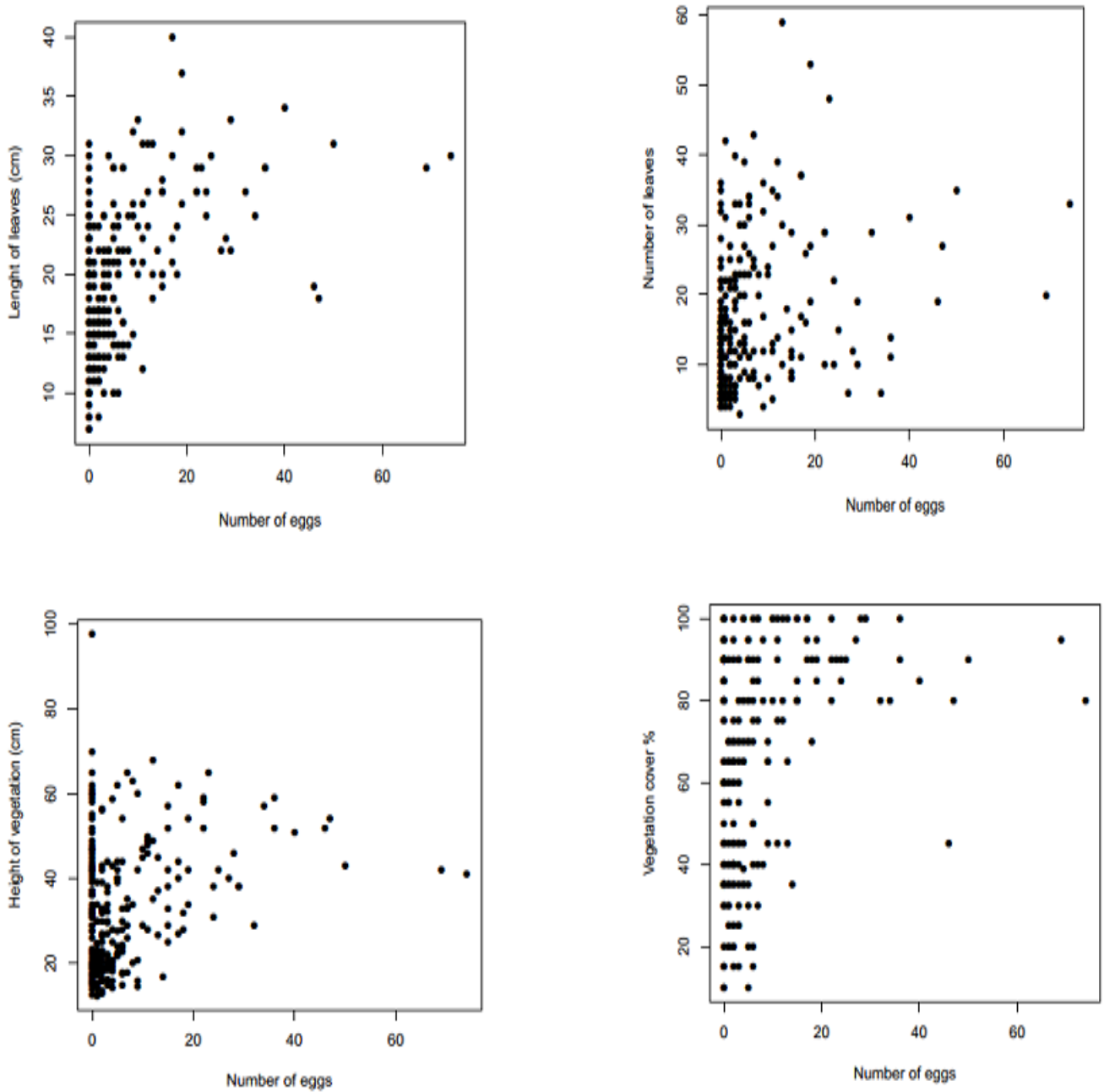


Fig. 1. The effect of the foodplant characteristics to the egg abundance

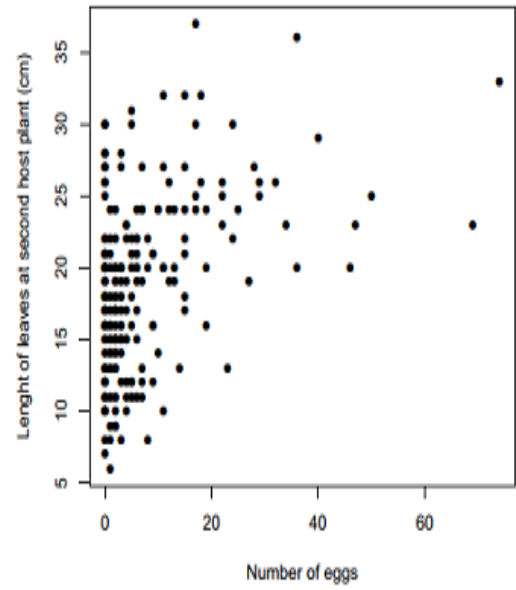
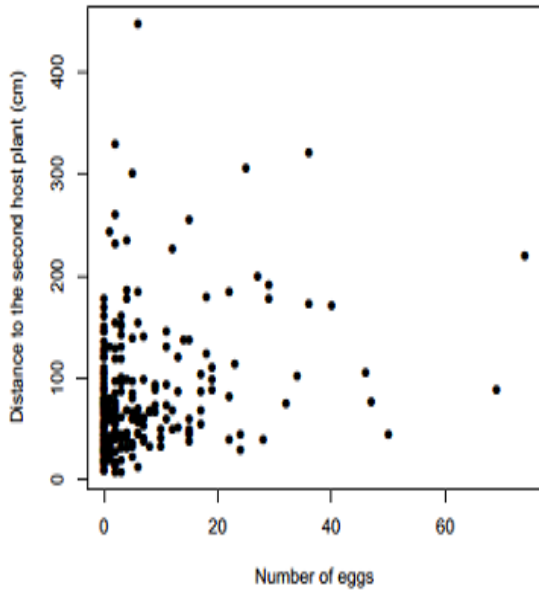
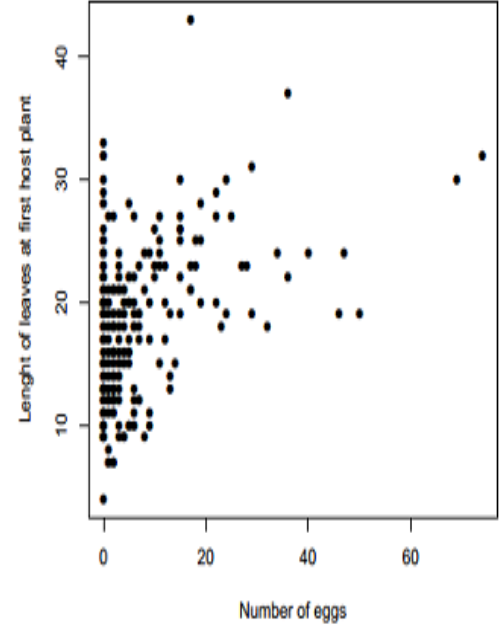
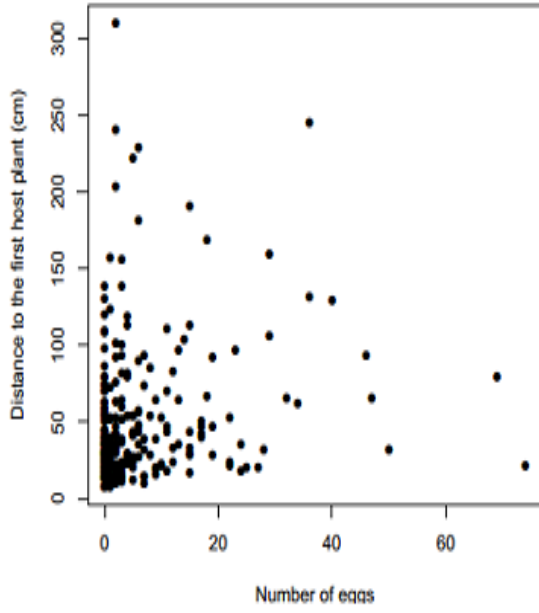


Fig. 2. The effect of the foodplant density to the egg abundance

4.2. Foraging strategies of ants at the *Kretania sephirus* habitats

We studied the ant community structure and the ant food preference in the case of two *Kretania sephirus* habitats. In the case of the ants there are two types of food source which are important. The carbohydrate source (Baker et al. 1985, Hahn and Wheeler 2002), and the animal originated protein source (Sudd and Franks 1987). The protein is necessary for their colonies and larvae's growth, however the carbohydrate is important energy supplement for the workers (Markin 1970, Cassill and Tschinkel 1999).

During the investigation we wanted the answer to the following questions:

1. What kind of community structure can be observed regarding different species of ants?
2. Do we notice any difference between the ant communities living in the habitat of two host plants?
3. Are there any noticeable differences in the food preferences of ant species regarding protein or carbohydrate rich food sources?

The ants are omnivorous, the main components of their feed are proteins and carbohydrates. The commonly used and well-proven method for the study of ant communities' structure is to use baits (Savolainen and Vepsäläinen 1988, Morrison 1996). During baiting experiments they use tuna as protein source, while for the carbohydrate source honey or sugar solutions are applied. To detect the structure of the ant community and determine the relations of dominance, we placed bait on the *A. monspessulanus* and on the *A. dasyanthus* habitats. On each area we placed baits randomly, with at least 2 m distance between the baits, in order to ensure the independence of the data. At every site we placed 40 baits with tuna (animal protein) and honey (polyfloral)

We distinguished six species groups occurring at baits. On the bait is hard to distinguish to the species, for that, in the case of - *Camponotus* species including *C. aethiops* and *C. piceus*, in *Lasius* spp. belong the *L. alienus* and *L. paralienus* species, the *Formica* spp. containing the *Formica cunicularia* and *F. rufibarbis* ant species – distinguished species group. Beside that, we

recorded *Tetramorium* cf. *caespitum*, *Plagiolepis pygmaea*, *Messor structor*, *Tapinoma subboreale* (3 individuals) and *Myrmica sabuleti* (8 individuals).

At both study sites *Camponotus* species were present in the highest abundance at baits, they were followed by *Lasius* species at *A. monspessulanus* habitat, and *Tetramorium* cf. *caespitum* (Fig. 3.). At the *A. dasyanthus* habitats after the *Camponotus* species, *Tetramorium* cf. *caespitum* was present in the largest numbers, which were followed by *Lasius* species (Fig. 3.).

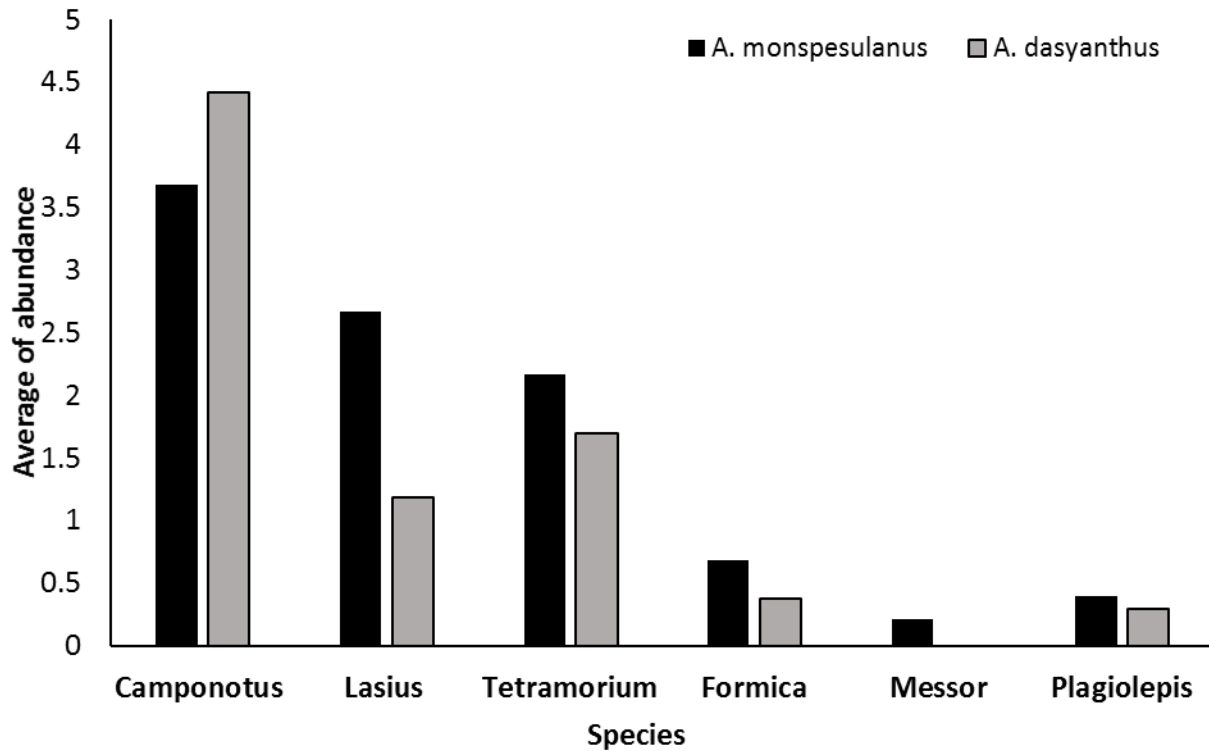


Fig. 3. Average number of different ant species at the two different habitats at baits.

The ant species diversity (Shannon-Wiener, log₂) on the baits, in the case at both study sites are similar in the case of *Camponotus* spp. and *Plagiolepis pygmaea* species

Food preferences in the studied ant communities

At the habitat of *A. dasyanthus* *Camponotus* spp., *Lasius* spp. and *Tetramorium* cf. *caespitum*, *Formica* spp. and *Tapinoma subboreale* preferred the animal protein food source and in the case of *Messor* cf. *structor* did not found a food preferences (Fig. 4.).

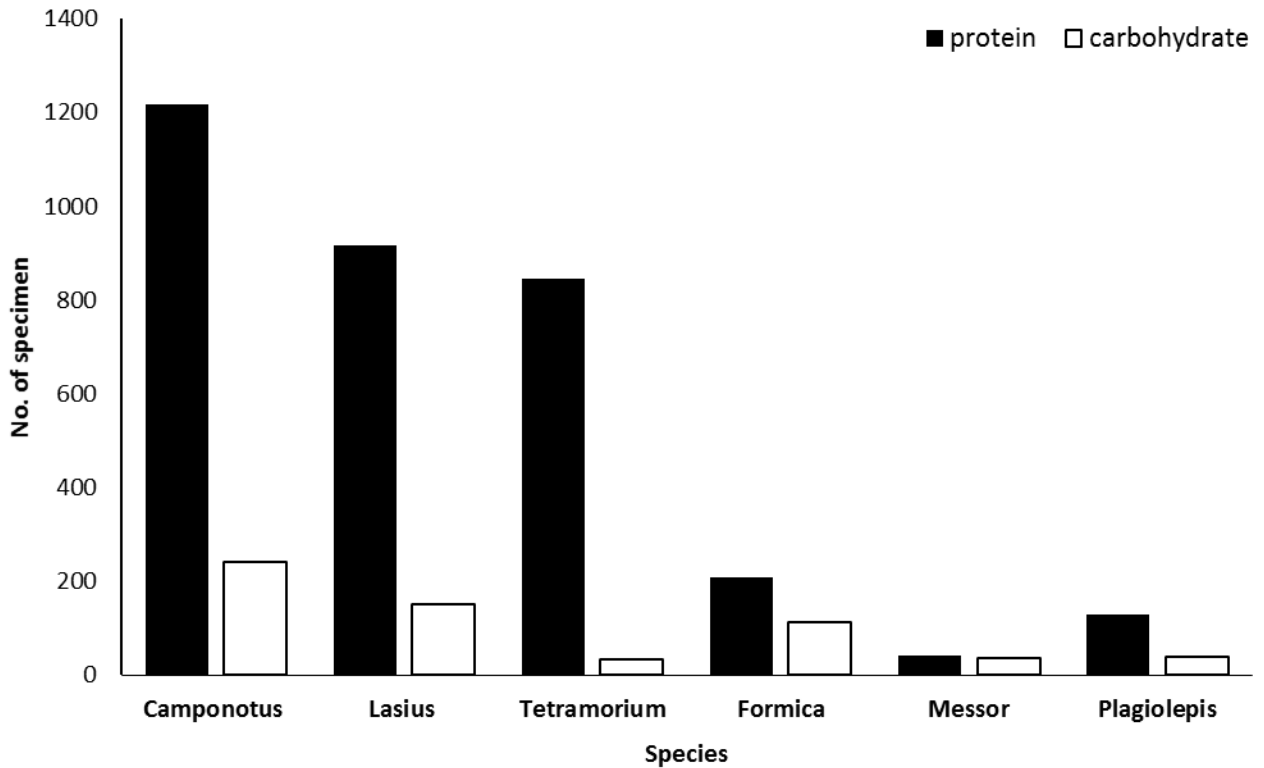


Fig. 4. Food preference of the *A. dasyanthus* habitat.

At the *A. monspessulanus* habitat *Camponotus* spp. was again the dominant group at the animal protein food source. It was followed by *Lasius* spp., *Tetramorium* cf. *caespitum* and in the case of *Formica* spp., and *Plagiolepis pygmaea* did not found a food preferences (Fig. 5.).

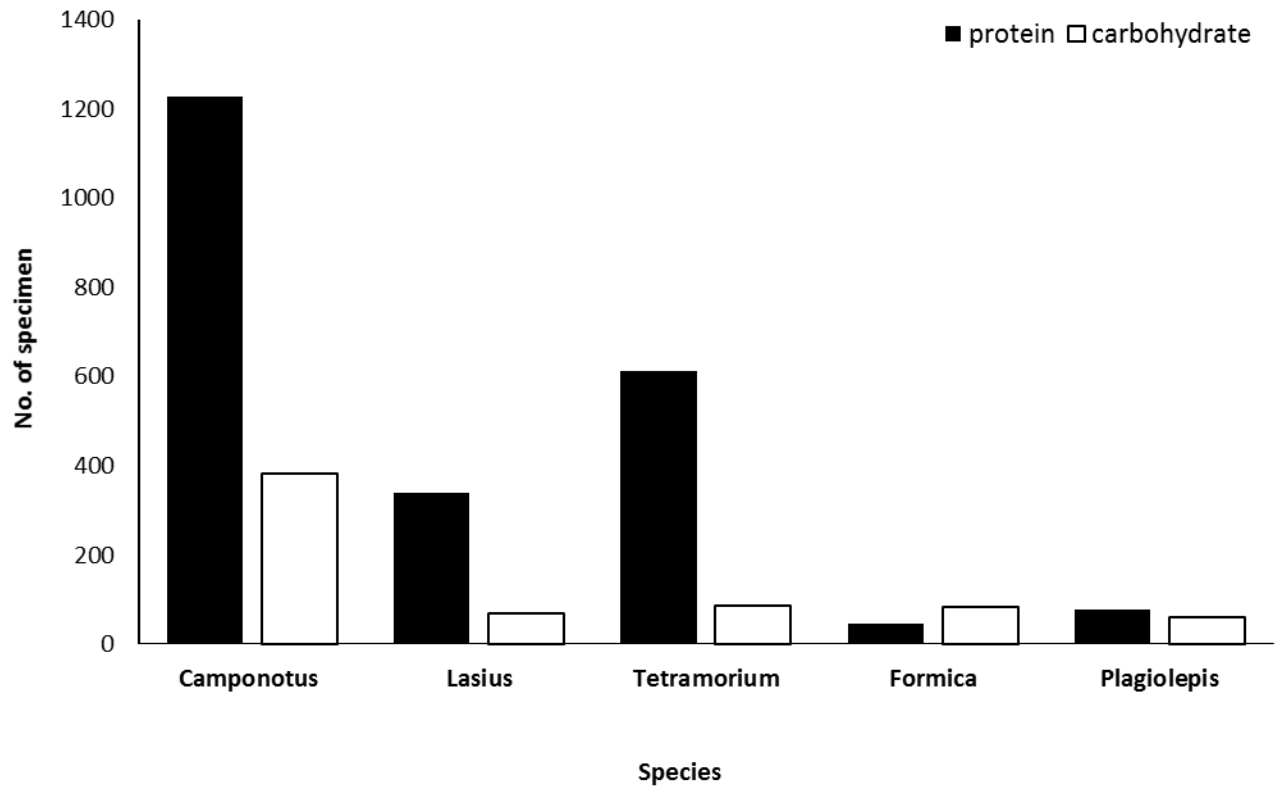


Fig. 5. Food preference of the *A. monspessulanus* habitat.

With the GLMM approach there was a detectable effect of the food types to the number of individuals in the case of *Camponotus* species group. At both habitats, we found significantly less *Camponotus* individuals on the honey than on animal protein source: (a) *A. monspessulanus* habitats ($t = -16.247$, $P < 0.001$); (b) *A. dasyanthus* habitats ($t = -765.6$, $p < 0.001$).

4.3. Structure of ant communities at *Kretania sephirus* habitats

For the myrmecophilous larvae is very important the ant community structure and the presence of the dominant ant species in these habitats. Pitfall trapping is a common used method were give a good picture about the ant community structure (species richness, the abundance of foragers).

During the investigations we wanted to answer to the following questions:

1. What kind of community structure can be observed regarding different species of ants?
2. Do we notice any differences between ant communities of the habitats of the two host plants?
3. Which species are the dominant ones?

Pitfall trapping is a common and well-proven method used for the study of epigeic ant communities (Gallé 1991, Majer 1980, Southwood 1984, Punttila et al. 1994, Majer 1996, Holec and Frouz 2005, Dauber and Wolters 2005, Ottonetti et al. 2006, Vele et al. 2011). Altogether 15 traps (200 ml, 8 cm diameter), filled with ethylene glycol solution as conserving material, and were placed at each sampling site arranged in a 3×5 grid, where each trap was separated by 5 m from the next one. Traps were active for 10 days during summer (from 25th august to 5th September) 2013 – at Cojocna (*A. exscapus* habitats) and (Căianu Vamă) (*A. monspessulanus* habitats) and summer (from 15th to 25 July) of 2014 – at Bărați (the *A. monspessulanus*) and at Vișea (*A. dasyanthus* habitats) and their control sites.

Altogether 11 ant species were collected; most of them were common, occurring on all sites, they belonged to three subfamilies: Myrmicinae, Formicinae and the Dolichoderinae. Interesting species were also found, as the sub-mediterranean *Messor* cf. *structor* (Sclick-Steiner et al. 2006). During the sampling in 2013 the number of ant specimen was very low at the *A. exscapus* and *A. monspessulanus* habitats. We found five species in low abundance at the *A. monspessulanus* habitat and four species on the *A. exscapus* habitat.

We did not find large differences among the ant communities of studied sites based on the diversity of ant communities. We noticed that the *A. monspessulanus* (Bărați) habitat and the *A.*

dasyanthus (Vișea) could be characterized almost with the same diversity, meanwhile the other two communities showed a lower diversity (Fig. 6.).



Fig. 6. The study sites diversity

Camponotus aethiops was present with high diversity at all study sites, while *Messor structor* is present on the lowest diversity. The distribution of *Tetramorium* cf. *caespitum* is present also in a high diversity, but it was present only at the *A. dasyanthus* and the *A. monspessulanus 2* (Bărați) habitats (Fig. 7.).

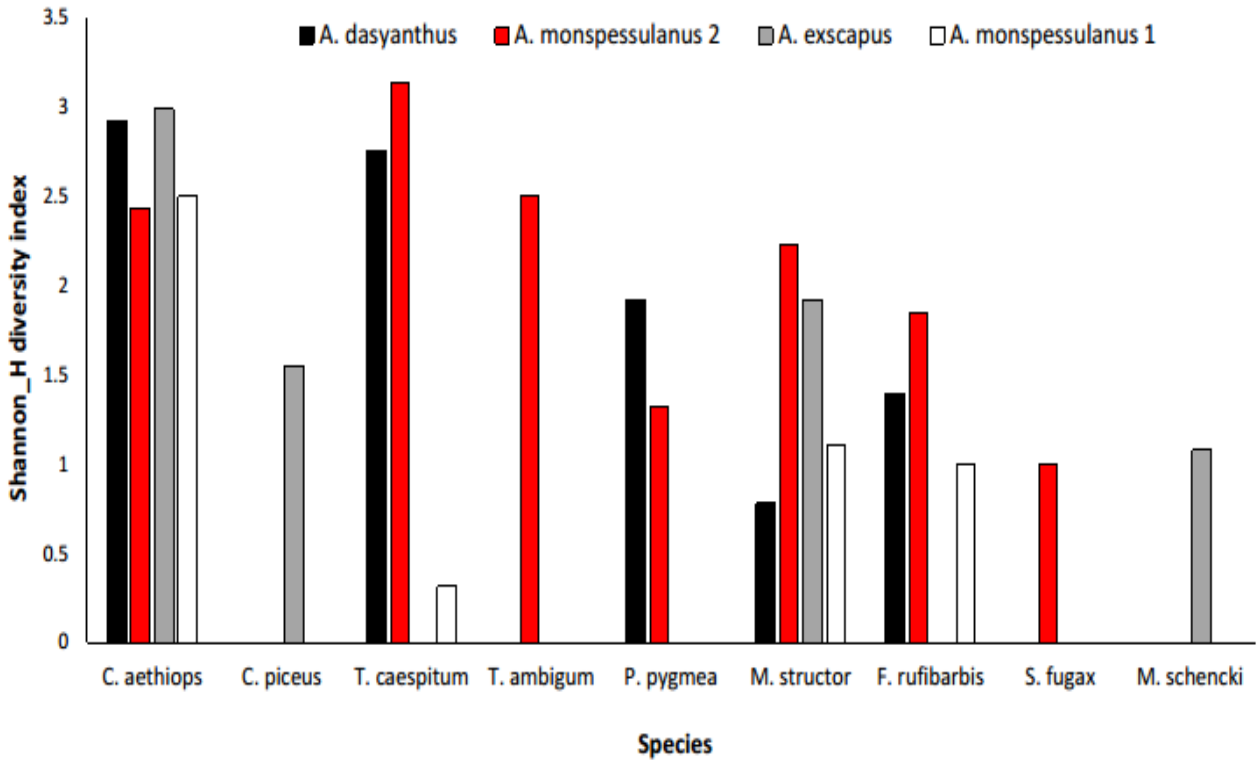


Fig. 7. The ant species diversity on the different habitats

Community structure

We can observe a relatively sharp separation in the NMDS space between the ant communities of the *A. dasyanthus* (Vișea) and *A. monspessulanus 2* (Bărați) habitats and their control sites. However, the two study sites with the host plants are located relatively close to each other in the

NMDS space, and these communities are mainly characterized by *C. aethiops*, *P. pygmaea*, *T. subboreale* and *M. cf. structor* species (Fig. 8.).

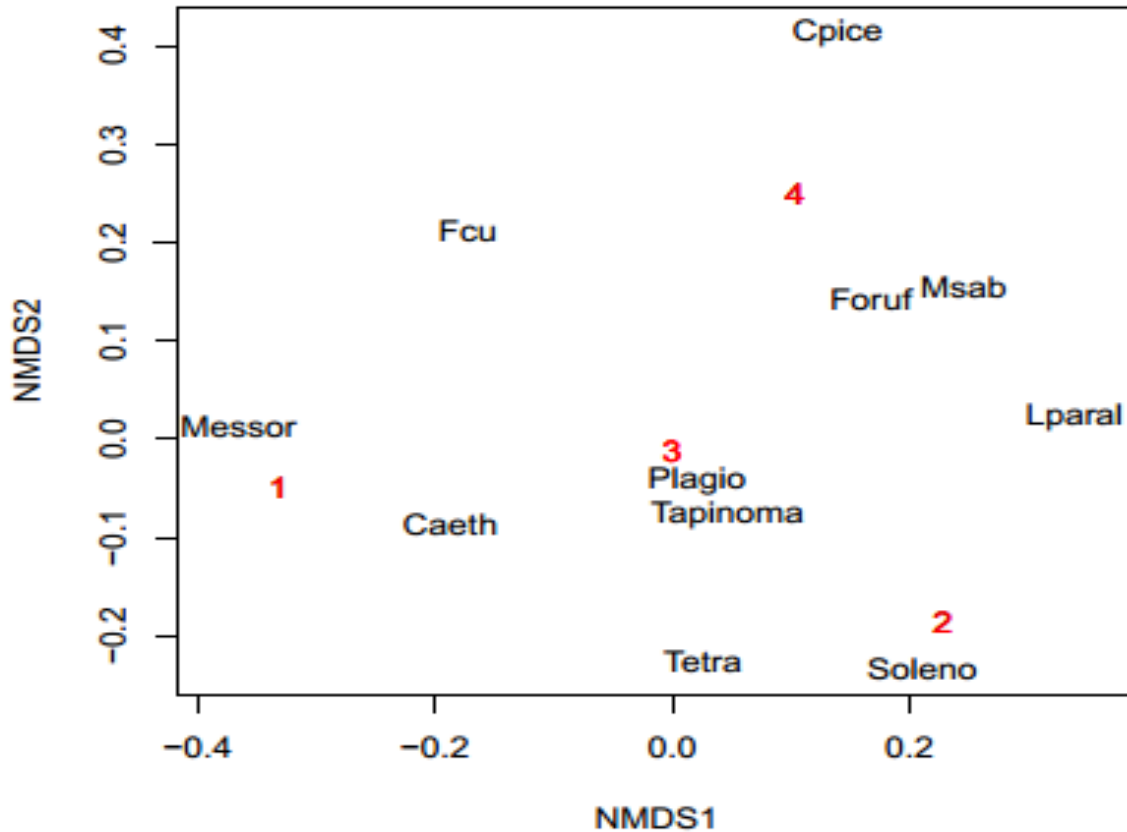


Fig. 8 NMDS ordination of the different ant communities (Bray-Curtis similarity index, stress = 0.019). Abbreviations: 1. Vișa – *A. dasyanthus* habitat; 2. Vișa – control site for *A. dasyanthus*, 3. Bărați – *A. monspessulanus* habitat, 4 - Bărați – control site for *A. monspessulanus*; Foruf – *Formica rufibarbis*, Fcu – *Formica cunicularia*, Msab – *Myrmica sabuleti*, Messor – *Messor cf. structor*, Tetra – *Tetramorium cf. caespitum*, Caeth. – *Camponotus aethiops*, Plagio – *Plagiolepis pygmaea*, Tapinoma – *Tapinoma subboreale*, Soleno – *Solenopsis fugax*, Cpice – *Camponotus piceus*.

4.4. Myrmecophilous relationships and host plant preference of the protected Zefir blue (*Kretania sephirus*)

The European Zephyr Blue *Kretania sephirus* is also a rare and protected myrmecophilous species. Earlier research has shown that the Zephyr Blue has a mutualistic relation with certain ant species (*Tetramorium cf. caespitum*, *Formica pratensis*, *Camponotus aethiops*, *Lasius alienus*) (Fiedler and Bálint 1994), which indeed are protecting the caterpillars from predators and parasites. The relationship is a typical food-for-protection type, where the caterpillar rewards the ants with honeydew. According to the assumptions of Fiedler and Bálint (1994) egg-laying female butterflies even oviposit on those host plants (*Astragalus* spp.) on which certain ant species have higher abundance. The tightness of this relationship is characterized by the fact that in some cases the caterpillars may even overwinter at the entrances of the ant nest (Fiedler and Bálint 1994). Despite this it is assumed that the relationship between the caterpillar and the ant partners is facultative implying that the survival of larvae does not entirely depend on the presence of ants, but their mortality is decreased in the presence of ants

Currently, due to changes in land management techniques (e.g. land abandonment, disappearance of traditional agriculture, decrease in livestock etc.), habitat modifications are threatening many Zephyr Blue populations the invasion of shrubs being a major threat to the survival of *Astragalus* host plants. In addition its habitats are relatively small and isolated, while the butterfly has low mobility. Habitat modifications on the other hand may cause severe alterations in the structure of ant communities, while the protective nature of the myrmecophilous relationship becomes more valuable with shrinking population size.

Therefore, the aim of the current study is to answer the following questions: (1) which characteristics of host plant influence the presence of the butterfly larvae? (2) are there specific ant species tending the caterpillar?

The study was carried out in the first part of May 2015 where the occurrence of *Kretania sephirus* was recorded previously in spring (the first part of May) 2014. On each site we marked 100 randomly chosen food plants, with a minimum of 2 m distance between neighbouring host plants. We recorded the plants' physical conditions (height, number of leaves, and number of inflorescences – in the case of *A. monspessulanus*), the presence of *Kretania* larvae, the presence

of ants on the plant and on the larvae and their species and abundance. In addition, we also recorded the maximum height of the vegetation (cm) and the vegetation cover (%) surrounding the host plants within a 1 m² plot.

Interaction assays were carried out in laboratory conditions between butterfly larvae and potentially tending ants found on the host plants, as e.g. *Lasius paralienus* and *Camponotus aethiops*. As controls we also tested the behavior of the ant species *Tetramorium cf. caespitum*, which, usually, does not visit the host plant but it is frequent on the study sites, and the reaction of the ant species *Myrmica scabrinodis*, that does not occur at all on the study sites, but it is a known host species for many myrmecophilous Lycaenid butterflies, as *Maculinea* species in the region (see Tartally and Varga 2008, Witek et al. 2014).. All interactions occurring were recorded and categorized as follows: (a) mandible gaping, (b) antennation, and (c) honeydew request. The first type of event was considered aggressive, the second as neutral, and the third was treated as positive.

Host plant characteristics and ant visitation

Altogether 12 *Kretania sephirus* larvae were recorded in the *A. dasyanthus* site and 9 in the *A. monspessulanus* site.

The most abundant and stable ant species on the food plant in the *A. dasyanthus* habitat were *Plagiolepis pygmaea* and *Camponotus aethiops*, while in the *A. monspessulanus* habitat the most abundant ant species were *Plagiolepis sp.*, *Lasius paralienus* and *Camponotus aethiops*.

According to the results of the GLM analysis the recorded variables did not display any significant effect on the presence of the *Kretania sephirus* larvae in the case of the *A. dasyanthus* site: height; no. of leaves; presence of the ants; vegetation height; vegetation cover; ant abundance; number of ant species.

Similar non-significant effects were obtained for *A. monspessulanus*: height; no. of leaves; no. of the inflorescences; no. of ant species; presence of ants; abundance of ants; height of the vegetation; and vegetation cover.

The abundance of ants was significantly influenced by the no. of leaves and the vegetation cover had a negative effect, but the host plant height, the vegetation height and the presence of the *Kretania* larvae did not display any significant effect in the *A. dasyanthus* site.

On the other hand, in the case of *A. monspessulanus* the abundance of ants on the host plant was significantly positively influenced by such characteristics as the no. of inflorescences, the maximum height of surrounding vegetation, whereas vegetation cover had a significant negative influence. The no. of leaves, the host plant height and the presence of *Kretania* larvae did not have any significant influence on ant abundance.

Also the simple presence of ants was not influenced by the height of food plants, by the no. of leaves, and by the vegetation cover, by the presence of *Kretania* larvae and by the height of surrounding vegetation in case of *A. dasyanthus*.

For *A. monspessulanus* the no. of inflorescences and the maximum height of surrounding vegetation had a significant positive effect on the presence of ants, while the vegetation cover has a significant negative effect. The height of food plant, the no. of leaves and the presence of *Kretania* larvae did not prove to be of significance to the ants' presence.

Interaction assays

During our observation in a single case, in the case of *Camponotus aethiops*, we recorded aggressive behavior. We observed myrmecophylous interaction (honeydew request) in the case of *C. aethiops* and *L. paralienus* (Fig. 9.). *Myrmica scabrinodis* and *Tetramorium* cf. *caespitum* were neutral (antennations) towards the caterpillar (Fig. 10.).

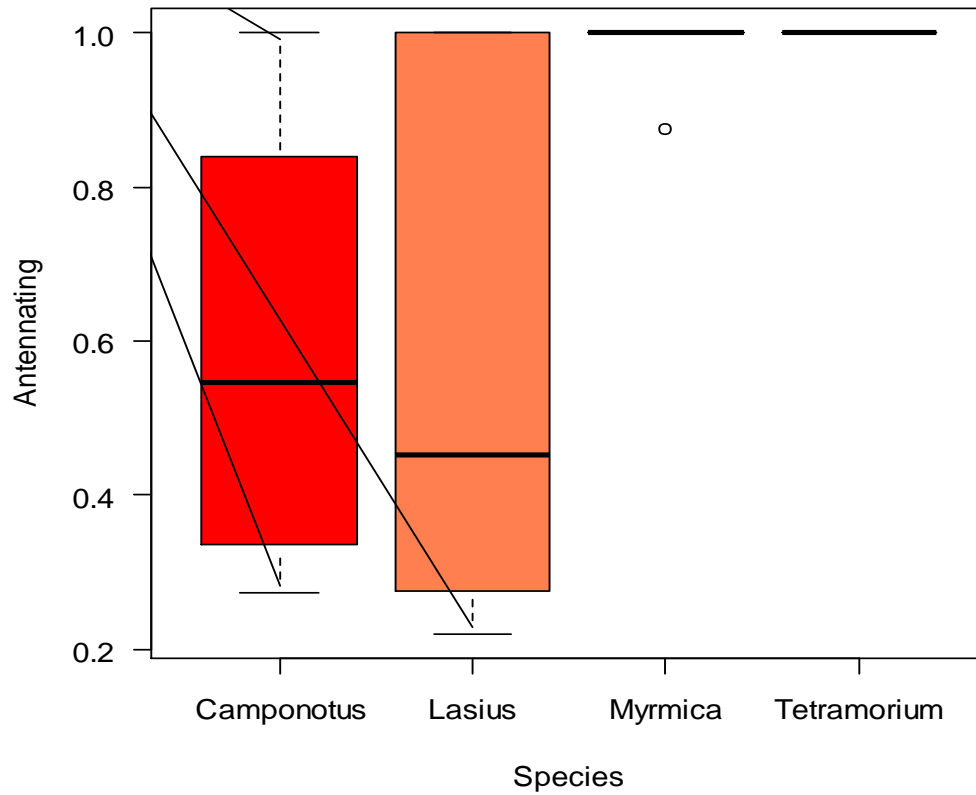


Fig. 9. The antennation index of ant species (Abbreviations: T – *Tapinoma subboreale*, L – *Lasius paralienus*, C – *Camponotus aethiops*, M – *Myrmica scabrinodis*)

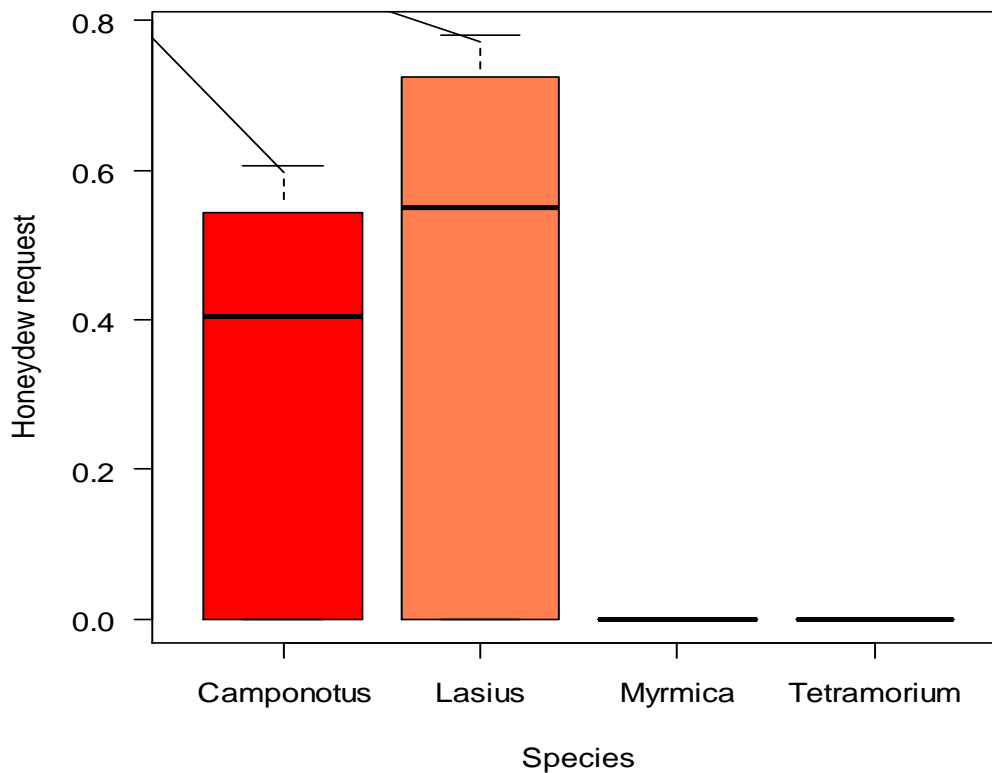


Fig. 10. The myrmecophilous (honeydew request) index of ant species (Abbreviations: T – *Tapinoma subboreale*, L – *Lasius paralienus*, C – *Camponotus aethiops*, M – *Myrmica scabrinodis*)

For the neutral and myrmecophilous behaviour in other ants species, GLMMs could be performed for the NI and MI index values. Significant differences in the NI were revealed between *Camponotus aethiops* and *Myrmica scabrinodis*, *C. aethiops* and *Tetramorium cf. caespitum*, also we observed significant differences between the *Lasius paralienus* and *Myrmica scabrinodis* and between the *L. paralienus* and *Tetramorium cf. caespitum*. Significant differences in the MI were revealed only between *Camponotus aethiops* and *Lasius paralienus*. Altogether a separation among the different ant species could be observed based on their behaviour towards the larvae (Fig. 11.). Thus, *L. paralienus* and *C. aethiops* were grouped together being characterized by frequent neutral and positive interactions with the larvae, while

T. cf. caespitum and *M. scabrinodis* were similar in being less interactive with the larvae (Fig. 31.).

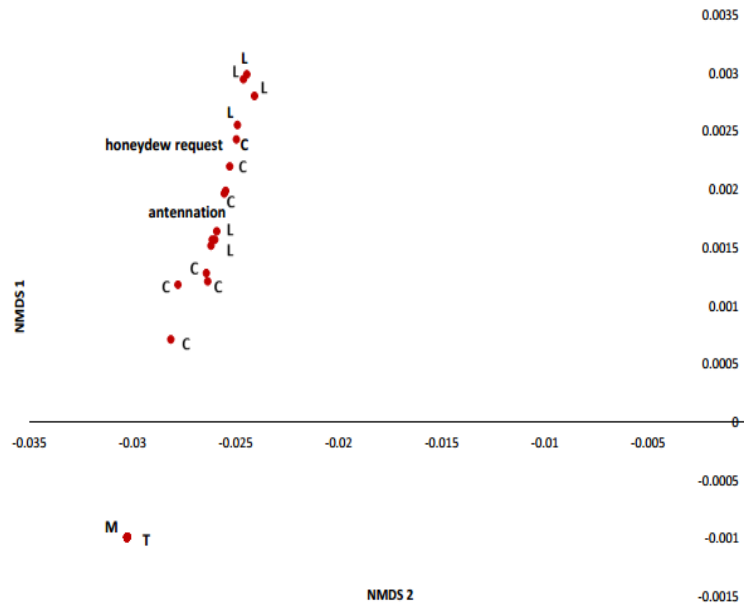


Fig. 11. NMDS graph of the ant species behavior towards the larvae (stress = 0.004). (Abbreviations: T – *Tapinoma subboreale*, L – *Lasius paralienus*, C – *Camponotus aethiops*, M – *Myrmica scabrinodis*)

4.5. Host plant and ant preference in the protected and endemic myrmecophylous Transylvanian Blue (*Pseudophilotes bavius hungarica*) butterfly (Lepidoptera: Lycaenidae)

The Transylvanian Blue (*Pseudophilotes bavius hungarica*) is an endemic, protected butterfly inhabiting forest-steppe patches of the Transylvanian, Romania that engages in facultative myrmecophilic relationships with different ant species (Jutzeler and Rakosy 1997, Crişan et al. 2011). The relationship seemingly involves the grooming and protection of the larvae by the ants, while in return the larvae rewards the ants with honeydew (Crişan et al. 2011). Despite its conservation value we do not have any information about the larvae's exact relationship with ants. Currently only sporadic field observations are available concerning the identify of ant partners (Jutzeler and Rákosy 1997, Crişan et al. 2012), no consistent studies have been performed in order to elucidate the extent of generality of larval host preference. Such information would help conservation management of this species. Therefore the aim of the current study is to answer the following questions: (1) which are those host plant characteristics that could predict the presence of the butterfly larvae and potentially caterpillar tending ants on the host plant? (2) how specific is the myrmecophily of the caterpillar, are there specific ant species that tend the caterpillar, which could also differ in their behaviour towards the caterpillar compared to other non-tending ants?

We selected randomly 215 plants of *Salvia nutans* on the study area between June 16-18, 2014, for recording various characteristics of the food plants: height, number of inflorescence, the presence of *Pseudophilotes* larvae, the presence and abundance of ants on the plant, and separately on the caterpillar, while also recording their species, and finally the presence and abundance of aphids. The abundance of aphids was visually estimated based on the stem length occupied by 10 aphid individuals (rounded to the closest 10).

Interaction assays between the butterfly larvae and different ant species were carried out in laboratory conditions. Due to its protected status, we used only 21 *Pseudophilotes* larvae collected from the study area and four species of ants. Two of the tested ant species, *Lasius paralienus* Seifert, 1992 and *Camponotus aethiops* (Latreille, 1798), were recorded previously on host plants.

Host plant characteristics and ant visitation

Only 28 *Pseudophilotes* larvae were detected on the 215 investigated plants (11.62% of all host plants), and each larvae was found on a separate host plant. The most abundant and also most stable ant species on the food plants and on the aphids were *Palgiolepis pygmaea*, *Lasius paralienus* and *Camponotus aethiops*.

The presence of ants had a significantly positive influence, while their abundance, and also the presence of aphids had a significant negative effect on the caterpillar's presence, but the height of host plant, the number of inflorescences and the abundance of aphids did not display significant effect in this case.

The presence of ants was not significantly influenced by the presence of caterpillars, and aphids, and not even by the height of host plants, the abundance of aphid and the number of inflorescence. However, their abundance was significantly positively influenced by the presence of aphids, and negatively by the presence of the caterpillar, but the abundance of aphids, the height of the food plant, and the number of inflorescences did not display any significant effect in this case as well.

Interaction assays

Generally, all tested ant species, with the exception of *Tapinoma subboreale* were neutral towards the caterpillar, only *T. subboreale* showed an elevated aggressive behaviour towards the larvae (Fig. 12), as also shown by the output of the NMDS analysis (Fig. 13), since all aggressive interactions were associated with *T. subboreale*. Due to the lack of aggression in other ant species, GLMMs could not be performed for the AI values, only for the ANI outputs. Based on the results of the analysis significant differences in the ANI were revealed only between *Camponotus aethiops* and *T. subboreale*.

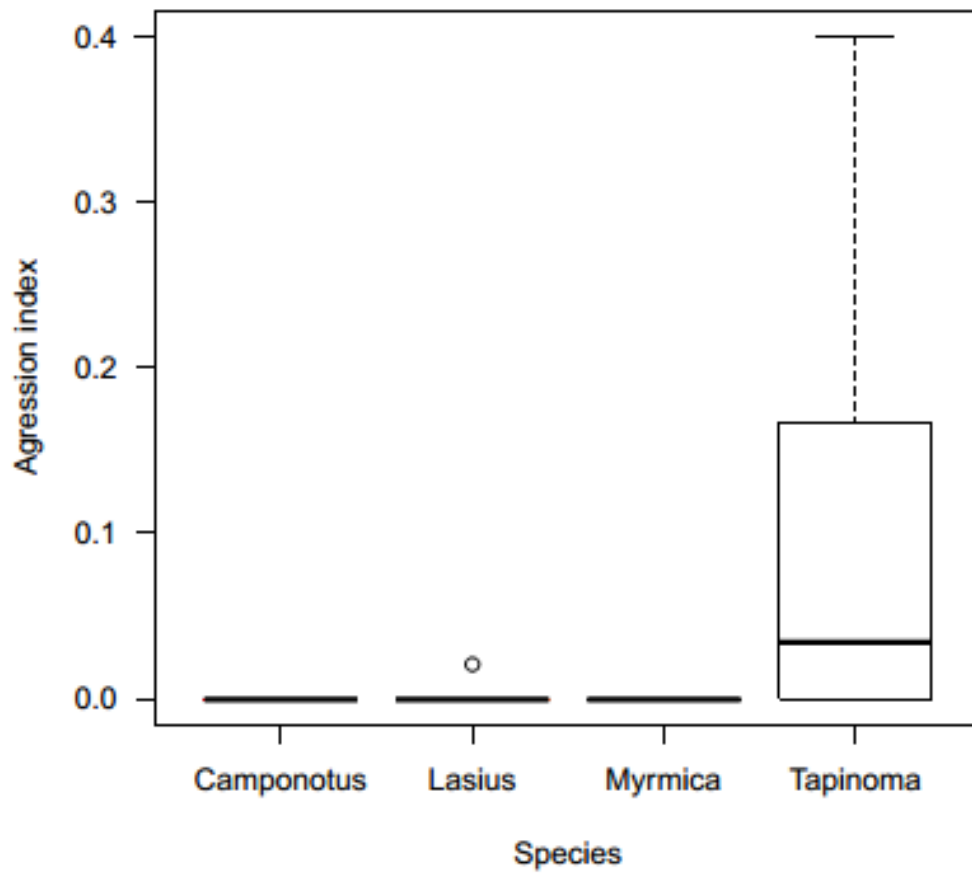


Fig. 12. The aggression indices of ant species (Abbreviations: Tapinoma – *Tapinoma subboreale*, Lasius – *Lasius paralienus*, Camponotus – *Camponotus aethiops*, Myrmica – *Myrmica scabrinodis*)

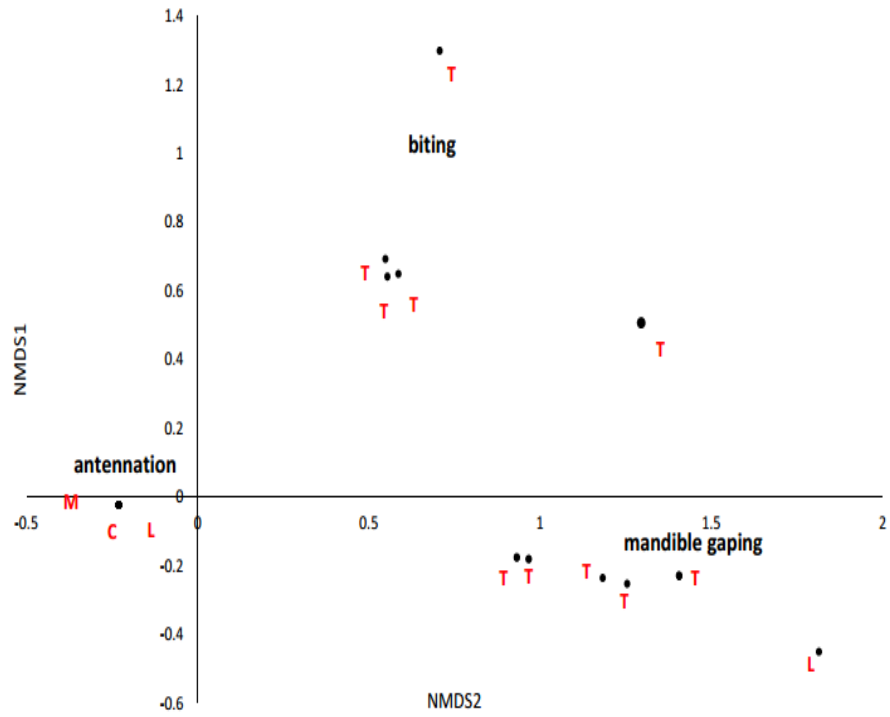


Fig. 13. NMDS graph of the ant species behavior towards the larvae (stress = 0.004). (Abbreviations: T – *Tapinoma subboreale*, L – *Lasius paralienus*, C – *Camponotus aethiops*, M – *Myrmica scabrinodis*)

5. CONCLUSIONS

Our findings have important effect to understanding of facultative myrmecophilous trade-off, of ants and *Kretania sephirus* and *Pseudophilotes bavius hungarica* butterflies.

Kretania sephirus

The abundance of eggs in *Kretania sephirus* is determined rather by the food plant characteristics (the length of leaves, number of leaves), many study support, that the female lay eggs on the food plant with a good physical characteristics (Nowicki et al. 2005, Árnayas et al. 2006, Czekes et al. 2014). The vegetation height has a positive, while the vegetation cover has a negative effect on the number of eggs present in all four studied populations.

In the case of the investigated populations, according to the parameters we have measured (height of food plant, no. of leaves, height and cover of vegetation), also we did not found any significant effect of the food plant characteristics on the presence of larvae. Our results have shown that the females of *Kretania sephirus* use a similar egg laying strategy in the case of *A. exscapus* and *A. dasyanthus* host plants, but their strategy differs in the case of *A. monspessulanus*.

According to our results animal protein sources were the most preferred sources by the *Camponotus* spp. the *Lasius* group, *Tetramorium* cf. *caespitum*, *Plagiolepis pygmaea* and *Myrmica* spp., the carbohydrate food source was of secondary interest. According to this results we could say that, the facultative myrmecophilous is an important relationship, because, however the ant species were present in the *Kretania sephirus* habitats, preferred more the protein source, despite they do not attack the larvae.

We studied the ant communities by the baits, on the butterflies habitats, and we observed a differences among the ant communities, the dominant and stable ant species was the *Camponotus* spp. on both studied sites (*A. dasyanthus* and also *A. monspessulanus* habitats).

Studied the ant community with the pitfall traps, we found some differences between the ant communities regarding the abundance of different species, *Camponotus aethiops* had the highest abundance at all of the areas. The ant communities of the four study areas differ from each other significantly. Based on these we could affirm that most probably *Camponotus* spp. is a very good candidate as a potential host ant of *Kretania* larvae. *Camponotus* spp. are important and known partners of *Lycaenidae* butterflies (Fiedler 2006), because *Camponotus* spp. do not attack the *Lycaenidae* larvae (Kaminski et al. 2010, Bächtold et al. 2014).

In addition, each *Kretania sephirus* larvae we found, was in the company of *Camponotus aethiops* ants. We have observed that *Camponotus aethiops* and *Lasius paralienus* pays great attention to the *Kretania* larva not only when making contact (antennation), but also when requesting the honeydew. The whole we can say, *Camponotus aethiops* is the host ant species of the *Kretania sephirus* larvae. According to our results, this trade-off is a relative closed relation, and from the parts of the larvae it is present a host specificity.

Pseudophilotes bavius hungarica

The egg laying strategy of *Pseudophilotes bavius hungarica* females is not influenced by the physical characteristics of host plants. The absence of aphids and the presence of different ant species are much more important factors, although the presence of ants is only beneficial when they are present in small numbers. The fungal infection on the host plant is much more frequent in the presence of aphids, due to the sugary liquid secreted by them, this jeopardizing the quality of the larval food plants (Stadler and Dixon 2008) therefore the aphid infestation on the food plant generally have a negative effect on the oviposition of the females (Stadler and Dixon 2008, Árnýas et al. 2009).

Among ant species occurring in the butterfly's habitat *Camponotus aethiops* seems to be the most preferred, while *Tapinoma subboreale* being the least preferred species. Our results suggest, that similarly to other *Lycaenidae* butterflies, the presence of ants on the host plants is advantageous for *P. bavius*. This can be attributed to the fact, that most ant species are partners

in myrmecophilous associations, and they seldom attack butterfly larvae (Pierce et al. 2002, Bächtold et al. 2014).

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