Final report on the project titled
'*Wolbachia* infestation in butterflies in the Carpathian Basin'
(K109223)

Microbial symbionts have great evolutionary and ecological influences on their eukaryotic hosts. To better understand the nature of these microbial symbioses, we studied the intracellular *Wolbachia* participating in diverse symbiont-host interactions. The main aim of our research was to understand how *Wolbachia* influence speciation patterns of *Maculinea* and *Melitaea* butterflies. To achieve this goal, we primarily focused on (i) the infestation level and pattern in each of the target species, (ii) the plausible effects of *Wolbachia* on speciation patterns of their butterfly hosts and (iii) the possible horizontal transfer routes of *Wolbachia* among these butterflies and other insects associated with them ecologically.

In parallel with the study of *Wolbachia* infestation, we revealed the basic phylogeographical and population genetic structure in the case of *Maculinea* and *Melitaea* species since without this knowledge we cannot identify the effects of *Wolbachia* on the studied species.

In addition, we also studied the influences of *Wolbachia* in Common pill bug (*Armadillidium vulgare*) and in Marsh fritillary (*Euphydryas aurinia*).

Altogether, we published ten papers based on our results, two manuscripts are under review and we are planning to write five further manuscripts publishable in international journals (see the attached publication list and the results in this final report for the details).

THE MAIN RESULTS IN THE GENUS *MACULINEA*

We studied all European *Maculinea* species (the two ecotypes of *M. alcon*, the two phenological forms of *M. arion*, *M. nausithous* and *M. teleius*) in 20 geographical regions. Altogether 924 individuals were screened for *Wolbachia* (1-25 individuals/population).

The studied *Maculinea* species strongly differed in the level and the pattern of *Wolbachia* infestation. On a large geographical scale, the prevalence of *Wolbachia* was 100% both in *M. alcon* and *M. arion* irrespective of phenology or differential food plant usage while the infection level was 36.2% in *M. nausithous* and 14.4% in *M. teleius*. In *Wolbachia* infestation of the latter two species we could not detect any geographical pattern.

All *M. alcon* individuals were infected with a single *Wolbachia* strain. Interestingly, 19 populations of *Euphydryas aurinia* in the United Kingdom proved to be also infected in 100% with the same strain as *M. alcon* in our study. Similarly, all *M. arion* individuals were infected with a single strain. The WSP (*Wolbachia* surface protein) allele of this strain was described by us and submitted as allele No. 685 to the *Wolbachia* MLST database. *M. nausithous* harboured various *Wolbachia* strains. Additionally, highly divergent strains occurred even in a single population of this species. Similarly, *M. teleius* bears different bacterial lineages. Interestingly, the parameters of *Wolbachia* gene diversity were proved to be higher in *M. teleius* than in *M. nausithous*.

In parallel, we surveyed the genetic diversity of *Maculinea* butterflies using mitochondrial (COI) and nuclear (GAPDH, MDH, wg) sequences as well as 11 allozyme loci. The highest mitochondrial variability was detected in *M. nausithous*. At the same time, we found a single mitotype in all *pneumonanthe* type specimens of *M. alcon*. The spring form of *M. arion* showed very similar pattern as the mitochondrial haplotype of all specimens was identical. Based on COI sequences, the split of *M. nausithous* and *M. teleius* was practically without statistical support. In *M. nausithous* strong geographical pattern was recognized. At the same time, the resolution of COI sequences in the other three *Maculinea* species proved to be very low. *M. nausithous* and *M. teleius* specimens infected with *Wolbachia* did not form separate clades. In general, the diversity of nuclear markers was low but we could not detect mito-nuclear discordance (i.e. incongruence between the patterns of mitochondrial and nuclear sequences). The highest variability was detected in *M. arion* and the lowest in *M. teleius*. In contrast with the mitochondrial marker, the combined dataset (mitochondrial and nuclear genes together) were suitable to separate all *Maculinea* species with high statistical support.

In the case of *M. alcon* and *M. arion,* we obtained similar results as other European researchers concerning the *Wolbachia* infestation patterns in these butterflies. In contrast with their results, we could not detect mito-nuclear discordance or find evidence for *Wolbachia*-induced selective sweep.

Previously, cryptic speciation has been hypothesised for *M. teleius* and *M. nausithous,* based on deep mitochondrial splits in each of these species. At the same time, the haplotypes previously attributed to cryptic species were part of *Wolbachia*-infected clades thus deep intraspecific divergences found in DNA barcode studies coincide with specific infection patterns at Palaearctic level. In contrast with the previous results, in the Carpathian Basin mitochondrial haplotypes of infected specimens formed separate clades neither in *M. nausithous* nor in *M. teleius*. Although the genetic diversity was the highest in *M. nausithous,* the phylogenetic and allozyme patterns mainly reflect the biogeographical history of the species since the great part of the variability arises from the differentiation of the large geographic regions which coincides the disjunct distribution of *M. nausithous*. Based on our results, we cannot hold only *Wolbachia* responsible for the restricted genetic diversity of *Maculinea* in the Carpathian Basin. Probably several factors shape together the level and pattern of genetic variability in *Maculinea* butterflies.

(**See for more details:** Bereczki et al. 2015. Controversial patterns of *Wolbachia* infestation in the social parasitic *Maculinea* butterflies (Lepidoptera: Lycaenidae) *Organisms Diversity & Evolution* 15(3): 591-607.)

Besides, we studied the genetic structure in samples from 9 *M. alcon* and 16 *M. teleius* sites obtained from the Carpathian Basin using 16 polymorphic enzyme markers. Genetic differentiation was generally higher among *M. alcon* populations as compared to *M. teleius*. Moreover, the results of Bayesian clustering indicated a clear regional pattern with three well-defined cluster regions in *M. alcon*. In contrast, the regional pattern was less evident in *M. teleius* where we found three comparatively less distinct cluster regions. Putatively adaptive genetic differences were detected among cluster regions exploiting different host ants in *M. alcon*, while they were less evident in *M. teleius*.

(**See for more details:** Pecsenye et al. 2015. Contrasting genetic structure in cuckoo and predatory *Maculinea* butterflies. *Conservation Genetics* 16(4): 939-954.)

Furthermore, we completed the previous studies on the taxonomy of the *Phengaris-Maculinea* complex applying a geometric morphometric approach on male genitalia. Strong phylogenetic signal was detected in the shape of valva. *Phengaris* s.str. and *Maculinea* s.str. could not been separated perfectly owing to the intermediate position of *Phengaris xiushani* having '*Maculinea*-like' valva shape. Our investigation emphasizes the need of a more comprehensive phylogenetic survey including all *Phengaris* species. At the same time, it also suggests that the synonymization of the two genera seems to be reasoned under the name *Phengaris* as senior synonym.

(**See for more details:** Bereczki et al. 2017. *Maculinea* or *Phengaris*? New insights from genitalia morphometry. *Zoologischer Anzeiger – A Journal of Comparative Zoology* 270: 57-59.)

In connection with *M. alcon* and *M. arion*, we performed further detailed studies since one of the main aims of the project was to reveal what level of differentiation exists between the two ecotypes of *M. alcon* or the two phenological forms of *M. arion* and whether *Wolbachia* play any role in the separation of these forms.

Maculinea alcon

In the case of *M. alcon*, we studied the differentiation between the two forms using different host plants (*Gentiana cruciata* versus *G. pneumonanthe*) using multiple gene sequences, microsatellites and allozymes completed with *Wolbachia* screening, together with wing and male genitalia morphometry. The variability of nuclear genes was very low but applied microsatellites showed high level of variability (altogether with 132 alleles). However, we could not detect any differentiation between the two forms of *M. alcon* but the topotypic populations of 'Hirschke's *rebeli*' from the high altitude of the Styrian Alps with much controversial taxonomic position (which was studied genetically at the first time) were highly distinct from the other populations not only at the highly variable microsatellites, but also at standard genes and clearly represents a well-separated unit (potentially subspecies) within *M. alcon*. As all individuals were infected with a single *Wolbachia* strain, this is possibly an ancient infection with high maternal transmission rate. It also suggests that the separation of the topotypic populations was probably a relatively recent event which could be promoted by genetic drift or specific conditions at high altitudes and maintained by restricted gene-flow.

We found differences between the two forms neither based on genitalia nor wing morphometry. At the same time, these forms (even the samples collected in the beginning and at the end of the flight period in a Transylvanian site where the two phenologically different host plant species co-occur) clearly differentiated based on allozymes, mostly the esterase locus, whose role has been previously revealed in connection with the effectiveness of plant consumption in *Colias* species. Both the genetic and the morphometric studies supported that the geographical origin of the populations have a higher effect on the structure of variation than the food plant use. Moreover, we detected more genetic lineages than could be defined by differential host plant usage.

(**See for more details:** Bereczki et al. 2018. *Maculinea rebeli* (Hirschke) – a phantom or reality? Novel contribution to a long-standing debate over the taxonomic status of an enigmatic Lycaenidae butterfly. *Systematic Entomology* 43(1): 166-182.)

Due to the collected samples on our recent Balkan expedition, we could complete the study of the phylogeographical pattern in *M. alcon*. The preliminary results suggest that one of the main refugial areas for this species was located in the Balkans from where a genetic lineage reached the Carpathian Basin through Transylvania (Fig. 1.).

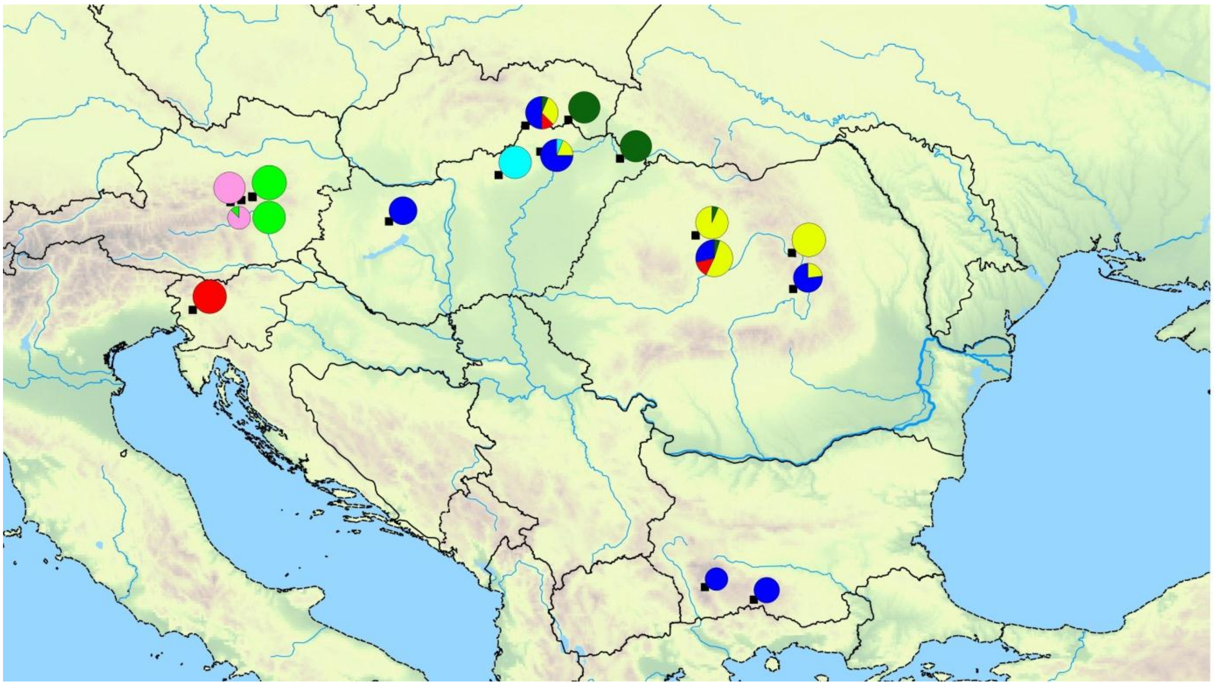


Figure 1. Results of the Bayesian-clustering Structure analysis based on ten microsatellite loci. Black squares represent the sampling sites. The size of the pie charts is proportional with the number of studied specimens. The number of genetically different clusters: K=7. (unpublished)

Furthermore, we performed a landscape genetic study on 176 *M. alcon* specimens (*cruciata* type) from 11 populations in the Aggtelek Karst region (Hungary) using two marker sets (13 variable enzyme loci and 10 polymorphic microsatellites) completed with *Wolbachia* screening. All individuals proved to be infected with a single strain (as on a large geographical scale). The resolution of the two different marker sets was distinct: microsatellites had much higher resolution than enzymes. Although three genetic clusters were separated based on both marker sets (Fig. 2A and B), the classification of individuals into each cluster was uncertain based on enzymes. The populations classified into each cluster were isolated from each other by forested areas (Fig. 2C). We are planning to publish these results in an international journal.

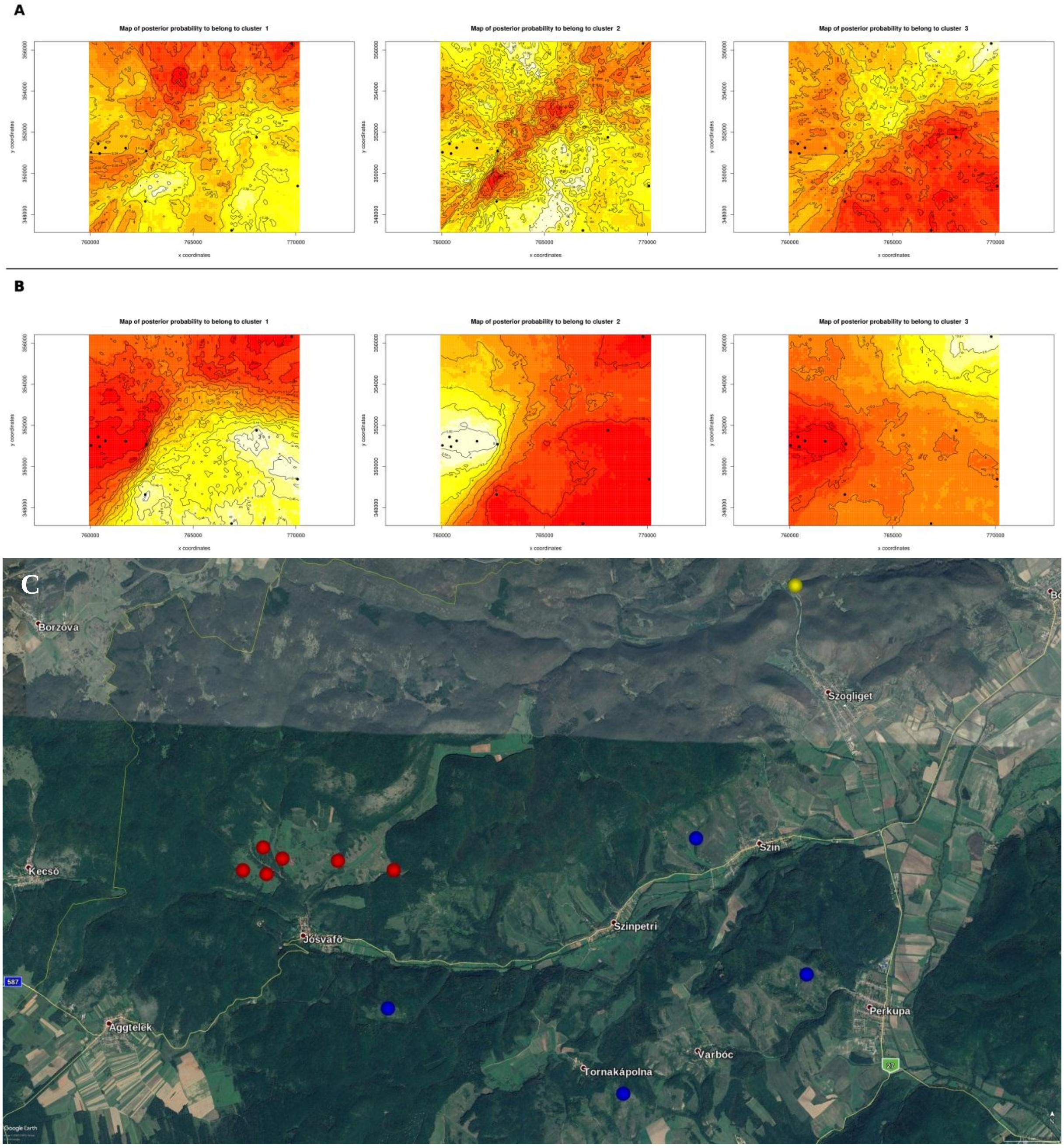


Figure 2. The results of landscape genetic study on *M. alcon*: A – enzymes; B – microsatellites. Black points represent the sampling sites. C – The classification of populations into each cluster based on microsatellites. The different clusters are indicated by distinct colours. (unpublished)

Maculinea arion

One of the main aims of the project is to reveal whether reproductive incompatibility exists between the two phenological forms of *M. arion*. Using four standard gene regions and allozymes, we could not detect any genetic difference between the spring and summer type of *M. arion*. At the same time, these forms clearly differentiated from each other based on morphological traits (genitalia and wings).
(**See for more details:** Bereczki et al. 2014. Multilevel studies on the two phenological forms of Large Blue (*Maculinea arion*) (Lepidoptera: Lycaenidae). *Journal of Zoological Systematics and Evolutionary Research* 52(1): 32-43.)

The applied genetic markers, however, had very low variability thus we studied four population pairs of the two forms originated from the Aggtelek Karst region using 9 microsatellite loci. These microsatellites proved to be highly polymorphic (altogether with 122 alleles) and the two forms differentiated from each other based on these loci (Fig. 3 and 4). At the same time, the signs of restricted gene flow between them were also recognized, which was an expected result according to the periodically overlapping flight period of the two forms. We also found significant differences between the two phenological forms of the studied syntopic populations based on male genitalia morphometry (Fig. 5).

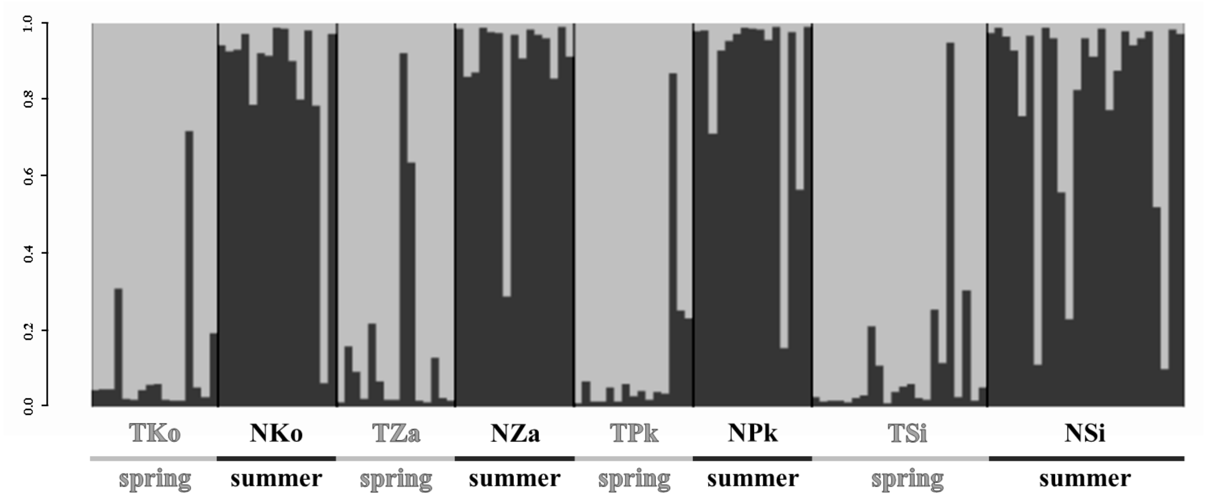


Figure 3. Results of the Bayesian-clustering Structure analysis based on nine microsatellite loci. Syntopic population pairs from the Aggtelek Karst region (Hungary): TKo, NKo – Korlát hill, TZa, NZa – Zabanyik, TPk, NPk – Perkupa, TSi, NSi – Szin. (The letter T at the beginning of the names refers to spring *arion*, N does summer *arion*). (unpublished)

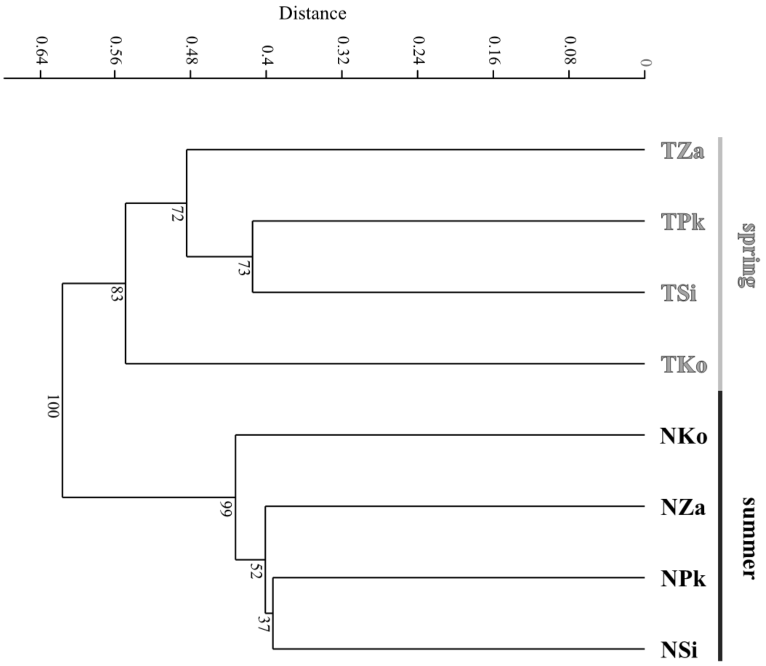


Figure 4. UPGMA dendrogram based on allele frequencies of nine microsatellite loci. Syntopic population pairs from the Aggtelek Karst region (Hungary): TKo, NKo – Korlát hill, TZa, NZa – Zabanyik, TPk, NPk – Perkupa, TSi, NSi – Szin. (The letter T at the beginning of the names refers to spring *arion*, N does summer *arion*). (unpublished)

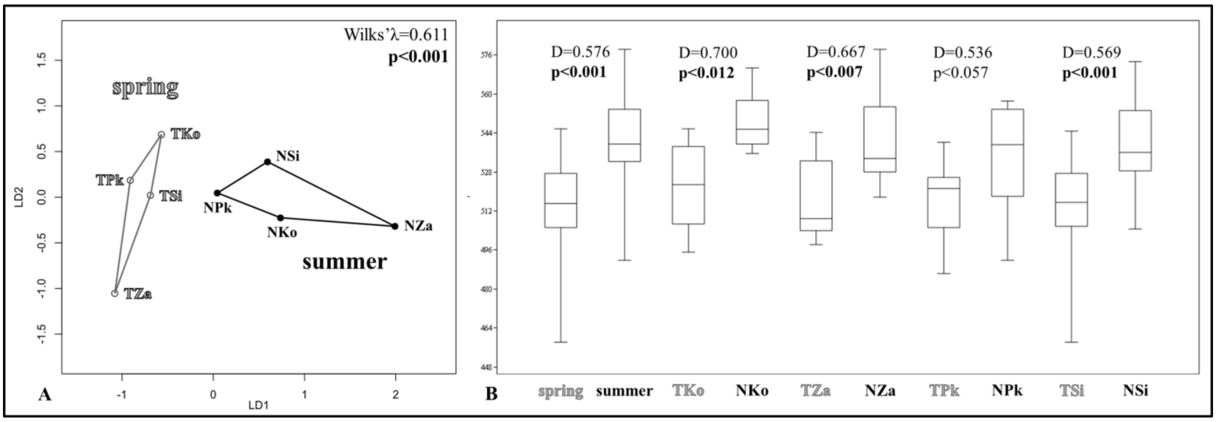


Figure 5. Differences between the spring and the summer type of *M. arion* by outline-based male genitalia morphometry. **A** – differences in the shape of valva, **B** – differences in the size of valva. Syntopic population pairs from the Aggtelek Karst region (Hungary): TKo, NKo – Korlát hill, TZa, NZa – Zabanyik, TPk, NPk – Perkupa, TSi, NSi – Szin. (The letter T at the beginning of the names refers to spring *arion*, N does summer *arion*). (unpublished)

Besides we studied the *Wolbachia* infestation in these population pairs. On this narrower geographical scale, the infestation level was 98.6%. We could not detect *Wolbachia* in two spring type specimens. All infected samples harboured a single strain that was also found on the larger geographical scale. The infestation level seemed to be different in the spring and the summer type of *M. arion* checking the PCR products of *Wolbachia* screening on agarose gels since only faint bands were observed in the spring form (Fig. 6A). This difference was quantified by RT-Q-PCR method and the summer type contained 6-7 times more *Wolbachia* on the average than the spring form (Fig. 6B). However, certain specimens collected in spring have rather summer type genetic background and vice versa (based on microsatellites, see Fig. 3). We assume that the difference in the quantity of *Wolbachia* between the two forms of *M. arion* is resulted by environmental factors as it could not be linked to the genetic background (microsatellite cluster) of each specimen but the flight time (Fig. 3).

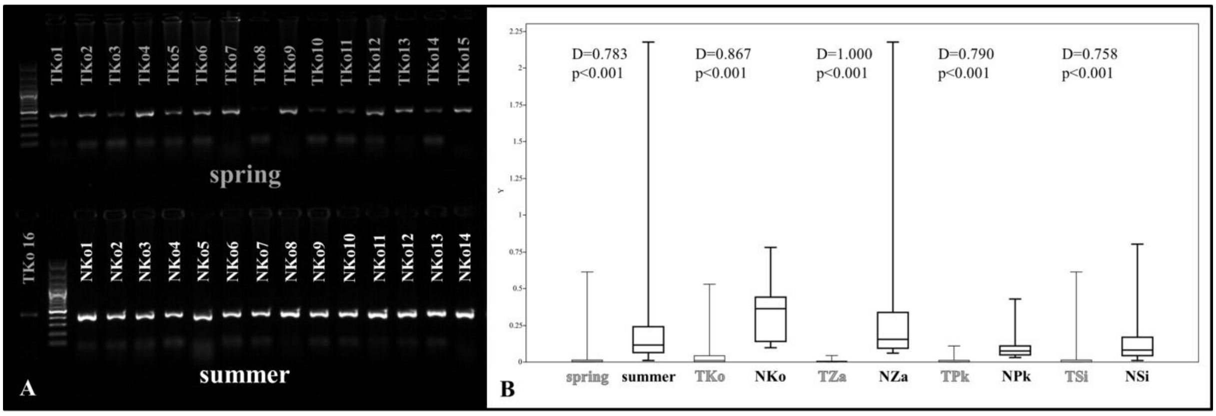


Figure 6. Differences in *Wolbachia* infestation between the spring and the summer type of *M. arion*. **A** – differences between the spring and the summer type specimens from Korlát hill in the *Wolbachia* quantity on 1% agarose gel, **B** – differences in the infestation level on box-plots between the spring and summer *arion* in four syntopic population pairs from the Aggtelek Karst region (Hungary): TKo, NKo – Korlát hill, TZa, NZa – Zabanyik, TPk, NPk – Perkupa, TSi, NSi – Szin. (The letter T at the beginning of the names refers to spring *arion*, N does summer *arion*). (unpublished)

Besides, we also studied the *Myrmica* fauna in these habitats and provided the first host ant data of *M. arion* in the Carpathian Basin. A spring *arion* pupa was found in a *My. scabrinodis* nest and a summer *arion* prepupa was found in a *My. specioides* nest.
(**See for more details:** Tartally et al. 2017. First data on the host ant usage of Large Blue from the Carpathian Basin. *Sociobiology* 64(1): 122-124.)

Altogether, four ant species were identified in *M. arion* habitats (*My. scabrinodis*, *My. sabuleti*, *My. specioides*, *My. schencki*) using an identification system establishing by us which is based on the combination of barcode sequences and geometric morphometrical data. We also screened the *Wolbachia* infestation in 41 ant nests (including the two nests harbouring *M. arion* pupae), out of which 24 nests were infected with a single bacterial strain (Fig. 7). The *My. specoides* nest which contained a *M. arion* prepupa was also infected with *Wolbachia* but not with the same strain as *M. arion* prepupa. Based on these results, we could not prove the horizontal transfer between *M. arion* and their ant hosts, instead of this, we found high maternal transmission rate in these ant species.

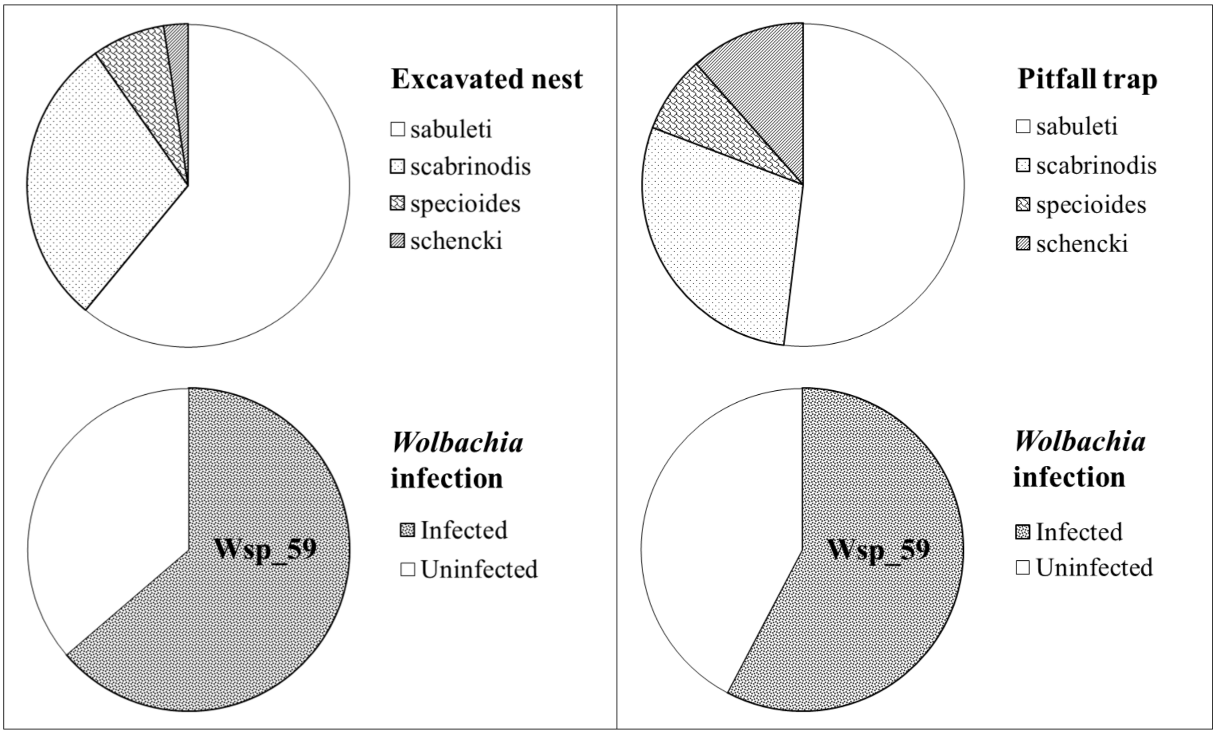


Figure 7. Species identification and *Wolbachia* screening of *Myrmica* ants from excavated nests and pitfall traps in four *M. arion* habitats of Aggtelek Karst region (Hungary). (unpublished)

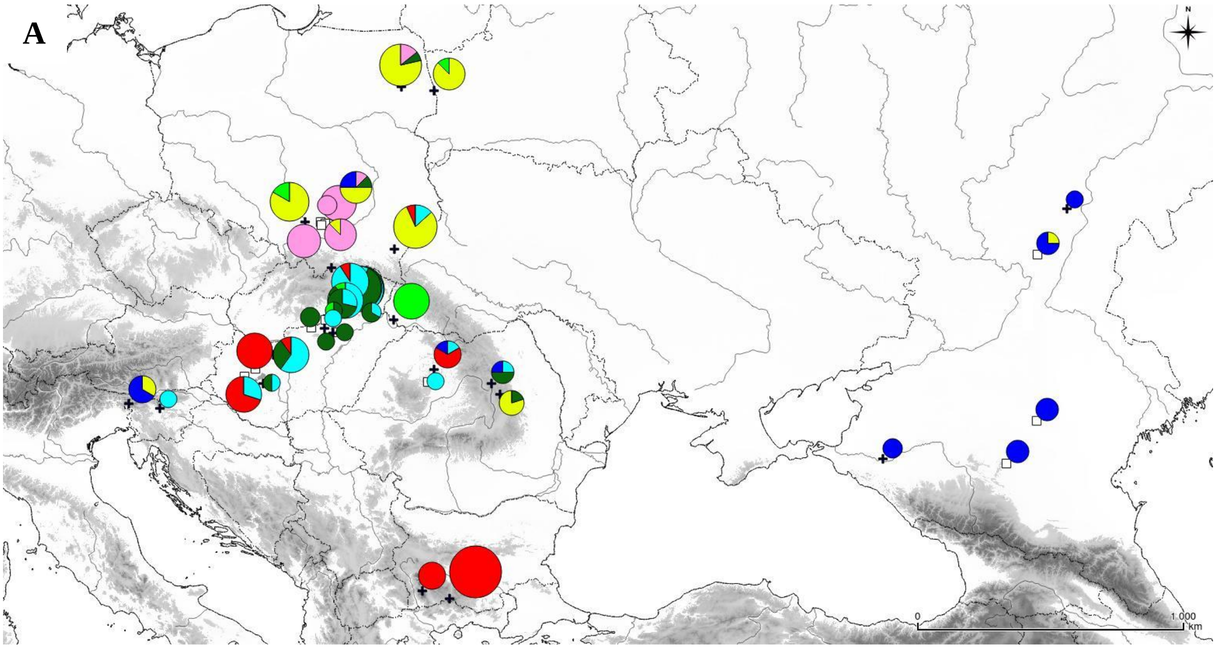
Additionally, we expanded our studies by screening other intracellular bacteria and we found *Lariskella arthropodarum* in *M. arion* which is the first occurrence data of this bacterium in lepidopterans. Additionally, we revealed the presence of sex ratio distorter *Spiroplasma* bacteria in *Myrmica* ants.

Certain parts of these results are included in a Hungarian study paper (Tóth et al. 2014.[1]) and in a Hungarian conference talk (Bereczki et al. 2016.[2]) but we are also planning to publish these results with some additions in an international journal.

(**See for more details**:
[1]Tóth, J.P., **Bereczki, J.** & Varga, Z. 2014. A nagypettyes hangyaboglárka (*Maculinea arion*) és a magyar tarkalepke (*Melitaea ornata kovacsi*) (Lepidoptera) az Aggteleki Nemzeti Park területén. [Large Blue (*Maculinea arion*) and Eastern Knapweed Fritillary (*Melitaea ornata kovacsi*) in Aggtelek National Park]. In: Tóth V. (szerk.): Kutatások az Aggteleki Nemzeti Parkban II. [Research in Aggtelek National Park and Biosphere Reserve II.] Aggteleki Nemzeti Park Igazgatóság, Jósvafő. p. /ANP Füzetek XI./. 119-132 p.)
[2]**Bereczki, J.**, Varga, Z., Váradi, A., Sramkó, G., Takács, A. & Tóth, J.P. 2016. Adaptáció és fajképződés két *Maculinea* faj példáján. [Adaptation and speciation in two *Maculinea* species] II. Országos Lepkész Találkozó, Szögliget. Abstract: p. 12.)

To better understand the background of the difference between the phenological forms of *M. arion*, using of phylogeographical tools is necessary. Due to international cooperation and our recent expeditions to Russia and to the Balkans, we could acquire several *M. arion* samples at Palearctic level. Therefore, we could investigate the phylogeographical patterns in this target species by 12 microsatellite loci. Similarly to the case of *M. alcon*, we found a genetic lineage originated from the Balkans which reached the Carpathian Basin from two directions (through Transylvania and from the western part of the Balkan Peninsula). At the same time, we also detected gene-flow from Asia (Fig. 8A). The separation of the spring and the summer *arion* was also confirmed (Fig. 8B) but surprisingly it showed a local adaptation pattern since neither the spring nor the summer *arion* is homogeneous and none of them could be trace back to special refugial origin.

We are planning to publish these results completed with molecular genetic study of 35 specimens from the Apennine Peninsula (which is in process) in an international journal.



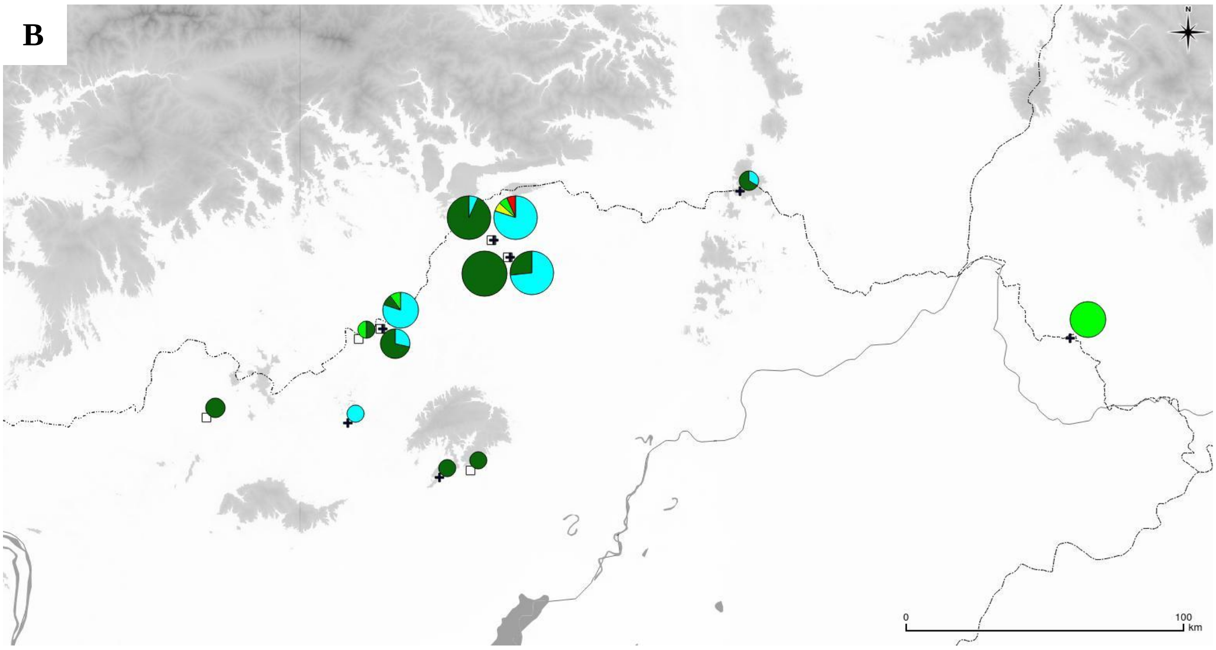


Figure 8. The phylogeography of *M. arion* based on 12 microsatellite loci. **A** – the phylogeographical pattern on a large geographical scale, **B** – the separation of syntopic population pairs of the spring and the summer *arion*. Empty squares represent the geographical locations of spring *arion* populations, plus signs indicate the locations of spring *arion* populations. (unpublished)

THE MAIN RESULTS IN THE GENUS *MELITAEA*

Several *Melitaea* species (*Me. phoebe*, *Me. ornata*, *Me. abbas*, *Me. arduinna*, *Me. ferghana*, *Me. sarvistana*, *Me. scotosia*) were screened but we have detected *Wolbachia* only in *Me. phoebe* and *Me. ornata* so far. These two species highly differed in the infestation pattern. The prevalence of *Wolbachia* was nearly 90% in *Me. phoebe* at Palaearctic level. At the same time, we found only tree infected *Me. ornata* individuals on the same geographical scale. The infected *Me. ornata* specimens were found in southern Italy, Hungary and Macedonia. We identified three different strains: the strain No. 694 previously published in the *Wolbachia* MLST database and two new strains described by us (No. 702 and 703). The infected *Me. ornata* individuals harboured the one of the new alleles. In parallel with the *Wolbachia* screening, a detailed phylogeographical reconstruction has been carried out on *Me. ornata* based on the mitochondrial cytochrome oxidase subunit I and four nuclear genes (EF-1a, MDH, RPS5 and wingless). We revealed that *Me. ornata* individuals split into two mitochondrial genetic lineages: the western clade clustered together with *Me. phoebe*, while the eastern clade is well-separated. On the contrary, both eastern and western clade of *Me. ornata* is well-separated from *Me. phoebe* based on the nuclear DNA. Thus, we identified the so-called ‘one barcode-two species phenomenon’ in the case of these closely related species. Since the level of *Wolbachia* infestation in the two species proved to be highly different, an on-going hybridisation between them is very unlikely which is also supported by the clear difference in the nuclear haplotypes between the two species. That is, the revealed mito-nuclear discordance seems to be the result of an ancient hybridisation event between *Me. phoebe* and *Me. ornata*, putatively in the Apennine Peninsula. Based on this large-scale study, we identified a *Wolbachia* strain causing ‘selective sweep’ in *Me. phoebe*.
(**See for more details:** Tóth et al. 2017. Mito-nuclear discordance helps to reveal the phylogeographic patterns of *Melitaea ornata* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society* 121(2): 267-281.)

Besides, we found a new population of *Me. ornata* in Transdanubia which is the known westernmost occurrence of this species in the Carpathian Basin. Simultaneously, we detected parasitoid occurrence (*Cotesia acuminata*) from this species in the first time. Additionally, *Me. ornata* co-occurs with *Me. phoebe* on this locality and the majority of the screened *Me. ornata* specimens (8 from 12) proved to be infected with the same *Wolbachia* strain as *Me. phoebe* whose infestation level is 100% on this site as well (based on 12 individuals). We found that the two closely related *Melitaea* species can feed on the same host plant (*Centaurea scabiosa*) on this locality and they can be found even on the same food plant in contrast with the previous results of Tóth et al. 2014* and 2015**. In addition, they could be parasitized by the same parasitoid wasp species (*Cotesia acuminata*). This is an interesting finding because in the Aggtelek Karst region, where these two species also co-occur, *Me. ornata* caterpillars develop only on *Cirsium pannonicum*. We could never detect parasitoid infection in *Me. ornata* in this region despite the large amount of screened individuals. We hypothesize that this observation could be the result of the effects of secondary metabolites of *C. pannonicum*.

Screening altogether 44 *Cotesia acuminata* wasps hatched from *Me. ornata* and *Me. phoebe* in the newly discovered Transdanubian site, we found a few individuals which seemed to be weakly infected with *Wolbachia* (faint bands on agarose gel). It refers to possible local horizontal transfer of *Wolbachia* via parasitoids. Additionally, we discovered a parasitoid wasp (*Hyposoter horticola*) and its hyperparasitoid (*Mesochorus* species) in *Me. phoebe* on this locality. This parasitoid system has been only known in *Me. cinxia* so far. Additionally, we found the same *Wolbachia* strain in *H. horticola* and *Me. phoebe* which also suggests horizontal *Wolbachia* transfer.

We are planning to publish these new results in an international journal.

(**See for more details:**

*Tóth et al. 2014. A nagypettyes hangyaboglárka (*Maculinea arion*) és a magyar tarkalepke (*Melitaea ornata kovacsi*) (Lepidoptera) az Aggteleki Nemzeti Park területén. [Large Blue (*Maculinea arion*) and Eastern Knapweed Fritillary (*Melitaea ornata kovacsi*) in Aggtelek National Park]. In: Tóth V. (szerk.): Kutatások az Aggteleki Nemzeti Parkban II. [Research in Aggtelek National Park and Biosphere Reserve II.] Aggteleki Nemzeti Park Igazgatóság, Jósvafő. p. /ANP Füzetek XI./. 119-132 p.);

**Tóth et al. 2015. Different host plant utilization ability of two closely related *Melitaea* species (Lepidoptera: Nymphalidae). *European Journal of Entomology* 112(1): 120-125.)

We also studied the infestation level of *Me. phoebe* on a smaller geographical scale. We screened *Wolbachia* in altogether 230 *Me. phoebe* individuals from 47 localities in Hungary (Fig. 9A) and only two specimens proved to be uninfected (all the other infected individuals harboured a single strain). Out of 14 females which laid their eggs under laboratory conditions, only one female and its offspring was uninfected. Most of the infected females have also infected offspring except one whose descendants were infected only in 33%. We could detect the infestation in all developmental stages. Based on these results, it seems that the maternal transmission rate is very high but not 100% in every case which is also supported by the occurrence of some rare, uninfected individuals. On the contrary, *Me. ornata* occurring locally in Hungary had much lower infection rate (based on 65 individuals). We detected *Wolbachia* in this species only in the newly discovered Transdanubian site (Fig. 9B).

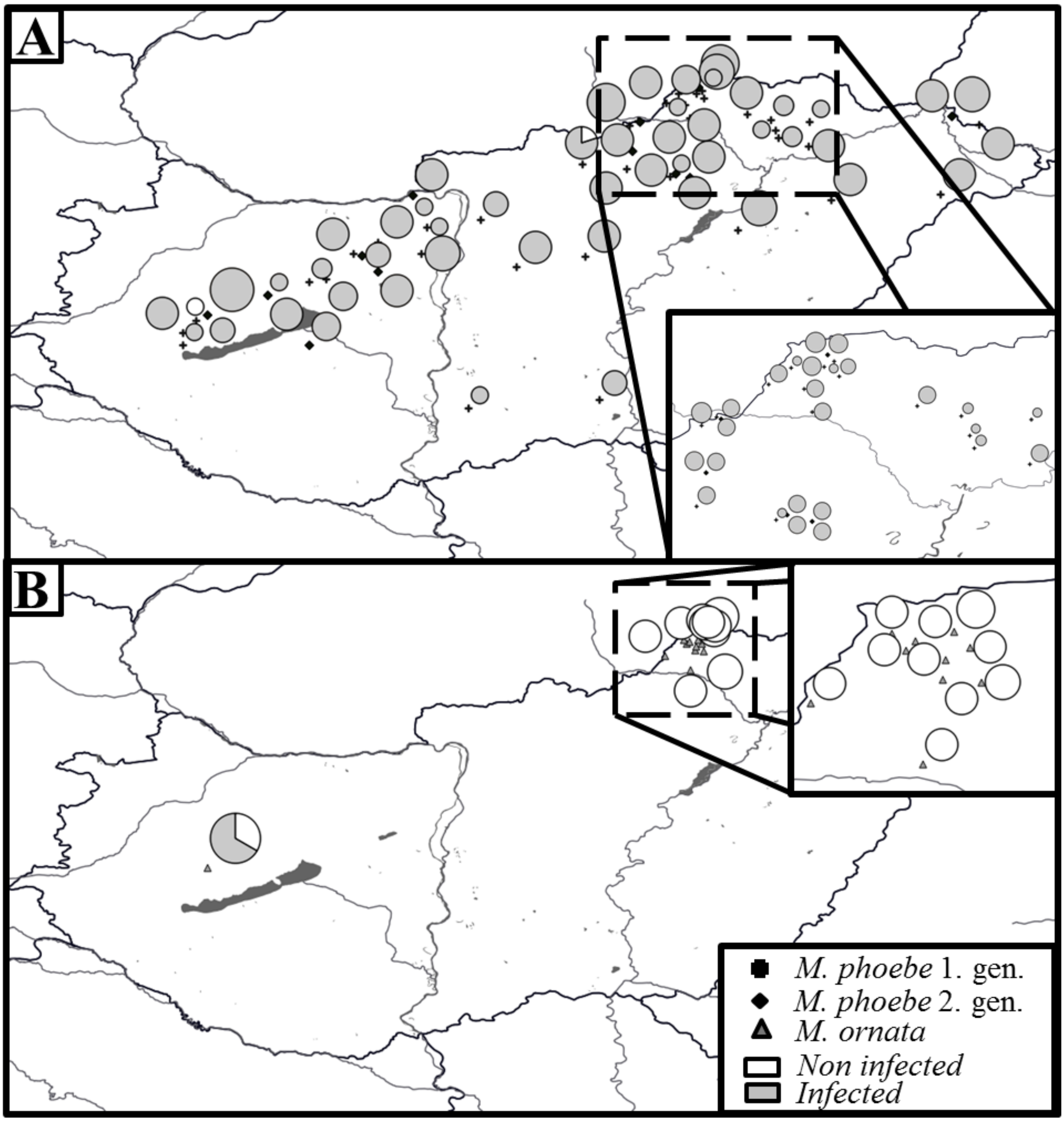


Figure 9. The *Wolbachia* infestation pattern in *Me. phoebe* **(A)** and *Me. ornata* **(B)** in Hungary. The size of the pie charts is proportional with the number of studied specimens. (unpublished)

In *Me. ornata* and *Me. phoebe*, we have studied the possible horizontal transfer routes screening the insects feeding on *Cirsium pannonicum* as well as their parasitoids. We identified different lepidopteran (*Zygaena brizae*, *Jordanita subsolana*, *Diachrysia chrysitis*) and coleopteran (e.g. Curculionidae, Chrysomelidae) species consuming *C. pannonicum*. We detected *Wolbachia* infestation only in *Z. brizae* and *J. subsolana* but with different strains (one new allele and allele No. 63, respectively) than in *Melitaea* species. At the same time, we discovered new parasitoid tachinid fly species in *Me. phoebe* which have been unknown up till now (*Drino inconspicua*, *Phryxe vulgaris*, *Erycia festinans*, *Erycia furibunda*). We found one case in which the horizontal transfer is likely between *Me. phoebe* and its parasitoid fly (*Erycia* sp.) as they contained the same strain. We identified two parasitoid fly species in *Z. brizae* (*Alsomyia capillata*, *Phryxe magnicornis*), one of which was infected with *Wolbachia*

but not with the same strain as its lepidopteran host. We also found a parasitoid wasp species (*Cotesia zygaenarum*) hatched from *Z. brizae* but it was uninfected. We are planning to publish these results in an international journal.
(**See for more details:** Váradi et al. 2015. Preliminary results on *Wolbachia* infestation in two closely related *Melitaea* species. XIX. European Congress of Lepidopterology, Dresden (Germany). Abstract (poster): pp. 88.)

We also clear up the taxonomy and biogeography of the endemic Iranian *Me. abbas* and reconstruct its potential range in climatically different epochs using the combination of genetic markers, geometric morphometry and climatic niche modelling. Our results show that this species is clearly separated from all taxa of the *Melitaea phoebe* species group and only known from Iran and Azerbaijan so far. Molecular markers and distribution modelling show consistently that this species should have had a long-term survival in this area and its range could have been slightly larger during the LGM than currently. Based on the studied molecular markers, three main groups (putative subspecies) in *M. abbas* can be recognised. We could not detect *Wolbachia* infection in this species.
(**See for more details:** Tóth et al. 2016. Long-term survival and diversification of an endemic *Melitaea* species in mountains of Iran and adjacent areas *Journal of Zoological Systematics and Evolutionary Research* 54(2): 106-115.)

OTHER *WOLBACHIA* STUDIES

(1) In collaboration with the Behavioural Ecology Research Group of Eötvös Lóránd University, we studied the effects of *Wolbachia* infestation on the behaviour of Common pill-bug (*Armadillidium vulgare*). We identified five different strains in the experimental animals. *Wolbachia* infection seemed to influence the behaviour of these arthropods decreasing their boldness.
(**See for more details:** Horváth et al. 2018. Roll with the fear: environment- and state-dependence of pill bug (*Armadillidium vulgare*) personalities. *Behavioural Processes* – manuscript under review.)
(2) We surveyed the infestation level in nine populations of Marsh fritillary butterfly (*Euphydryas aurinia*). We found 72% of the screened individuals to be infected with a single strain which was the same as detected in *Maculinea alcon* (in accordance with the results based on populations in the United Kingdom).
(**See for more details:** Pecsenye et al. 2018. Surprising diversity in the Pannonian populations of Marsh Fritillary (*Euphydryas aurinia*, Lepidoptera: Nymphalidae): morphometric and molecular aspects. *Journal of Zoological Systematics and Evolutionary Research* – revised manuscript under review.)

PUBLICATION LIST

PUBLISHED ARTICLES

1. **Bereczki, J.**, Pecsenye, K., Varga, Z., Tartally, A. & Tóth, J.P. 2018. *Maculinea rebeli* (Hirschke) – a phantom or reality? Novel contribution to a long-standing debate over the taxonomic status of an enigmatic Lycaenidae butterfly. ***Systematic Entomology*** 43(1): 166-182. **IF: 4.474**
   http://real.mtak.hu/id/eprint/64549
2. **Bereczki, J.**, Varga, Z. & Tóth, J.P. 2017. *Maculinea* or *Phengaris*? New insights from genitalia morphometry. ***Zoologischer Anzeiger – A Journal of Comparative Zoology*** 270: 57-59. **IF: 1.200**
   http://real.mtak.hu/id/eprint/73604
3. Tartally, A., Tóth, J.P., Váradi, A. & **Bereczki, J.** 2017. First data on the host ant usage of Large Blue from the Carpathian Basin. ***Sociobiology*** 64(1): 122-124. **IF: 0.699**
   http://real.mtak.hu/id/eprint/64550
4. Tóth, J.P., Varga, Z., Verovnik, R., Wahlberg, N., Váradi, A. & **Bereczki, J.** 2017. Mito-nuclear discordance helps to reveal the phylogeographic patterns of *Melitaea ornata* (Lepidoptera: Nymphalidae). ***Biological Journal of the Linnean Society*** 121(2): 267-281. **IF: 1.984**
   http://real.mtak.hu/id/eprint/64551
5. Tóth, J.P., Varga, Z. & **Bereczki, J.** 2016. Long-term survival and diversification of an endemic *Melitaea* species in mountains of Iran and adjacent areas ***Journal of Zoological Systematics and Evolutionary Research*** 54(2): 106-115. **IF: 2.444**
   http://real.mtak.hu/41379/
6. **Bereczki, J.**, Rácz, R., Varga, Z. & Tóth, J.P. 2015. Controversial patterns of *Wolbachia* infestation in the social parasitic *Maculinea* butterflies (Lepidoptera: Lycaenidae) ***Organisms Diversity & Evolution*** 15(3): 591-607. **IF: 1.734**
   http://real.mtak.hu/id/eprint/64552
7. Pecsenye, K., **Bereczki, J.**, Juhász, E., Tartally, A. & Varga, Z. 2015. Contrasting genetic structure in cuckoo and predatory *Maculinea* butterflies. ***Conservation Genetics*** 16(4): 939-954. 10.1007/s10592-015-0713-5 **IF: 2.040**
   http://real.mtak.hu/id/eprint/64553
8. **Bereczki, J.** 2015. *Wolbachia*-baktériumok és gazdáik. A manipuláció mesterei. ***Élet és Tudomány*** LXX(1): 16-18.
   http://real.mtak.hu/27888/
9. Tóth, J.P., **Bereczki, J.**, Végvári, Zs., Juhász, E. & Varga, Z. 2015. Different host plant utilization ability of two closely related *Melitaea* species (Lepidoptera: Nymphalidae). ***European Journal of Entomology*** 112(1): 120-125. **IF: 0.954**
   http://real.mtak.hu/id/eprint/64554
10. Tóth, J.P., **Bereczki, J.** & Varga, Z. 2014. A nagypettyes hangyaboglárka (*Maculinea arion*) és a magyar tarkalepke (*Melitaea ornata kovacsi*) (Lepidoptera) az Aggteleki Nemzeti Park területén. [Large Blue (*Maculinea arion*) and Eastern Knapweed Fritillary (*Melitaea ornata kovacsi*) in Aggtelek National Park]. *In*: Tóth V. (szerk.): Kutatások az Aggteleki Nemzeti Parkban II. [Researches in Aggtelek National Park and Biosphere Reserve II.] Aggteleki Nemzeti Park Igazgatóság, Jósvafő. p. /ANP Füzetek XI./. 119-132 p.
    http://real.mtak.hu/id/eprint/64604

11. **Bereczki, J.**, Tóth, J.P., Sramkó, G. & Varga, Z. 2014. Multilevel studies on the two phenological forms of Large Blue (*Maculinea arion*) (Lepidoptera: Lycaenidae). ***Journal of Zoological Systematics and Evolutionary Research*** 52(1): 32-43. **IF: 1.677**
    http://real.mtak.hu/id/eprint/64556
    (OTKA identification number is not indicated in the Aknowledgements)

TALK

Bereczki, J., Varga, Z., Váradi, A., Sramkó, G., Takács, A. & Tóth, J.P. 2016. Adaptáció és fajképződés két *Maculinea* faj példáján. [Adaptation and speciation in two *Maculinea* species] II. Országos Lepkész Találkozó, Szögliget. Abstract: p. 12.

POSTER

Váradi, A., Tóth, J.P. & **Bereczki, J.** 2015. Preliminary results on *Wolbachia* infestation in two closely related *Melitaea* species. XIX. European Congress of Lepidopterology, Dresden (Germany). Abstract: pp. 88.
http://real.mtak.hu/id/eprint/73606

MANUSCRIPTS UNDER REVIEW

1. Pecsenye, K., Tóth, A., Tóth, J.P., **Bereczki, J.**, Katona, G. & Varga, Z. 2018. Surprising diversity in the Pannonian populations of Marsh Fritillary (*Euphydryas aurinia*, Lepidoptera: Nymphalidae): morphometric and molecular aspects. ***Journal of Zoological Systematics and Evolutionary Research*** (revised manuscript under review)
2. Horváth, G., **Bereczki, J.**, Urszán, T.J., Balázs, G., Garamszegi, L.Zs. & Herczeg, G. 2018. Roll with the fear: environment- and state-dependence of pill bug (*Armadillidium vulgare*) personalities. ***Behavioural Processes*** (manuscript under review)