Wolbachia infection and parasitoid occurrence among plant-feeding caterpillars of the endangered butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) in southern Poland

Ewa B. Śliwińska, Rafał Martyka, Mariusz Cichoń & Piotr Tryjanowski

Śliwińska, E. B., Martyka, R., Cichoń, M. & Tryjanowski, P. 2019: *Wolbachia* infection and parasitoid occurrence among plant-feeding caterpillars of the endangered butterfly *Phengaris teleius* (Lepidoptera: Lycaenidae) in southern Poland. — Entomol. Fennica 30: 20–27. https://doi.org/10.33338/ef.79902

Parasites are an important component of ecological communities, as they shape host population dynamics and interfere with interspecific competition in ecosystems. Here, we studied *Wolbachia* infection and parasitoid occurrence among caterpillars of the endangered *Phengaris teleius* butterfly in five populations inhabiting southern Poland. The knowledge about potential parasites of *P. teleius* may be of particular importance for understanding forces regulating population processes of this species. Our study showed lack of *Wolbachia* infection and endoparasitoids in the sample of 91 4th instar *P. teleius* caterpillars. However, we found larvae of an unidentified hymenopteran ectoparasitoid on 17 3rd and 4th instar *P. teleius* caterpillars. We compare our results to findings from other populations of *P. teleius,* and its sister species in Europe and Asia, and discuss possible causes of observed patterns of parasite occurrence.

E. B. Śliwińska & R. Martyka, Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31–120 Kraków, Poland; E-mails: sliwinska@iop.krakow.pl, martyka@iop.krakow.pl
M. Cichoń, Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30–387 Kraków, Poland; E-mail: mariusz.cichon@uj.edu.pl
P. Tryjanowski, Institute of Zoology, Poznań University of Life Sciences, Wojska Polskiego 71C, 60–625 Poznań, Poland; E-mail: piotr.tryjanowski@gmail.com

Received 16 October 2017, accepted 14 May 2018

1. Introduction

Wolbachia (Hertig & Wolbach, 1924) (Rickettsiales: Rickettsiaceae) is a bacterial parasite of invertebrate animals that causes several problems, particularly in the conservation management of Lepidoptera (Hamm *et al.* 2014). *Wolbachia* is an intracellular α-proteobacterium that has the ability to manipulate the biology of its invertebrate hosts. In Lepidoptera, *Wolbachia* infection may induce feminization of genetic males, kill the male progeny of infected females and cause cytoplasmic incompatibility (i.e. inability of infected males to successfully mate with females lacking the same *Wolbachia* strain; Werren *et al.* 2008). Typically, *Wolbachia* spreads vertically in populations and is inherited maternally due to its presence in the cytoplasm of female gametes (Werren *et al.* 2008). The presence of *Wolbachia* may decrease the effective population size of Lepidop-

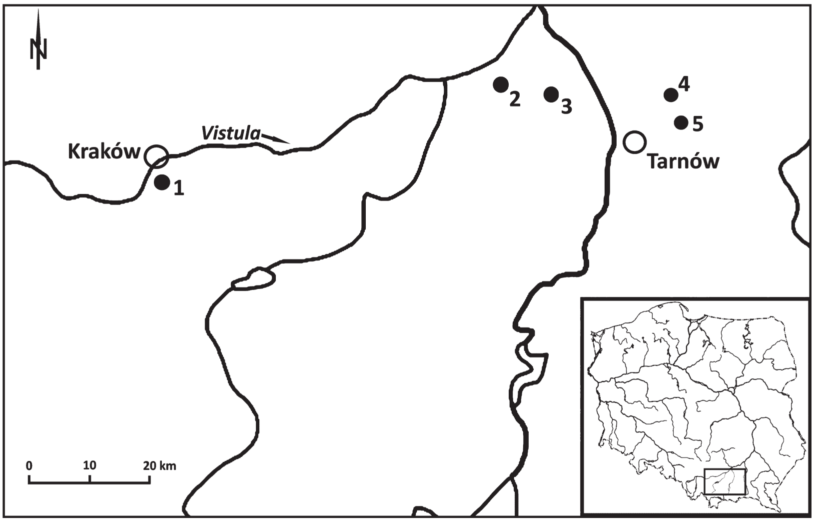

Fig. 1. Map of the study area with locations of the sampled *Phengaris teleius* populations (black dots). Localities: 1 – Kraków-Kostrze, 2 – Barczków, 3 – Jadowniki Mokre, 4 – Żukowice Stare, 5 – Zaczarnie.

tera and therefore poses a serious risk for threatened butterfly species (Hamm *et al.* 2014).

Parasitoid wasps (from the suborder Apocrita) are an example of parasites specialized in utilizing different arthropod species, including butterflies (Hinz 1983, Goulet & Huber 1993, Quicke 1997). Adult parasitoids attack Lepidoptera as eggs, larvae or pupae, laying their eggs inside the insects (endoparasitoids) or on their cuticulae (ectoparasitoids). Parasitoids can be used in pest control (e.g. van Lenteren & Woets 1988, van Lenteren 2000), but may negatively influence endangered populations of their hosts, as even a few dozen parasitoid species may attack the same host species (Godfray & Charles 1994).

In this study, we assessed the occurrence of *Wolbachia* infection among caterpillars of the scarce large blue butterfly *Phengaris teleius* (Bergsträsser, 1779), originating from populations located in southern Poland. In the course of sampling, we also recorded the presence of parasitoid larvae on *P. teleius* caterpillars. *Phenagris teleius* is a threatened butterfly (van Swaay & Warren 1999, van Swaay *et al.* 2012) that is considered to be a flagship species for nature conservation in Europe (Thomas 1995, Thomas & Settele 2004).

Identifying the potential parasites of *P. teleius* may be important for understanding population processes in this species (Dobson & Hudson 1986), with potential significance for conservation management of the butterfly (e.g. McCallum & Dobson 1995, Shaw & Hochberg 2002, Hamm *et al.* 2014).

2. Materials and methods

2.1. Study species, site and general procedures

The *P. teleius* butterfly is characterized by a complicated life cycle. Its caterpillar is a monophagous herbivore that feeds exclusively on the great burnet *Sanguisorba officinalis* L. As a 1st to 3rd instar caterpillar, it feeds inside a single flower bud until leaving the plant (Thomas 1984). 4th instar larvae drop to the ground, remaining there to wait for foraging *Myrmica* Latreille, 1804 ants. If foraging worker ants come across such a caterpillar, they take it to their ant colony in a process called adoption (Thomas 1984). The predatory *P. teleius* caterpillar then spends 11 or 23 months in the *Myrmica* nest, feeding on the ant brood (Thomas 1995, Witek *et al.* 2006). It pupates in late spring/early summer and leaves the colony as an adult butterfly between June and August (Thomas 1995, Witek *et al.* 2006).

We searched for *Wolbachia* infection and recorded parasitoid presence among caterpillars originating from five separate *P. teleius* populations in the western part of the Sandomierz Basin, southern Poland, in the years 2013–2014 (Fig. 1). In both seasons, the study was conducted in Au-

Table 1. Data gathered from five populations during two years of study, including number of inspected food plants, total number of caterpillars and number of caterpillars with ectoparasitoid larvae.

Year	Population	No. of plants inspected	No. of caterpillars	
			collected	with ecto-parasitoid larva
2013	Barczków	60	56	3
	Jadowniki Mokre	80	118	3
	Żukowice Stare	30	102	9
2014	Barczków	60	194	1
	Jadowniki Mokre	40	104	0
	Żukowice Stare	26	60	0
	Zaczarnie	22	26	0
	Kraków-Kostrze	43	118	1

gust when caterpillars, in their 4th larval instar, are most likely to be found on food plants. In each population, we randomly gathered a set of food plants that were later inspected under laboratory conditions to find *P. teleius* caterpillars.

After detecting a caterpillar, we confirmed its species and determined its larval instar, based on the identification table in Śliwińska *et al.* (2006), using a Nikon microscope SMZ 1500 (magnification 10–20×). In total, we found and investigated 778 *P. teleius* caterpillars at different larval instars, from 361 food plant stems (for details see Table 1). Afterwards, to kill and preserve the caterpillars, they were submerged in a solution of RNA Later (20 mM sodium citrate, 10 mM EDTA, 70% ammonium sulphate, pH 5.2; RNA Later solution also stabilizes DNA) and frozen at –30 °C until further examination.

To determine the presence of *Wolbachia* infection, we examined 4th instar caterpillars (91 caterpillars in total). As *Wolbachia* was not detected in 4th instar caterpillars (see below), we did not find it necessary to include younger larvae in our sample.

2.2. Molecular determination of *Wolbachia* infection

To test for *Wolbachia* infection in *P. teleius* caterpillars, we performed PCR of the 16S rDNA fragment using PCR protocols available in Patricelli *et al.* (2013) and W-Specf and W-Specr primers from Werren and Windsor (2000). Additionally, we used universal arthropod primers for 28S rDNA (as in Nice *et al.* 2009) to verify the negative results of the 16S rDNA *Wolbachia* PCR. For each sample, one or two PCRs were then performed. First, all samples were screened for *Wolbachia* (16S rDNA PCR) and afterwards, the samples with a negative result were analysed for arthropod 28S rDNA to check for overall PCR quality. In cases where the quality of 28S rDNA PCR was poor, the DNA sample was sequentially diluted, following Nice *et al.* (2009), and *Wolbachia* PCR was performed again to confirm the negative result. DNA isolation was performed as follows. A whole caterpillar body was macerated in 50 µl of TE buffer, and 1 µl of Proteinase K (Thermo Scientific, 14–22 mg/ml) was added. The mixture was then placed in a thermoblock for 2 h at 56 °C. After protein digestion, 100 µl of 5% CHELEX (chelating material, BioRad) solution was added and the mixture was intensively vortexed for 1 min. After that, the mixture was placed in a thermoblock at 95 °C with an intensive shake (1,400 rpm) for 10 min and then centrifuged at 13,000 rpm for 10 min. The supernatant with purified DNA was taken to the PCR chamber. PCR was performed in a SensoQuest Labcycler. The PCR products were visualized on 1% agarose gels.

2.3. Inspection for parasitoids

At the moment of extraction from inflorescences, each caterpillar, from 1st to 4th instars, was inspected for ectoparasitoid larvae feeding on the surface of their bodies. Furthermore, all 4th instar *P. teleius* caterpillars were checked for the presence of endoparasitoid larvae. We examined only 4th instar caterpillars, as visual detection of younger endoparasitoids is unreliable in *P. teleius* (Anton *et al.* 2007b, Anton, personal inform.). Thus, each 4th instar caterpillar was dissected, after thawing under sterile conditions (on a single-use microscope slide, cut with a single-use sterile scalpel and sterile microscope needle), in order to find the parasitoid larvae inside the body using a Nikon microscope SMZ 1500 (magnification 10–20×). After inspection, the caterpillar was again placed in RNA Later solution for further genetic analyses of *Wolbachia* infection.

3. Results

All 4th instar caterpillars of *P. teleius* were found to be free from *Wolbachia* infection. In addition, no endoparasitoids were found in our sample, either. In contrast, we detected larvae of ectoparasitoid wasps that, however, remained unidentified. We were unable to rear the parasitoids to the adult stage, and any attempts to assign the larvae, even to a family on morphological grounds, would have remained uncertain (Burks 2003). Unfortunately, we also lost the genetic material of the ectoparasitoid larvae, due to difficulties associated with preservation of DNA samples, so that DNA barcoding could not be applied either. Infested caterpillars were paralyzed, i.e. all muscles of a caterpillar were loosened, and it did not move, although it remained alive during the observation (Fig. 2). In total, we found 17 caterpillars (3rd and 4th instars) with ectoparasitoid larvae, in the four studied *P. teleius* populations (for details see Table 1).

4. Discussion

In our study, we found no *Wolbachia* infection among the screened *P. teleius* caterpillars originating from five studied populations, located in the western part of the Sandomierz Basin, in southern Poland. The lack of *Wolbachia* infection was confirmed by the most appropriate and sensitive available molecular methods. Therefore, we are confident in our results. Interestingly, Ritter *et al.* (2013) analysed *P. teleius* individuals from four populations in Poland – Wólka near Warsaw, Kosyń near Włodawa, Wiesiółka near Zawiercie and Widacz near Krosno. However, all individuals from these populations were also free from *Wolbachia* infection.

Our study was performed on populations located between Wólka and Zawiercie (Ritter *et al.* 2013), providing information about *Wolbachia* infection in another part of the Polish range of *P. teleius.* In contrast, a recent genetic study conducted on *P. teleius* revealed the occurrence of *Wolbachia* infection (lineage B) in Mongolian, Russian (Altai region), Belarusian and French *P. teleius* populations (Ritter *et al.* 2013), as well as (lineage A and B) in Hungary and Romania (Bereczki *et al*. 2015). In total, 13% of screened individuals were infected within the investigated range of *P. teleius* occurrence in Ritter *et al.* (2013) and 14% of examined individuals were reported to be infected in the Carpathian Basin (Bereczki *et al*. 2015).

Other butterfly species from the *Phengaris* clade have also shown differential *Wolbachia* infection. So far, *Wolbachia* has been found in *P. nausithous* (Bergsträsser, 1779) populations in Kazakhstan, Russia (Volgograd region), Slovakia and Czechia (*Wolbachia* super-group B; Ritter *et al.* 2013) as well as in Hungary and Romania (super-group A and B, Bereczki *et al.* 2015). *Wolbachia* infection has also been documented in *P. alcon* (Denis & Schiffermüller, 1775) from Lithuania, Poland, Austria, Hungary and Romania (*Wolbachia* supergroup B, Sielezniew *et al.* 2012, Bereczki *et al.* 2015) and in *P. arion* (Linnaeus, 1758) from Poland, Italy, Hungary and Romania (*Wolbachia* supergroup A, Patricelli *et al.* 2013, Bereczki *et al.* 2015).

Our study showed that populations of *P. teleius* from southern Poland are attacked by an unidentified species of ectoparasitoid wasps. To our knowledge, this is the first observation of ectoparasitoid larvae feeding on caterpillars of *Phengaris* butterflies. At the same time, we failed

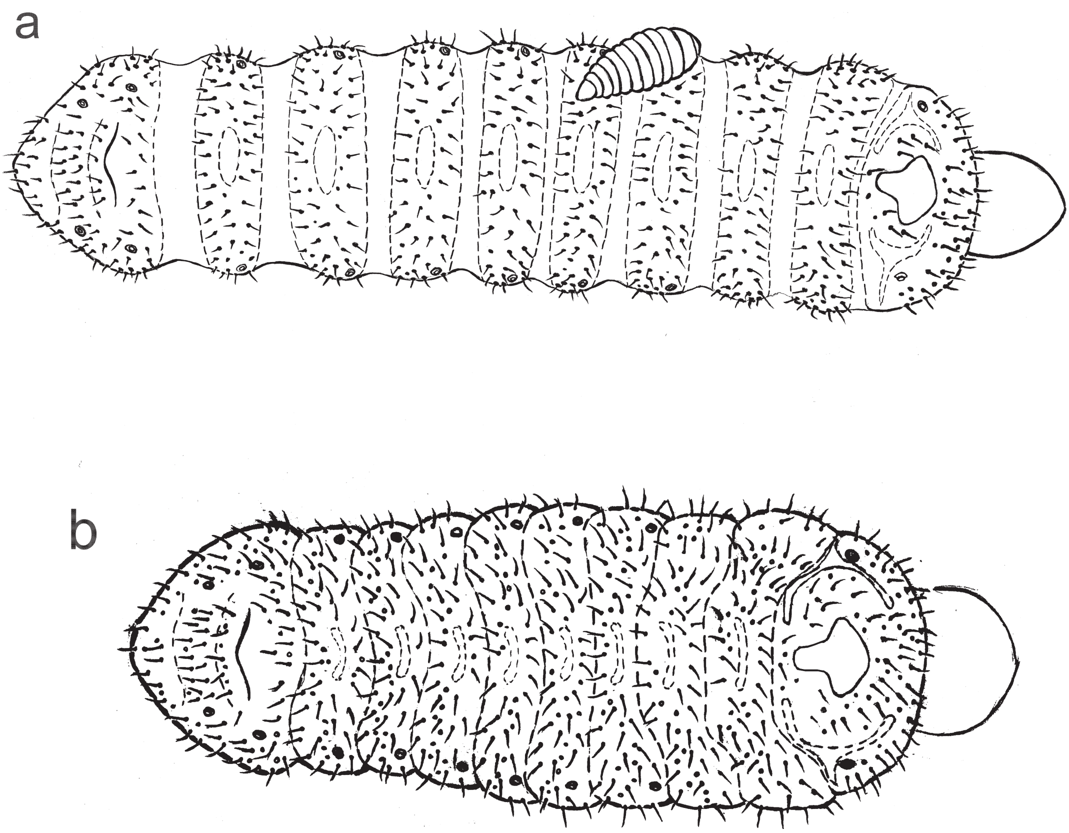

Fig. 2. Sketches of a 3rd instar caterpillar of *Phengaris teleius*. – a. A paralyzed caterpillar infested by an ectoparasitoid larva. – b. An uninfested caterpillar.

to find the larvae of any endoparasitic wasps in *P. teleius* caterpillars from the same region of southern Poland. The latter finding is concordant with that of Anton *et al.* (2007a), who studied two populations of *P. teleius* (similarly, by dissecting *P. teleius* caterpillars feeding on plants; Anton, unpublished data) in the Upper Rhine Valley, southwestern Germany.

In general, various endoparasitic *Neotypus* (Ichneumonidae) species attack the predatory myrmecophilous species of *Phengaris* (*P. teleius, P. nausithous* and *P. arion*). In particular, in Hungary, larvae of the parasitoid wasp, *N. melanocephalus* Gmelin, 1790 (= *N. pusillus* Gregor, 1940) have been found in a *P. teleius* pupa, originating from *Myrmica* nests (Tartally 2005). This suggests that *N. melanocephalus* is the parasitoid of *P. teleius* in the Carpathian Basin, but its frequency is very low (only one pupa with a parasitoid larva was detected among eight sites of *P. teleius* in the Carpathian Basin, Tartally 2005). Probably, *N. melanocephalus* is a specialist parasitoid of *P. nausithous*, the sister species of *P. teleius*, and it is recorded from Poland (Stankiewicz *et al.* 2004) and southwestern Germany (Anton *et al.* 2007a). Therefore, the observations of *N. melanocephalus* attacking *P. teleius* caterpillars might be based on accidental events. In turn, *N. coreensis* Uchida, 1930 has been shown to attack the predatory *P. arion* (Sielezniew *et al.* 2010).

In contrast, *Ichneumon* sp. attacks *P. alcon,* a *Phengaris* species with the “cuckoo” lifestyle (Thomas & Elmes 1993, Sielezniew & Stankiewicz 2004, Stankiewicz *et al.* 2004, Tartally 2005, 2008, Tartally *et al.* 2013, 2014, Timu *et al.* 2013). So far, there is one known case of predatory *P. teleius* getting parasitized by *Ichneumon* sp. (Tartally 2008). *Ichneumon* sp. has numerous adaptations to infiltrate *Myrmica* colonies and to find and oviposit into *Phengaris* larvae. Therefore, *Phengaris* cuckoo species are attacked by parasitoids only within *Myrmica* ant colonies (Thomas & Elmes 1993, Witek *et al.* 2014).

The presence and diversity of parasites within an ecosystem is a sign of its health (Hudson *et al.* 2006). So, what is the implication of the scarce number of parasites attacking a given species in local populations? The potential reasons of very low frequency of parasites in the studied *P. teleius* butterfly (including *Wolbachia*) may be in density-dependent effects occurring in host populations across the study region (Cronin 2004, Hancock *et al*. 2016), but also in biotic and abiotic factors, such as natural enemies of parasites, or microclimatic conditions (Ram *et al*. 2008, Heard *et al*. 2015).

The host population turnover and decrease in host densities may have a negative effect on the persistence of parasitoid populations, as well (e.g. Cronin 2004). Populations of *P. teleius* located in our study area have been described as stable and weakly influenced by weather conditions (Nowicki *et al*. 2005, 2009), as well as resistant to natural catastrophes (i.e. flood and fire, Kajzer-Bonk *et al*. 2013, Nowicki *et al*. 2014).

However, during the two years of our study, we witnessed the disappearance of several subpopulations of *P. teleius*, most likely due to succession and a lack of proper habitat management within respective habitat patches (Batáry *et al*. 2007, Dierks & Fischer 2009, van Swaay *et al*. 2012). In the context of frequent subpopulation turnovers, like those observed for *P. teleius* in southern Poland, the conditions for the persistence of the populations of parasites may not be met. Otherwise, the lack of *Wolbachia* infection among inspected *P. teleius* caterpillars may be due to other factors than frequent population turnovers.

In fact, *P. arion* and *P. alcon* have infestation levels of 100% (e.g. Particelli *et al.* 2013, Bereczki *et al*. 2015) even though their population parameters are similar to *P. teleius*. Therefore, the potential mechanisms that cause low levels of *Wolbachia* infection in *P. teleius* remain unknown. To fully understand the factors that determine parasitoid occurrence and *Wolbachia* infection in *P. teleius* populations, a large scale, long-term study is needed, which would take into account habitat changes and the abundance dynamics of butterflies in local populations.

Acknowledgements. Specimens of *P. teleius* caterpillars were collected with permission of the General Directorate for Environmental Protection in Poland (DOP-oz. 6401.01.52.2013.JRO). The study was financially supported by the Polish National Science Centre via a post-doctoral fellowship (DEC-2012/04/S/NZ8/00215) and partly by statutory funds of the Institute of Nature Conservation of the Polish Academy of Sciences. We thank anonymous reviewers for useful commentaries that helped to improve the manuscript.

References

Anton, C., Musche, M. & Settele, J. 2007a: Spatial patterns of host exploitation in a larval parasitoid of the predatory dusky large blue *Maculinea nausithous*. — Basic and Applied Ecology 8: 66–74. doi: https://doi.org/10.1016/j.baae.2006.03.006

Anton, C., Zeisset, I., Musche, M., Durka, W., Boomsma, J. J. & Settele, J. 2007b: Population structure of a large blue butterfly and its specialist parasitoid in a fragmented landscape. — Molecular Ecology 16: 3828–3838. doi: https://doi.org/10.1111/j.1365-294X.2007.03441.x

Batáry, P., Örvössy, N., Kőrösi, Á., Vályinagy, M. & Peregovits, L. 2007: Microhabitat preferences of *Maculinea teleius* (Lepidoptera: Lycaenidae) in a mosaic landscape. — European Journal of Entomology 104: 731–736. doi: https://doi.org/10.14411/eje.2007.093

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: 591–607. doi: https://doi.org/10.1007/s13127-015-0217-7

Burks, R. A. 2003: Key to the Nearctic genera of *Eulophidae*, subfamilies *Entedoninae*, *Euderinae* and *Eulophinae* (Hymenoptera: Chalcidoidea). — World Wide Web electronic publication. URL http//cache.ucr.edu/%7Eheraty/Eulophidae/ (Site visited on 10 October 2017.)

Cronin, J. T. 2004: Host-parasitoid extinction and colonization in a fragmented prairie landscape. — Oecologia 139: 503–514. doi: https://doi.org/10.1007/s00442-004-1549-8

Dierks, A. & Fischer, K. 2009: Habitat requirements and niche selection of *Maculinea nausithous* and *M. teleius* (Lepidoptera: Lycaenidae) within a large sympatric metapopulation. — Biodiversity and Conservation 18: 3663–3676. doi: https://doi.org/10.1007/s10531-009-9670-y

Dobson, A. P. & Hudson, P. J. 1986: Parasites, disease and the structure of ecological communities. — Trends in Ecology and Evolution 1: 11–15. doi: https://doi.org/10.1016/0169-5347(86)90060-1

Godfray, H. & Charles, J. 1994: Parasitoids: Behavioral and Evolutionary Ecology. — Princeton University Press, Princeton. 473 pp.

Goulet, H. & Huber, J. T. 1993: Hymenoptera of the world: an identification guide to families. — Ottawa, Ont, Centre for Land and Biological Resources Research.

Hamm, C. A., Handley, C. A., Pike, A., Forister, M. L. Fordyce, J. A. & Nice, C. C. 2014: *Wolbachia* infection and Lepidoptera of conservation concern. — Journal of Insect Science 14: 6. doi: https://doi.org/10.1093/jis/14.1.6

Hancock, P. A., White, V. L., Ritchie, S. A., Hoffmann, A. A. & Godfray, H. C. J. 2016: Predicting *Wolbachia* invasion dynamics in *Aedes aegypti* populations using models of density-dependent demographic traits. — BMC Biology 14: 96. doi: https://doi.org/10.1186/s12915-016-0319-5

Heard, G. W., Thomas, C. D., Hodgson, J. A., Scroggie, M. P., Ramsey, D. S. & Clemann, N. 2015: Refugia and connectivity sustain amphibian metapopulations afflicted by disease. — Ecology Letters 18: 853–863. doi: https://doi.org/10.1111/ele.12463

Hinz, R. 1983: The biology of the European species of the genus *Ichneumon* and related species (Hymenoptera: Ichneumonidae). — Contribution of the American Entomological Institute 20: 151–152.

Hudson, P. J., Dobson, A. P. & Lafferty, K. D. 2006: Is a healthy ecosystem one that is rich in parasites? — Trends in Ecology and Evolution 21: 381–385. doi: https://doi.org/10.1016/j.tree.2006.04.007

Kajzer-Bonk, J., Nowicki, P., Bonk, M., Skórka, P., Witek, M. & Woyciechowski, M. 2013: Local populations of endangered *Maculinea* (Phengaris) butterflies are flood resistant. — Journal of Insect Conservation 17: 1105–1112. doi: https://doi.org/10.1007/s10841-013-9591-7

McCallum, H. & Dobson, A. 1995: Detecting disease and parasite threats to endangered species and ecosystems. — Trends in Ecology and Evolution 10: 190–194. doi: https://doi.org/10.1016/S0169-5347(00)89050-3

Nice, C. C., Gompert, Z., Forister, M. L. & Fordyce, J. A. 2009: An unseen foe in arthropod conservation efforts: The case of *Wolbachia* infections in the Karner blue butterfly. — Biological Conservation 142: 3137–3146. doi: https://doi.org/10.1016/j.biocon.2009.08.020

Nowicki, P., Bonelli, S., Barbero, F. & Balletto, E. 2009: Relative importance of density-dependent regulation and environmental stochasticity for butterfly population dynamics. — Oecologia 161: 227–239 doi: https://doi.org/10.1007/s00442-009-1373-2

Nowicki, P., Marczyk, J. & Kajzer-Bonk, J. 2014: Metapopulations of endangered *Maculinea* butterflies are resilient to large-scale fire. — Ecohydrology 8: 398–405. doi: https://doi.org/10.1002/eco.1484

Nowicki, P., Witek, M., Skórka, P., Settele, J. & Woyciechowski, M. 2005: Population ecology of the endangered butterflies *Maculinea teleius* and *M. nausithous*, and its implications for conservation. — Population Ecology 47: 193–202. doi: https://doi.org/10.1007/s10144-005-0222-3

Patricelli, D., Sielezniew, M., Ponikwicka-Tyszko, D., Ratkiewicz, M., Bonelli, S., Barbero, F., Witek, M., Buś, M. M., Rutkowski, R. & Balletto, E. 2013: Contrasting genetic structure of rear edge and continuous range populations of a parasitic butterfly infected by *Wolbachia*. — BMC Evolutionary Biology 13: 14. doi: https://doi.org/10.1186/1471-2148-13-14

Quicke, D. L. J. 1997: Parasitic wasps. — Chapman & Hall Ltd., London. 470 pp.

Ram, K., Preisser, E. L., Gruner, D. S. & Strong, D. R. 2008: Metapopulation dynamics override local limits on long-term parasite persistence. — Ecology 89: 3290-3297. doi: https://doi.org/10.1890/08-0228.1

Ritter, S., Michalski, S. G., Settele, J., Wiemers, M., Fric, Z. F., Sielezniew, M., Šašić, M., Rozier, Y. & Durka, W. 2013: *Wolbachia* infections mimic cryptic speciation in two parasitic butterfly species, *Phengaris teleius* and *P. nausithous* (Lepidoptera: Lycaenidae). — PLoS ONE 8(11): e78107. doi: https://doi.org/10.1371/journal.pone.0078107

Shaw, M. R. & Hochberg, M. E. 2002: The neglect of parasitic hymenoptera in insect conservation strategies: the British fauna as a prime example. — Journal of Insect Conservation 5: 253–263. doi: https://doi.org/10.1023/A:1013393229923

Sielezniew, M., Rutkowski, R., Ponikwicka-Tyszko, D., Ratkiewicz, M., Dziekanska, I. & Švitra, G. 2012: Differences in genetic variability between two ecotypes of the endangered myrmecophilous butterfly *Phengaris* (= *Maculinea*) *alcon* – the setting of conservation priorities. — Insect Conservation and Diversity 5: 223–236.

Sielezniew, M. & Stankiewicz, A. M. 2004: Simultaneous exploitation of *Myrmica vandeli* and *M. scabrinodis* (Hymenoptera: Formicidae) colonies by the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae). — European Journal of Entomology 101: 693–696. doi: https://doi.org/ 10.14411/eje.2004.091

Sielezniew, M., Włostowski, M. & Dziekańska, I. 2010: *Myrmica schencki* (Hymenoptera: Formicidae) as the primary host of *Phengaris* (*Maculinea*) *arion* (Lepidoptera: Lycaenidae) at heathlands in eastern Poland. — Sociobiology 55: 1–12.

Stankiewicz, A. M., Sielezniew, M. & Sawoniewicz, J. 2004: *Neotypus pusillus* Gregor, 1940 (Hymenoptera, Ichneumonidae) endoparasite of *Maculinea nausithous* (Bergsträsser, 1779) (Lepidoptera, Lycaenidae): new data on distribution in Poland with remarks on its biology. — Fragmenta Faunistica 47: 115-120.

Śliwińska, E. B., Nowicki, P., Nash, D. R., Witek, M., Settele, J. & Woyciechowski, M. 2006: Morphology of caterpillars and pupae of European *Maculinea* species (Lepidoptera: Lycaenidae) with an identification table. — Entomologica Fennica 17: 351–358.

Tartally, A. 2005: *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae): first record of a parasitoid wasp attacking *Maculinea teleius* (Lycaenidae). — Nota Lepidopterologica 28: 21–23.

Tartally, A. 2008: Myrmecophily of *Maculinea* butterflies in the Carpathian Basin (Lepidoptera: Lycaenidae).

PhD thesis. — University of Debrecen, Debrecen. 25 pp.

Tartally, A., Koschuh, A. & Varga, Z. 2014: The re-discovered *Maculinea rebeli* (Hirschke, 1904): Host ant usage, parasitoid and initial food plant around the type locality with taxonomical aspects (Lepidoptera, Lycaenidae). — ZooKeys 406: 25–40. doi: https://doi.org/10.3897/zookeys.406.7124

Tartally, A., Rodrigues, M. C., Brakels, P. & Arnaldo, P. S. 2013: *Myrmica aloba* (Hymenoptera: Formicidae) hosts isolated populations of a hoverfly, a butterfly and an ichneumon species in NE-Portugal. — Journal of Insect Conservation 17: 851–855. doi: https://doi.org/10.1007/s10841-013-9575-7

Thomas, J. A. 1984: The behaviour and habitat requirements of *Maculinea nausithous* (the dusky large blue butterfly) and *M. teleius* (the scarce large blue) in France. — Biological Conservation 28: 325–347. doi: https://doi.org/10.1016/0006-3207(84)90040-5

Thomas, J. A. 1995: The ecology and conservation of *Maculinea arion* and other European species of the large blue butterfly. — In: Pullin A. S. (ed.), Ecology and Conservation of Butterflies: 180–197. Chapman & Hall, London. 363 pp.

Thomas, J. A. & Elmes, W. 1993: Specialised searching and the hostile use of allomones by a parasitoid whose host, the butterfly *Maculinea rebeli* inhabits ant nests. — Animal Behavior 45: 593–602. doi: https://doi.org/10.1006/anbe.1993.1069

Thomas, J. A. & Settele, J. 2004: Butterfly mimics of ants. — Nature 432: 283–284. https://doi.org/10.1038/432283a

Timu, N., Constantineanu, R. & Rákosy, L. 2013: Ichneumon balteatus (Hymenoptera: Ichneumonidae) – a new parasitoid species of *Maculinea alcon* butterflies (Lepidoptera: Lycaenidae). — Entomologica Romanica 18: 31–35.

Van Lenteren, J.C. 2000: Success in biological control of arthropods by augmentation of natural enemies. — In: Gurr G. & Wratten S. (eds), Biological Control: Measures of Success: 77–104. Kluwer Academic Publishers, Dordrecht. 428 pp. doi: https://doi.org/10.1007/978-94-011-4014-0_3

Van Lenteren, J. C. & Woets, J. 1988: Biological and integrated pest control in greenhouses. — Annual Review of Entomology 33: 239. doi: https://doi.org/10.1146/annurev.en.33.010188.001323

van Swaay, C., Collins, S., Dušej, G., Maes, D., Munguira, M.L., Rakosy, L., Ryrholm, N., Šašić, M., Settele, J., Thomas, J. A., Verovnik, R., Verstrael, T., Warren, M., Wiemers, M. & Wynhoff, I. 2012: Dos and Don'ts for butterflies of the Habitats Directive of the European Union. — Nature Conservation 1: 73–153. doi: https://doi.org/10.3897/natureconservation.1.2786

van Swaay, C. A. M. & Warren, M. S. 1999: Red data book of European butterflies (Rhopalocera). — Nature and Environment 99: 129–134.

Werren, J. H., Baldo, L. & Clark, M. E. 2008: *Wolbachia*: master manipulators of invertebrate biology. — Nature Reviews Microbiology 6: 741–751. doi: https://doi.org/10.1038/nrmicro1969

Werren, J. H. & Windsor, D. M. 2000: *Wolbachia* infection frequency in insects: evidence of a global equilibrium? — Proceedings of the Royal Society of London B 267: 1277–1285. doi: https://doi.org/10.1098/rspb.2000.1139

Witek, M., Barbero, F. & Markó, B. 2014: Myrmica ants host highly diverse parasitic communities: from social parasites to microbes. — Insectes Sociaux 61: 307–323. doi: https://doi.org/10.1007/s00040-014-0362-6

Witek, M., Śliwińska, E. B., Skórka, P., Nowicki, P. & Woyciechowski, M. 2006: Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in myrmecophilous insects. — Oecologia 148: 729–733. doi: https://doi.org/10.1007/s00442-006-0404-5