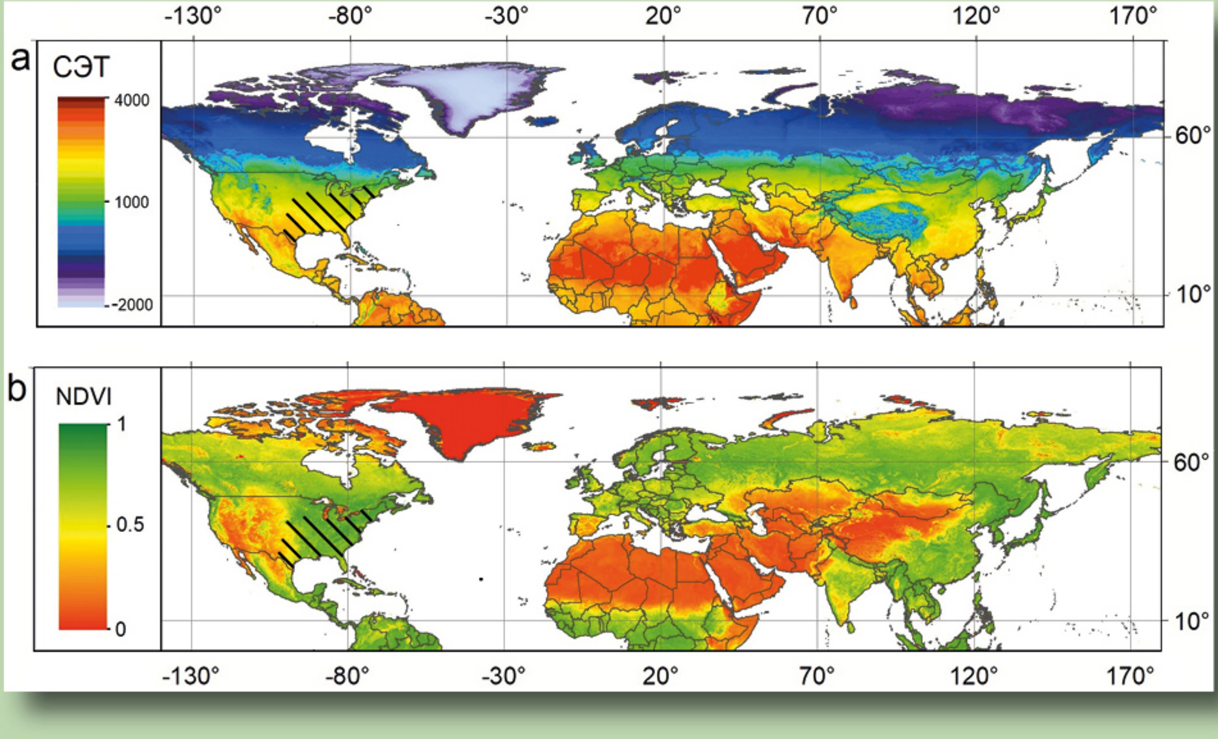
ISSN 1727-1320 (Print),
ISSN 2308-6459 (Online)

ВЕСТНИК
ЗАЩИТЫ РАСТЕНИЙ
PLANT PROTECTION NEWS

2021 ТОМ VOLUME 104 ВЫПУСК ISSUE 1



Санкт-Петербург
St. Petersburg, Russia

OECD+WoS: 1.06+IY (Entomology) https://doi.org/10.31993/2308-6459-2021-104-1-14945

Full-text article

IS *APORIA CRATAEGI* AN UNSUITABLE HOST OF *WOLBACHIA* SYMBIONTS?

R.A. Bykov*, G.V. Yurlova, M.A. Demenkova, Yu.Yu. Ilinsky

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

**corresponding author, e-mail: bykovra@bionet.nsc.ru*

The Black-veined White *Aporia crataegi* (Lepidoptera: Pieridae) is a trans-Palearctic species causing damage to various fruit and berry crops. Here we analyzed *Wolbachia* infection in *A. crataegi* populations. *Wolbachia* bacteria are maternally transmitted intracellular symbionts of many arthropods, including numerous Lepidoptera. We have studied 376 samples of *A. crataegi* collected in 10 regions of Russia from the Far East to Kaliningrad. *Wolbachia* prevalence was very low; only eight *Wolbachia*-positive specimens of *A. crataegi* were detected in Yakutia, Republic of Buryatia, Sverdlovsk and Kaliningrad Provinces, and no infection was found in other localities. Two *Wolbachia* haplotypes, ST-19 and ST-109, from A and B supergroups respectively, were identified using the multilocus sequence typing (MLST) protocol. These haplotypes were also previously reported in different lepidopteran species. Both *Wolbachia* haplotypes were associated with the same mtDNA haplotype (as inferred from the cytochrome oxidase subunit I gene) of *A. crataegi*, and ST-19 with two mtDNA haplotypes. This incongruence of maternally inherited agents indicates independent cases of the bacteria acquisition in *A. crataegi* history. The above data suggest that *Wolbachia* can infect *Aporia crataegi* but cannot establish in the host populations.

Keywords: *Wolbachia*, Pieridae, Lepidoptera, *Aporia*, mtDNA

Received: 10.01.2021 *Accepted: 30.03.20211*

Introduction

The Black-veined White *Aporia crataegi* L. (Lepidoptera: Pieridae) is a pest of various fruit and berry crops. The larvae damage the species of *Prunus*, *Crataegus*, *Rosa*, *Pyrus*, *Padus*, *Sorbus* and several other genera (Emmet, Heath 1989; Gorbunov, Kosterin, 2003). Population outbreaks result in complete defoliation of trees (Ilyinskiy, Tropin, 1965; Maximov, Marushchak, 2012). This butterfly is a trans-Palearctic species with high migratory activity (Tolman and Lewington 2008). The abundance of *A. crataegi* varies in different regions, for instance, in Russia it is rare in Ural, Amurland and Primorye, but abundant in most of West Siberia (Gorbunov, Kosterin, 2003). In some regions, populations of *A. crataegi* fluctuate greatly from year to year, e.g., in Ural (Gorbunov, Kosterin, 2003) or have long-term fluctuations, e.g., in Finland (Kuussaari et al. 2007). Decreasing *A. crataegi* populations (Fokin, Korovin, 2001; Kim et al., 2015; Jugovic et al., 2017), have been observed in the territories of Northern, Central, Eastern and Southern Europe, and North Africa, primarily due to human activity (van Swaay et al., 2010; Todisco et al., 2020). Extinction of *A. crataegi* has been reported in England, Czech Republic, The Netherlands, and South Korea (Asher et al. 2001; van Swaay et al., 2010; Park et al., 2013; Kim et al., 2020).

Bacteria of the *Wolbachia* genus are maternally inherited intracellular symbionts found in many insects (Hilgenboecker et al., 2008; Zug, Hammerstein, 2012). *Wolbachia* can affect host biology in different ways. Reproductive abnormalities, such as male killing, feminization of males, thelytokous parthenogenesis and cytoplasmic incompatibility (CI) are the ways for *Wolbachia* to spread in a host population (Werren et al., 2008). *Wolbachia* can also be a mutualist by providing for essential nutrients, protecting from viruses and parasites or increasing lifespan and fecundity of the hosts (De Barro, Hart, 2001; Dong et al., 2007; Hosokawa et al., 2010; Nikoh et al., 2014; Van Nouhuys et al., 2016; Mariño et al., 2017). Such deep involvement of the symbiont in the host biology allowed considering *Wolbachia* a potential agent for pest control (Zabalou et al., 2008; Bourtzis, 2008). Laboratory experiments of *Wolbachia* transmission from *Rhagoletis cerasi* (Diptera: Tephritidae) to *Ceratitis capitata* (Diptera: Tephritidae), an important agricultural pest, resulted in total progeny death due to complete CI in the new host (Zabalou et al., 2008). Transmission of CI-induced *Wolbachia* strain from *Laodelphax striatellus* (Hemiptera: Delphacidae) to a dangerous rice pest *Nilaparvata lugens* (Hemiptera: Delphacidae) results in high levels of CI as well, resulting in rice protection from *Rice ragged stunt virus* transmitted by the pest (Gong et al., 2020). However, most of such studies currently are limited to laboratory tests.

Wolbachia are divided into 17 phylogenetic clades, namely 'supergroups' which are denoted from A to S, excluding G and R (Werren et al., 1995; Lo et al., 2002; Baldo, Werren, 2007; Augustinos et al., 2011; Glowska et al., 2015; Gerth, 2016; Lefoulon et al., 2020). Supergroups A and B are the most common in insects, while the others are not so widespread, and some lineages are specific to the certain insect host taxa. The same *Wolbachia* variants could be found in hosts belonging to different taxa, which implies horizontal transmission (HT) of the symbiont (Werren 1997; Vavre et al., 1999; Dedeine et al., 2005; Haine et al., 2005; Stahlhut et al., 2010; Zug, Hammerstein, 2012; Ahmed et al., 2016; Ilinsky, Kosterin, 2017; Shaikevich et al., 2019). In spite of numerous cases of

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HT, maternal transmission within a host is rather stable, and the co-evolution of the symbiont and host mtDNA lineages is observed (Rousset, Solignac, 1995; Marcade et al., 1999; Hinrich et al., 2002; Mercot, Charlat., 2004; Shoemaker et al., 2004; Hurst, Jiggins, 2005; Ilinsky, 2013; Cariou et al., 2017; Chen et al., 2017). MtDNA of *Wolbachia*-infected species may undergo indirect selection that lead to reduction or increase in mtDNA diversity, changes in mtDNA variation, and to paraphyly of mtDNA (Hurst, Jiggins, 2005).

Wolbachia are found in a wide range of Lepidoptera species, and its prevalence greatly varies from low levels to totally infected populations (Tagami, Miura, 2004; Salunke et al., 2012; Ahmed et al., 2015; Solovyev et al., 2015; Ilinsky, Kosterin, 2017; Tokarev et al., 2017; Bykov et al., 2020; Malysh et al., 2020). Genetic diversity of *Wolbachia* in Lepidoptera hosts has been studied in detail employing the MLST protocol (Russell et al., 2009; Ahmed et al., 2016; Ilinsky, Kosterin, 2017; Duplouy et al., 2020). This protocol uses five bacterial loci: *gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA*; and a combination of alleles forms a Sequence Type (ST) or a haplotype (Baldo et al., 2006). Lepidopteran hosts often harbour *Wolbachia* strains of ST-41 and other ST-41-related haplotypes which belong to the supergroup B (Ahmed et al., 2016; Ilinsky, Kosterin, 2017). Certain haplotypes of the supergroup A have been also found in Lepidoptera (Russell et al., 2009; Ahmed et al., 2016; Ilinsky, Kosterin, 2017; Duplouy et al., 2020). In some Lepidoptera, *Wolbachia* induce feminization, male killing, and CI (Hiroki et al., 2004; Charlat et al., 2006, 2007; Narita et al., 2007; Graham, Wilson, 2012; Salunkhe et al., 2014; Arai et al., 2019).

Previously, *Wolbachia* symbionts were found in some species of the Pieridae family, with high infection rates in *Leptidea*, *Colias* and *Eurema* species (Tagami, Miura, 2004; Solovyev et al., 2015; Ilinsky, Kosterin, 2017; Duplouy et al., 2020). For *A. crataegi, Wolbachia* infection was only noted in Novosibirsk population (see discussion in Ilinsky, Kosterin, 2017) without the data on the symbiont prevalence. Here, we analyzed *Wolbachia* prevalence in populations of *A. crataegi* throughout the Russian Federation from the Far East to Kaliningrad. Additionally, we studied mtDNA haplotypes and *Wolbachia* variants of *A. crataegi* to reveal their associations.

Materials and Methods

A total of 376 adults of *A. crataegi* were collected from 2001 to 2019 in 16 localities of 10 regions of Russian Federation from the Far East to Kaliningrad (Fig. 1; Table 1).

Total DNA was extracted from abdomens of air-dried or fresh samples in CTAB buffer by standard protocol (see Bykov et al., 2020). The DNA extraction quality was determined by PCR with the primer set HCO2198/LCO1490 (Vrijenhoek et al., 1994) for the mitochondrial cytochrome-c oxidase subunit 1 gene (*COI*). *Wolbachia* infection was examined by PCR with primers for *coxA* gene (Baldo et al., 2006). Six out of eight *Wolbachia*-positive samples were genotyped according to MLST protocol (Baldo et al., 2006). Additionally, we sequenced the 658 bp part of *COI* gene for these six *Wolbachia*-infected samples and eight uninfected samples (one per region) to determine the mtDNA haplotypes of *A. crataegi*. Amplicons were purified with exonuclease I *E. coli* (New England Biolabs) and further were sequenced using BrilliantDye Terminator Cycle Sequencing kit v3.1 (Nimagen). All sequences were analyzed in FinchTV v1.4.0 (Geospiza Inc). All sequences were deposited to the GenBank database under accession numbers MW243570 - MW243583 for *COI* gene and MW246635 - MW246664 for MLST *Wolbachia* genes. The alignments were performed in MEGA 6 (Tamura et al., 2013). Phylogenetic reconstructions were performed in MEGA 6 by the maximum likelihood algorithm. The data on other populations of *A. crataegi* (Park et al., 2013; Kim et al., 2020; Todisco et al., 2020) with *A. hippia* as an outgroup taxon were used for mtDNA tree reconstruction.

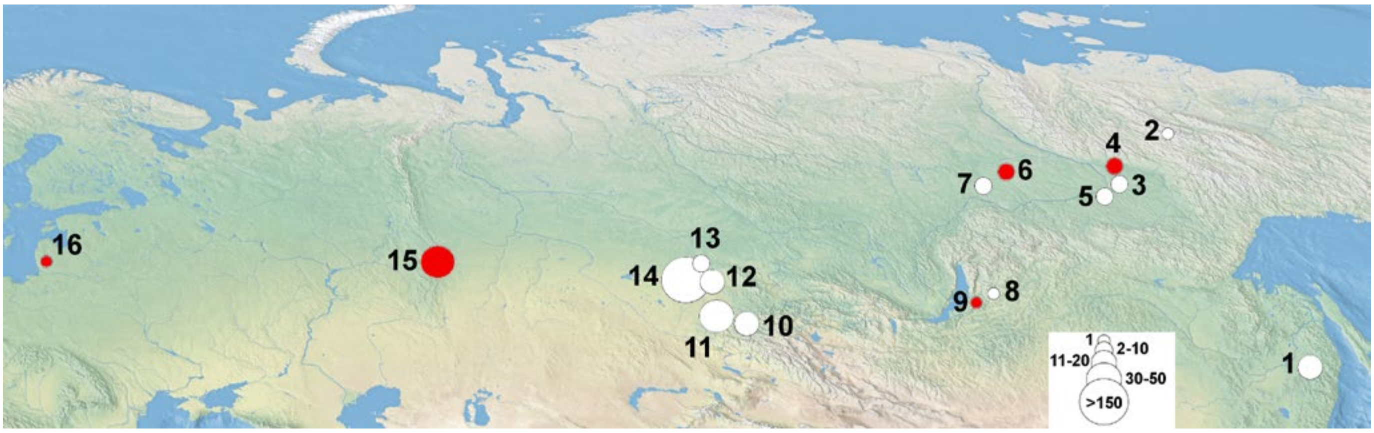

Figure 1. Sampling sites for *Aporia crataegi*: 1 – Khabarovsk Krai; 2 – Yakutia, Oymyakonsky District; 3 – Yakutia, Yakutsk; 4 – Yakutia, Namsky District; 5 – Yakutia, Khangalassky District; 6 – Yakutia, Suntarsky District; 7 – Yakutia, Lensky District; 8 – Republic of Buryatia, Yeravninsky District; 9 – Republic of Buryatia, Khorinsky district; 10 – Altai Republic; 11 – Altai Krai; 12 – Kemerovo Province; 13 – Tomsk Province; 14 – Novosibirsk Province; 15 – Sverdlovsk Province; 16 – Kaliningrad Province. Dot size indicates sample size. Filled dots indicate localities where *Wolbachia* infection was found

Table 1. *Wolbachia* infection in populations of *Aporia crataegi*

Region, locality	**Year**	**N_{w+}/N***
Khabarovsk Krai	2018	0/12
Yakutia, Oymyakonsky District	2017	0/1
Yakutia, Yakutsk	2003	0/2
	2015	0/1
Yakutia, Namsky District	2002	0/1
	2016	1/2
	2017	0/1
Yakutia, Khangalassky District	2001	0/4
	2016	0/1
Yakutia, Suntarsky District	2017	2/5
Yakutia, Lensky District	2012	0/4
Republic of Buryatia, Yeravninsky District	2018	0/1
Republic of Buryatia, Khorinsky district	2018	1/1
Kemerovo Province	2016	0/4
	2017	0/15
Tomsk Province	2019	0/6
Novosibirsk Province	2016	0/67
	2017	0/49
	2018	0/2
	2019	0/72
Altai Republic	2016	0/4
	2017	0/15
Altai Krai	2017	0/14
	2018	0/35
Sverdlovsk Province	2015	1/20
	2016	2/20
	2017	0/16
Kaliningrad Province	2017	1/1
Total:		**8/376**

* N_{w+} – number Wolbachia-positive specimens; N – total number of analyzed insects.

Results

Screening of 376 *A. crataegi* specimens from the vast territory revealed only eight cases of *Wolbachia* infection (2 %). No specific geographic pattern of *Wolbachia* infection in populations of *A. crataegi* has been found. The symbiont has been detected in Yakutia, Republic of Buryatia, Sverdlovsk, and Kaliningrad Provinces (Table 1). In other regions, *Wolbachia* symbionts were not found even in large samples from Novosibirsk Province and Altai Krai.

Analysis of *Wolbachia* genetic diversity based on the MLST protocol revealed two haplotypes of the symbiont. *Wolbachia* ST-19 was found in samples from Yakutia, Sverdlovsk, and Kaliningrad Provinces, and ST-109 – in the sample from Buryatia. These haplotypes belonged to different *Wolbachia* supergroups: ST-19 – to A, and ST-109 – to B-supergroup (Fig. 2B).

We found discordance between mtDNA haplotypes of *A. crataegi* and *Wolbachia* haplotypes. *Wolbachia* haplotype ST-19 associated with two different mtDNA haplotypes of the host, and ST-109 – with one haplotype shared with ST-19 (Fig. 2A, B). One of these mtDNA haplotypes associated with *Wolbachia* haplotypes belongs to the most common and widespread «Eurasian» haplogroup (Todisco et al., 2020). This mtDNA haplotype was found in infected samples from Sverdlovsk and Kaliningrad Provinces and in uninfected samples from Novosibirsk, Kemerovo and Tomsk Provinces, Altai Krai, Republic of Buryatia and Altai Republic. The other mtDNA haplotype was found in infected and uninfected samples from Yakutia, and in uninfected samples from the Khabarovsk Krai. This haplotype belongs to the haplogroup previously described in Central and East Asia and Yakutia (Todisco et al., 2020), and it is probably typical for Asian populations of *A. crataegi*.

Discussion

Wolbachia prevalence in *A. crataegi* populations was very low. Similar cases of low *Wolbachia* prevalence were previously described in *Pieris rapae* (Lepidoptera: Pieridae) populations, where 3.4 % infection prevalence was detected (Tagami, Miura, 2004). Possible explanation of such low *Wolbachia* prevalence may be the absence of any advantages given by the symbiont to its host and no reproductive abnormalities induced by *Wolbachia*. Besides, host immunity may be able to suppress the symbiont. There are species that are reported to be *Wolbachia* free based on hundreds of screened samples, such as *Lymantria dispar* (Lepidoptera: Lymantriidae) (Martemyanov et al., 2014; Ilinsky et al., 2017), *Agriocnemis pygmaea* (Odonata: Coenagrionidae) (Thipaksorn et al., 2003), *Aedes caspius* (Diptera: Culicidae) (Bozorg-Omid et al., 2020), *Anopheles gambiae* (Diptera: Culicidae) (Scholz et al., 2020). Reasons for *Wolbachia* absence in some species remain unclear.

In *A. crataegi,* we found two diverged *Wolbachia* haplotypes ST-19 and ST-109 that were also reported in different hosts. ST-109 (B supergroup) was found in *Colotis amata* (Pieridae), *Minois dryas* (Nymphalidae) and several Lycaenidae butterflies (Ahmed et al., 2016; Ilinsky, Kosterin, 2017). Haplotype ST-19 (A supergroup) was previously found in Pieridae, Pyralidae, Nymphalidae and Lycaenidae buttrerflies (Russell et al., 2009; Ahmed et al., 2016; Ilinsky, Kosterin, 2017; Duplouy et al., 2020), and also reported for Coleoptera and different Hymenoptera species, including parasitic wasps of the *Apanteles* and *Chelonus* genera (Russell et al., 2009; Tseng et al., 2020; pubMLST database https://pubmlst.org/organisms/wolbachia-spp). These wasps are parasitoids of different Lepidoptera, including *A. crataegi* (Wilbert, 1960); therefore, HT of *Wolbachia* between parasitic wasps and *A. crataegi* could not be ruled out. Reports of different *Wolbachia* supergroups in a single species are numerous (Tsutsui et al., 2003; Arthofer et al., 2009; Chai, Duo, 2011; Wiwatanaratanabutr, Zhang, 2016; Duplouy, Brattström 2018). For instance, in *Homona magnanima* (Lepidoptera: Tortricidae) there were three *Wolbachia* strains, two from the supergroup A and one from the supergroup B (Arai et al., 2019).

Long-term *Wolbachia*-host association leads to a specific pattern of *Wolbachia* variants and mitochondrial DNA. When a particular *Wolbachia* variant is coinherited with a particular maternal lineage, co-cladogenesis of these inherited factors could be observed. Recent *Wolbachia* acquisitions would not demonstrate any specific pattern of coinheritance. Assuming the co-evolution of *Wolbachia* and host mtDNA, we expected to find similar mtDNA haplotypes in *A. crataegi* specimens

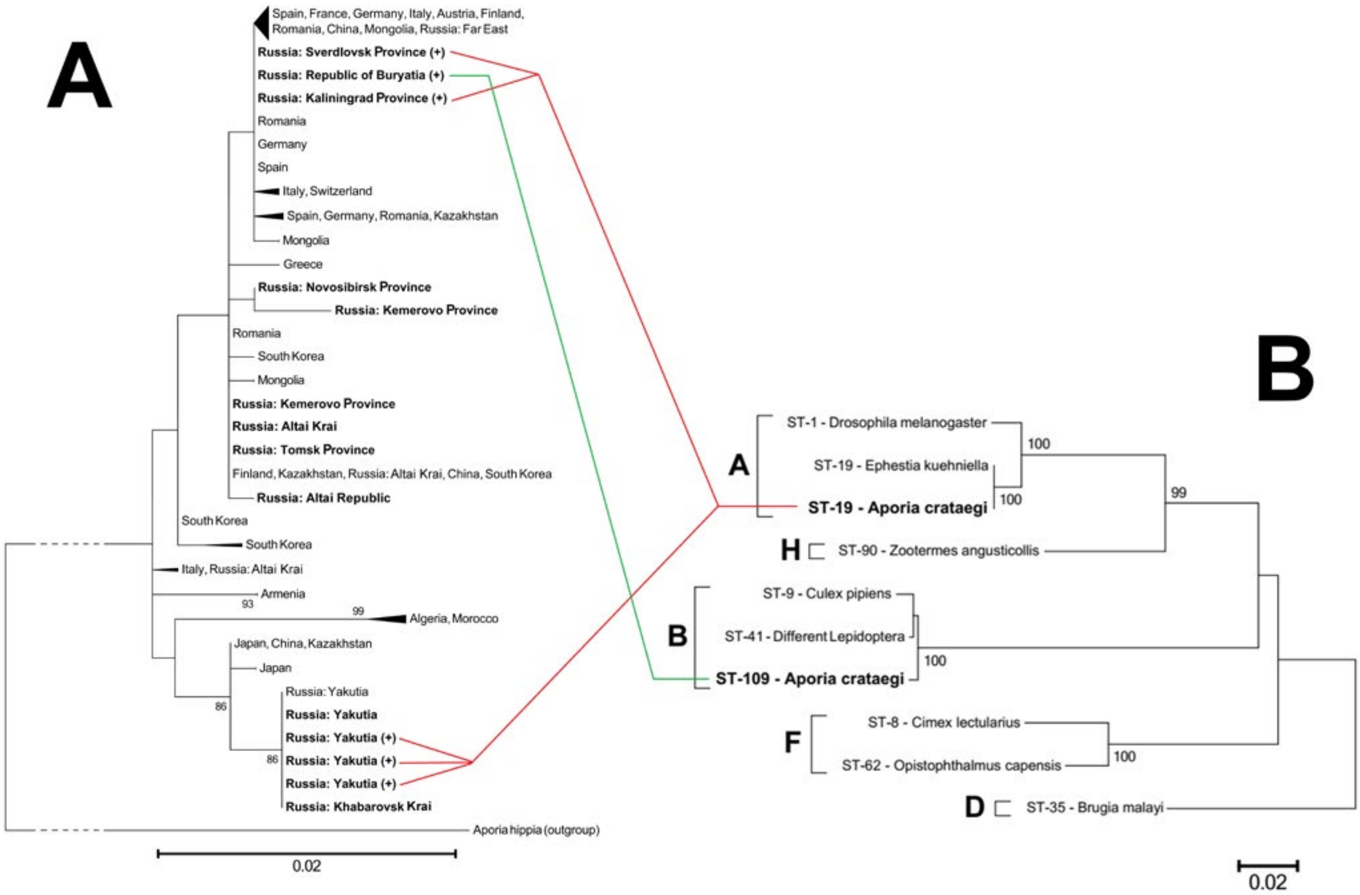

Figure 2. (A) – Maximum likelihood (ML) tree of *A. crataegi* mtDNA was reconstructed using the Tamura 3-parameter model of nucleotide replacement based on the 658bp region of the COI gene. Regions of collection are indicated. Samples investigated in this study are indicated in **bold**. *Wolbachia*-infected samples are indicated with (+); **(B)** – The ML tree of *Wolbachia* haplotypes was reconstructed based on concatenated sequences of five MLST genes using the GTR model of nucleotide replacement. Host species and *Wolbachia* haplotypes (STs) are indicated. Studied haplotypes ST-19 and -109 are in **bold**. Seven *Wolbachia* haplotypes (ST-1, -8, -9, -35, -41, -62, and -90) were used as references for the supergroups. Associations of *Wolbachia* haplotypes with mtDNA haplotypes of *A. crataegi* are indicated. Bootstrap values higher then 75 (1000 replicates) are indicated on both trees

infected with the same *Wolbachia* haplotype. However, two symbiont haplotypes were linked to the same host mtDNA haplotype and different mtDNA haplotypes co-occurred with ST-19 *Wolbachia* haplotype. Those *Wolbachia* haplotypes belonged to supergroups A and B, which diverged 58-200 Mya (Werren et al., 1995; Gerth, Bleidorn, 2017). Thus, we suppose that *Wolbachia* has recently emerged in *A. crataegi* populations.

Conclusion

Our data showed that widespread *Wolbachia* variants has recently infected *A. crataegi*, as inferred from the incongruence of *Wolbachia* and host mtDNA haplotypes. Low *Wolbachia* prevalence might indicate the difficulty of the symbiont establishment in *A. crataegi* populations, suggesting that *A. crataegi* is not a suitable host of *Wolbachia*.

Acknowledgments

The study was funded by the Russian Foundation for Basic Research (grants # 18-316-00099 and 19-04-00983) and the State Budgeted Project #0259-2021-0016*.

The authors express sincere gratitude to our colleagues who collected and kindly provided us with material from different regions: V.V. Dubatolov (Institute of Systematics and Ecology of Animals, SB RAS) – from the Khabarovsk Krai;
S.V. Shehovtsov (Institute of Cytology and Genetics, SB RAS) – from the Republic of Buryatia;
I.A. Kerchev (Institute of Systematics and Ecology of Animals, SB RAS) – from Tomsk;
A.P. Burnasheva (Institute for Biological Problems of Cryolithozone, SB RAS) – from Yakutia;
I.A. Solonkin and E.Yu. Zakharova (Institute of Plant and Animal Ecology, UB RAS) – from Sverdlovsk Province,
and to O.E. Kosterin (Institute of Cytology & Genetics, SB RAS) – from Novosibirsk.

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*acknowledgment of project # 0259-2021-0016 is lacking in the hardcopy version of the manuscript due to technical reasons

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Вестник защиты растений, 2021, 104(1), с.

OECD+WoS: 1.06+IY (Entomology) https://doi.org/10.31993/2308-6459-2021-104-1-14945

Полнотекстовая статья

APORIA CRATAEGI НЕУДОБНЫЙ ХОЗЯИН ДЛЯ *WOLBACHIA*?

Р.А. Быков*, Г.В. Юрлова, М.А. Деменкова, Ю.Ю. Илинский

Институт цитологии и генетики СО РАН, Новосибирск

**ответственный за переписку, e-mail: bykovra@bionet.nsc.ru*

Боярышница *Aporia crataegi* (Lepidoptera: Pieridae) – Транспалеарктический вид, который вредит различным плодово-ягодным культурам. Мы проводим анализ инфицированности *Wolbachia* популяций *A. crataegi*. Бактерии *Wolbachia* – это материнско-наследуемые внутриклеточные симбионты многих членистоногих, в том числе Чешуекрылых. Мы изучили 376 образцов *A. crataegi*, собранных в 10 регионах России от Дальнего Востока до Калининграда. Частота встречаемости *Wolbachia* была очень низкой, только восемь *Wolbachia*-положительных образцов *A. crataegi* было обнаружено в Якутии, Республике Бурятия, Свердловской и Калининградской областях, и не было выявлено инфекции в других локалитетах. Два гаплотипа *Wolbachia*, ST-19 и ST-109, из A и B супергрупп соответственно, были определены с использованием протокола мультилокусного генотипирования (MLST). Эти гаплотипы также встречаются у разных видов чешуекрылых. Оба гаплотипа *Wolbachia* ассоциированы с одним гаплотипом мтДНК *A. crataegi* (определенным на основании анализа гена первой субъединицы цитохром с-оксидазы), а ST-19 – с двумя гаплотипами мтДНК. Это несоответствие матерински наследуемых агентов указывает на случаи независимого приобретения бактерий в истории *A. crataegi*. Все вышеперечисленные данные позволяют предположить, что *Wolbachia* может инфицировать *Aporia crataegi*, но не способна закрепиться в популяциях хозяина.

Ключевые слова: *Wolbachia*, Pieridae, Lepidoptera, *Aporia*, мтДНК

Поступила в редакцию: 10.01.2021 *Принята к печати: 30.03.2021*
