Wolbachia, *Spiroplasma*, and *Rickettsia* symbiotic bacteria in aphids (Aphidoidea)

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Abstract. Aphids are a diverse family of crop pests. Aphids formed a complex relationship with intracellular bacteria. Depending on the region of study, the species composition of both aphids and their facultative endosymbionts varies. The aim of the work was to determine the occurrence and genetic diversity of *Wolbachia*, *Spiroplasma* and *Rickettsia* symbionts in aphids collected in 2018–2019 in Moscow. For these purposes, 578 aphids from 32 collection sites were tested by PCR using specific primers. At least 21 species of aphids from 14 genera and four families were identified by barcoding method, of which 11 species were infected with endosymbionts. *Rickettsia* was found in six species, *Wolbachia* in two species, *Spiroplasma* in one species. The presence of *Rickettsia* in *Impatientinum asiaticum*, *Myzus cerasi*, *Hyalopterus pruni*, *Eucallipterus tiliae*, *Chaitophorus tremulae* and *Wolbachia* in *Aphis pomi* and *C. tremulae* has been described for the first time. A double infection with *Rickettsia* and *Spiroplasma* was detected in a half of pea aphid (*Acyrthosiphon pisum*) individuals. For the first time was found that six species of aphids are infected with *Rickettsia* that are genetically different from previously known. It was first discovered that *A. pomi* is infected with two *Wolbachia* strains, one of which belongs to supergroup B and is genetically close to *Wolbachia* from *C. tremulae*. The second *Wolbachia* strain from *A. pomi* belongs to the supergroup M, recently described in aphid species. *Spiroplasma*, which we observed in *A. pisum*, is genetically close to male killing *Spiroplasma* from aphids, ladybirds and moths. Both maternal inheritance and horizontal transmission are the pathways for the distribution of facultative endosymbiotic bacteria in aphids.

Key words: aphids; endosymbionts; PCR; plant pests; mutualism.

Симбиотические бактерии *Wolbachia*, *Spiroplasma* и *Rickettsia* среди тлей (Aphidoidea)

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Аннотация. Тли – разнообразное семейство вредителей сельскохозяйственных культур. Тли сформировали сложную взаимосвязь с внутриклеточными бактериями, известными как эндосимбионты, которые оказывают как положительное, так и отрицательное влияние на хозяина, что может иметь практическое значение. В разных регионах мира состав факультативных симбионтов в популяциях тлей варьирует. Задачей работы было установить распространение и генетическое разнообразие симбионтов *Wolbachia*, *Spiroplasma* и *Rickettsia* в тлях, собранных в 2018–2019 гг. в Москве и Подмосковье. Для этого 578 тлей из 32 мест сбора тестировали методом ПЦР, используя специфические праймеры для мтДНК тлей, *Wolbachia*, *Spiroplasma* и *Rickettsia*. Методом молекулярно-генетического анализа определено не менее 21 вида тлей из 14 родов и четырех семейств. Одиннадцать видов оказались инфицированы эндосимбионтами, а именно: у шести видов обнаружены *Rickettsia*, у двух видов – *Wolbachia*, у одного – *Spiroplasma*. Впервые выявлено заражение бактерией *Rickettsia* у *Impatientinum asiaticum*, *Myzus cerasi*, *Hyalopterus pruni*, *Eucallipterus tiliae*, *Chaitophorus tremulae* и бактерией *Wolbachia* у *Aphis pomi* и *C. tremulae*. У половины особей гороховой тли *Acyrthosiphon pisum* установлено двойное заражение *Rickettsia* и *Spiroplasma*. Впервые выявлены риккетсии у шести видов тлей, которые генетически отличаются от известных ранее. Впервые обнаружено заражение яблонной тли *A. pomi* двумя штаммами

Wolbachia, причем один из штаммов относится к супергруппе B и генетически близок с *Wolbachia* из осиновой тли *C. tremulae*, а второй штамм относится к супергруппе M, недавно описанной у видов тлей. *Spiroplasma*, найденная нами у *A. pisum*, генетически близка *Spiroplasma*, вызывающей андроцид у тлей, божьих коровок и молей, и кластеризуется с *S. ixodetis*. Разнообразие ДНК симбионтов убедительно свидетельствует о том, что как материнское наследование, так и горизонтальный перенос являются путями распространения факультативных бактерий у тлей.

Ключевые слова: тля; эндосимбионты; ПЦР; вредители растений; мутуализм.

Introduction

Aphids (Aphidoidea) are an insect superfamily from the order Hemiptera, which includes about 10 families or subfamilies and about 5000 species. Aphids are widespread sap-feeding plant pests and can transmit at least 30 % of plant viruses (Augustinos et al., 2011). A complex interplay with intracellular bacteria also known as endosymbionts is commonly observed in aphids. Obligate associations with *Buchnera aphidicola* in aphids provide insect hosts with essential amino acids absent in sap (Douglas, 1998). Apart from that, there are nine species of facultative symbionts of aphids (see the review by Guo et al., 2017) coexisting with *Buchnera*, having both positive and negative effects on the hosts.

Depending on the species, facultative intracellular symbiotic bacteria may increase the resistance of aphids against heat stress, parasitoid wasps, and fungal infections, participate in the synthesis of essential nutrients for the host with the obligate symbiont, and facilitate the interaction of aphids with plants that they feed on (Guo et al., 2017). However, it has been shown that the *Rickettsia* bacteria have a negative effect on the fitness of the pea aphid *Acyrthosiphon pisum* and suppress the activity of *Buchnera aphidicola* (Sakurai et al., 2005). *Spiroplasma* bacteria in *A. pisum* shorten the life span of aphids and inhibit their reproduction (Simon et al., 2007, 2011), albeit have a (minor) protective effect against the parasitoid wasp *Aphidius ervi* (Mathé-Hubert et al., 2019). The role of *Wolbachia* in aphids is not yet fully understood (De Clerck et al., 2015; Manzano-Marín, 2019), but *Wolbachia* endosymbionts in the Asian citrus psyllid *Diaphorina citri* repress holin promoter activity in the alpha-proteobacteria 'Candidatus Liberibacter asiaticus', a citrus disease agent, thereby killing the bacteria and preventing the disease from spreading (Jain et al., 2017).

The present paper is dedicated to studying facultative endosymbionts *Wolbachia*, *Spiroplasma*, and *Rickettsia* in aphids. *Wolbachia* is the most common genus of symbiotic bacteria in insects, aphids not being an exception. *Wolbachia* infection has been recorded in 82 aphid species (Zytynska, Weisser, 2016). Two genera of symbionts, *Rickettsia* (Sakurai et al., 2005) and *Spiroplasma* (Fukatsu et al., 2001), were recorded in *A. pisum*. Both *Spiroplasma* and *Rickettsia* were recorded in the cowpea (*Vigna sinensis*) aphid *Aphis craccivora* (Brady et al., 2014) and the bean aphid *Aphis fabae* (Zytynska et al., 2016). *Spiroplasma* were also recorded in tropical aphids, specifically the citrus aphid *Aphis citricidus* and polyphagous *Aphis aurantii* (Guidolin, Cônsoli, 2018). *Rickettsia* were recorded in the blackberry aphid *Amphorophora rubi* (Haynes et al., 2003) and the cotton aphid *Aphis gossypii* (Jones et al., 2011). The symbiont composition varies in aphid populations around the world (Augustinos et al., 2011; Zytynska, Weisser,

2016; Guo et al., 2017; Guidolin, Cônsoli, 2018). For instance, *Wolbachia*, *Spiroplasma*, and *Rickettsia* were not recorded among facultative aphid symbionts in the Saratov Region, Russia (Malyshina et al., 2014).

Symbiont infection research in aphids is of practical value since such studies are instrumental in developing new strategies of controlling pathogen transmission in plants (Heck, 2018). Depending on the symbiont type, the information on infection may be used for symbiont elimination or transinfection with a specific strain of bacteria to inhibit the vector's ability for pathogen transmission.

The goal of the present study was to investigate the incidence and genetic diversity of the *Wolbachia*, *Spiroplasma*, and *Rickettsia* symbionts in the samples of aphids collected in Moscow and the cities of Zvenigorod and Lyubertsy in the Moscow Region. Thus, 578 aphids from 32 collection sites were PCR-tested using specific primers for *Wolbachia*, *Spiroplasma*, and *Rickettsia*. The taxonomic positions of aphid hosts and their symbionts were identified based on the sequences of aphid and bacterial genes.

Materials and methods

Aphids were collected in July–September 2018 and in May 2019 in Moscow, Zvenigorod, and Lyubertsy (Table 1). A total of 32 aphid samples were collected from 17 plant species. The collected adult aphids were preserved in 96 % ethanol.

Phenol-chloroform extraction was used to isolate total DNA from individual specimens (Sambrook et al., 1989). Amplification reactions were performed in a 25-µl volume using the universal Encyclo Plus PCR kit (Evrogen, Moscow) following the manufacturer's protocol. All the reactions were performed using the MiniAmp Plus thermocycler (Applied Biosystems). Aphid species were identified based on PCR using universal primers LCO1490 and HCO2198 complementary to the 5′ end of the mitochondrial cytochrome c oxidase subunit I gene (*COI*), as described earlier (Folmer et al., 1994).

Symbionts were identified using specific primers as follows: RicF141 and RicR548 for the *Rickettsia gltA* gene (Goryacheva et al., 2017), spi_f1 and spi_r3 for *Spiroplasma* 16S rRNA (Sanada-Morimura et al., 2013), ftsZ-F1 and ftsZ-R1 for the *Wolbachia ftsZ* gene (Baldo et al., 2006). The amplification procedure consisted of initial denaturation for 4 min 30 sec at 94 °C followed by 36 amplification cycles as follows: denaturation for 30 sec at 94 °C, annealing for 30 sec at 59 °C (53 °C for spi_f1 and spi_r3; and 56 °C for *ftsZ*), and extension for 40 sec (1 min for spi_f1 and spi_r3) at 72 °C. The PCR was finalized by an extension for 5 min at 72 °C.

The PCR results were visualized by agarose gel electrophoresis (1.5 %). DNA fragments were eluted from gel using the

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Table 1. Aphid and plant species and their dates and sites of collection

Aphid species (sample)		Collection site	Date	Plant species
Acyrthosiphon caraganae		55°41′37″ N, 37°51′55″ E	May, 2019	*Caragana arborescens*
Acyrthosiphon pisum		55°41′59″ N, 36°43′19″ E	July, 2018	*Pisum sativum*
Anoecia sp.	(1)	55°42′46″ N, 37°35′02″ E	September, 2019	*Cornus alba*
	(2)	55°42′51″ N, 37°35′14″ E		*Cornus sanguinea*
Anuraphis subterranea		55°41′22″ N, 37°51′34″ E	May, 2019	*Pyrus calleryana*
Aphis fabae		55°41′15″ N, 37°51′27″ E	May, 2019	*Philadelphus* sp.
Aphis pomi	(1)	55°42′17″ N, 37°31′30″ E	May, 2019	*Malus domestica*
	(2)	55°35′25″ N, 37°22′03″ E		*Cotoneaster* sp.
	(3)	55°41′37″ N, 37°34′15″ E		
Chaitophorus tremulae		55°35′13″ N, 37°22′20″ E	May, 2019	*Populus tremula*
Corylobium avellanae		55°41′49″ N, 37°33′54″ E	May, 2019	*Corylus avellana*
Dysaphis sp.		55°42′15″ N, 37°31′32″ E	May, 2019	*Malus domestica*
Dysaphis affinis		55°41′22″ N, 37°51′34″ E	May, 2019	*Pyrus calleryana*
Dysaphis devecta		55°42′19″ N, 37°31′26″ E	May, 2019	*Malus domestica*
Dysaphis plantaginea		55°41′18″ N, 37°51′24″ E	May, 2019	*Malus domestica*
Eucallipterus tiliae		55°41′45″ N, 37°33′52″ E	May, 2019	*Tilia* sp.
Hyalopterus pruni		55°42′46″ N, 37°37′16″ E	May, 2019	*Prunus cerasus*
Hyperomyzus lactucae		55°41′38″ N, 37°51′55″ E	May, 2019	*Ribes nigrum*
Impatientinum asiaticum	(1)	55°36′35″ N, 37°40′43″ E	September, 2018	*Impatiens parviflora*
	(2)	55°40′20″ N, 37°41′02″ E		
	(3)	55°39′51″ N, 37°40′05″ E		
	(4)	55°42′48″ N, 37°35′11″ E		
	(5)	55°50′30″ N, 37°38′04″ E		
Macrosiphum rosae		55°43′00″ N, 37°36′54″ E	May, 2019	*Rosa* sp.
Myzus cerasi	(1)	55°41′40″ N, 37°34′00″ E	May, 2019	*Prunus cerasus*
	(2)	55°42′45″ N, 37°37′17″ E		
Rhopalosiphum lonicerae		55°41′25″ N, 37°51′36″ E	May, 2019	*Lonicera tatarica*
Rhopalosiphum padi	(1)	55°42′03″ N, 37°34′35″ E	September, 2018	*Triticum* sp.
	(2)	55°41′31″ N, 37°51′25″ E	May, 2019	*Prunus padis*
	(3)	55°41′36″ N, 37°34′12″ E		
Schizaphis graminum	(1)	55°42′03″ N, 37°34′35″ E	September, 2018	*Triticum* sp.
	(2)	55°41′47″ N, 37°33′53″ E	May, 2019	

DNA isolation kit for agarose gels Cleanup Mini (Evrogen, Moscow) under the manufacturer's instructions. The purified PCR products were sequenced by Evrogen (Moscow). The newly acquired *COI* gene sequences were registered in the GenBank database under accession numbers MT302332–MT302357; *gltA Rickettsia*, under MT302358–MT302364; *ftsZ Wolbachia*, under MT302365–MT302368; and 16S *Spiroplasma*, under MT302369.

Sequence chromatograms were analyzed using the DNASTAR Lasergene 6 software (Clewley, 1995; Burland, 2000). The newly obtained sequences were compared to the previously available ones using the Barcode of Life (BOLD, http://www.barcodinglife.com/) and GenBank databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Dendrograms were plotted using the neighbor-joining method, Kimura evolutionary model, and 1000 bootstrap replications in the MEGA 6.06 software (Tamura et al., 2013). DNA sequences in the dendrograms were provided with the GenBank and PubMLST (in the case of *Wolbachia*) registration numbers on the right from the isolate name. The divergence between nucleotide sequences was calculated based on *p*-distances (per-site differences) using the MEGA 6.06 software (Tamura et al., 2013).

Results

Diversity of aphid species
To identify aphid species, the DNA barcoding method was used. The nucleotide sequences of *mitochondrial COI gene* were used to identify at least 21 aphid species in 32 samples, with 19 of them identified to a species level, and two, to a genus level, namely *Dysaphis* sp. from the apple tree *Malus domestica* and *Anoecia* sp. from the white dogwood *Cornus alba* and the common dogwood *Cornus sanguinea*. In latter cases, the sequence similarity was the highest with *Dysaphis apiifolia* (97 %) and *Anoecia fulviabdominalis* (96 %), respectively. The aphids collected represent four families as follows: Anoeciidae, Callaphididae, Chaitophoridae, and Aphididae (Fig. 1). Evolutionary divergence values amount to 6–16 % between the aphid genera, 0.8–6.6 % between the *Dysaphis* species and 6.3 % between *Aphis* species. Two mtDNA haplotypes were recorded in three species, namely *Aphis pomi*, *Chaitophorus tremulae*, and *A. pisum* (see Fig. 1). The differences between *COI* haplotypes were 0.2 % in *A. pomi*, 0.6 % in *C. tremulae*, and 0.16 % in *A. pisum*.

The apple aphid *A. pomi*, bird cherry-oat aphid *Rhopalosiphum padi*, black cherry aphid *Myzus cerasi*, and Asian balsam aphid *Impatientinum asiaticum* were found in two or more collection sites (see Table 1). Two aphid species were found in the same plant species in the following cases: *Hyalopterus pruni* and *M. cerasi* in cherry trees, *A. pomi* and *Dysaphis devecta* in apple trees, *Anuraphis subterranea* and *Dysaphis affinis* in pear trees, with the last aphid species coexisting on the leaves of the same tree (see Table 1). One aphid species was observed in different plant species in the following two cases: *R. padi* in bird cherry trees and wheat and *A. pomi* in apple trees and cotoneaster.
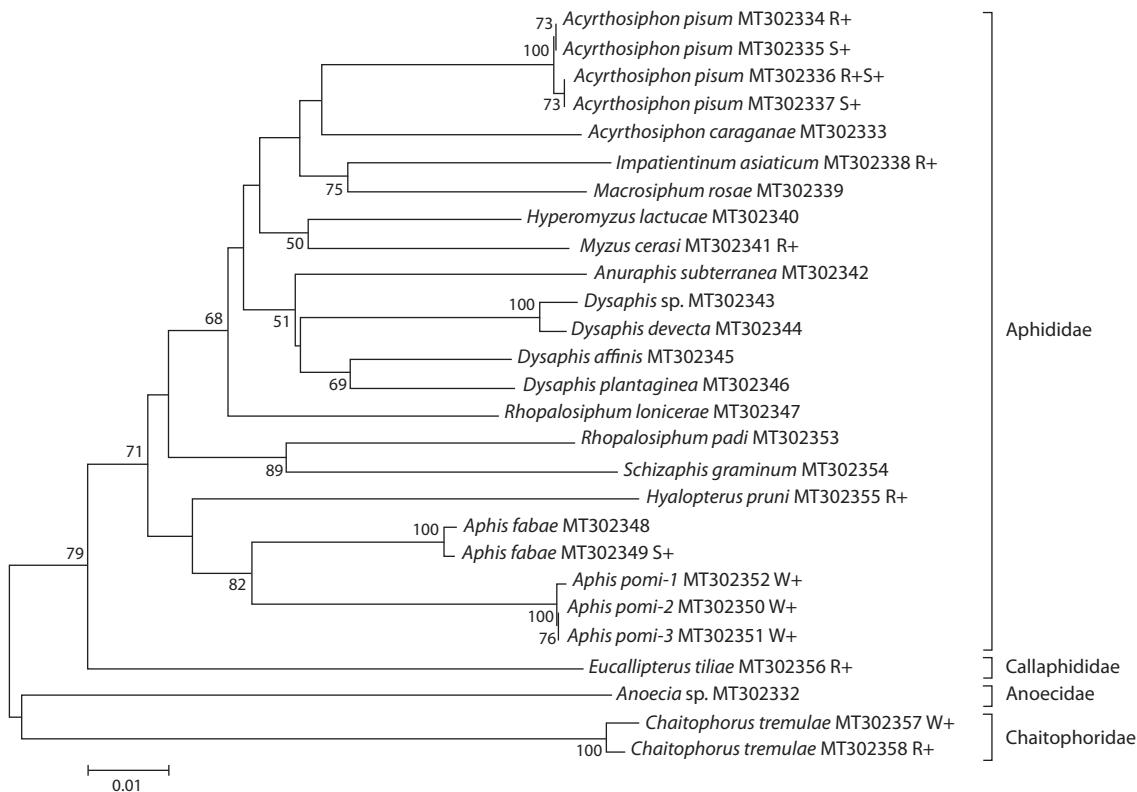

Fig. 1. Phylogenetic reconstruction of the studied aphids species based on the analysis of the 630-bp nucleotide sequences of the mitochondrial *COI* gene.

Individuals infected with *Wolbachia*, *Rickettsia* or *Spiroplasma* are designated W+, R+, S+, respectively.

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Table 2. The occurrence of the symbionts in aphids

Aphid species (sample)	N of individuals studied	Symbiont genus and the number of infective		
		Spiroplasma	*Rickettsia*	*Wolbachia*
Acyrthosiphon caraganae	20	0	0	0
Acyrthosiphon pisum	28	24	17	0
Anoecia sp. (1)	17	0	0	0
Anoecia sp. (2)	12	0	0	0
Anuraphis subterranea	11	0	0	0
Aphis fabae	40	0	0	0
Aphis pomi (1)	11	0	0	11
Aphis pomi (2)	36	0	0	36
Aphis pomi (3)	26	0	0	26
Chaitophorus tremulae	9	0	3	2
Corylobium avellanae	10	0	0	0
Dysaphis sp.	9	0	0	0
Dysaphis affinis	9	0	0	0
Dysaphis devecta	10	0	0	0
Dysaphis plantaginea	30	0	0	0
Eucallipterus tiliae	16	0	5	0
Hyalopterus pruni	10	0	10	0
Hyperomyzus lactucae	18	0	0	0
Impatientinum asiaticum (1)	18	0	11	0
Impatientinum asiaticum (2)	18	0	8	0
Impatientinum asiaticum (3)	18	0	14	0
Impatientinum asiaticum (4)	18	0	8	0
Impatientinum asiaticum (5)	18	0	7	0
Macrosiphum rosae	27	0	0	0
Myzus cerasi (1)	8	0	1	0
Myzus cerasi (2)	23	0	0	0
Rhopalosiphum lonicerae	12	0	0	0
Rhopalosiphum padi (1)	30	0	0	0
Rhopalosiphum padi (2)	12	0	0	0
Rhopalosiphum padi (3)	6	0	0	0
Schizaphis graminum (1)	18	0	0	0
Schizaphis graminum (2)	30	0	0	0
Total (%)	578 (100 %)	24 (4 %)	84 (14 %)	75 (13 %)

Symbiotic bacterial infection

The presence of *Wolbachia*, *Spiroplasma*, and *Rickettsia*
was analysed in 578 specimens of 21 aphid species and the
infections were detected in *A. pisum* (*Spiroplasma* and *Ri-
ckettsia*), *I. asiaticum*, *M. cerasi*, *H. pruni*, *Eucallipterus tiliae*
(*Rickettsia*), *A. pomi* (*Wolbachia*), and *Chaitophorus tremulae*
(*Rickettsia* and *Wolbachia*) (Table 2). The *C. tremulae* speci-
mens infected with *Rickettsia* and *Wolbachia* had different
mtDNA haplotypes (see Fig. 1).

Rickettsia were found in 84 aphid specimens representing
six species; *Wolbachia*, in 75 specimens of two species; and
Spiroplasma, in 24 specimens of one species (see Table 2).

Individual aphid specimens are typically infected by only one
type of symbiotic bacteria out of three. However, *Rickettsia/
Spiroplasma* coinfection was recorded in *A. pisum* in 13 of
28 individual specimens. In adult *A. pisum* specimens, 100 %
were infected with one or two types of endosymbionts. The
presence of *Rickettsia* in *I. asiaticum*, *M. cerasi*, *H. pruni*,
E. tiliae, and *C. tremulae* and *Wolbachia* in *A. pomi* and
C. tremulae has been described for the first time.

Bacteria of the genus *Rickettsia*. Aphid *Rickettsia* were
clustered with the *R. bellii* group (Fig. 2). *Rickettsia* in *E. tiliae*
and *C. tremulae* had species-specific *gltA* alleles. *Rickettsia*
in *A. pisum* had two alleles, *gltA¹* and *gltA²* (GenBank entries
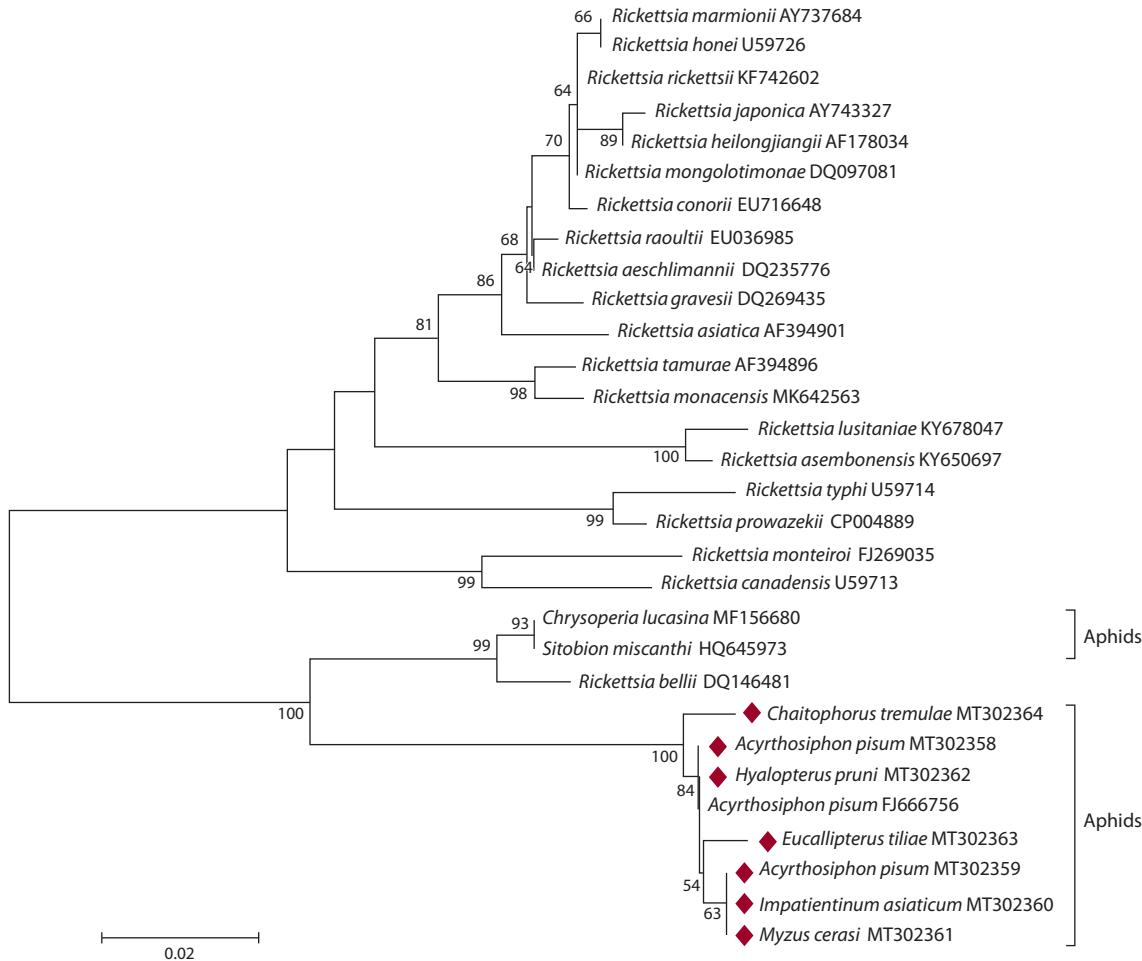
Fig. 2. Phylogenetic reconstruction of *Rickettsia* diversity according to the analysis of 343 bp of the *gltA* gene.

Bacteria from aphids are indicated by host species. The bacterial isolates obtained in this study are highlighted with diamonds.

MT302358 and MT302359), differing in one nucleotide substitution. The *gltA¹* allele was observed in one *A. pisum* specimen, and *gltA²*, in seven. The *gltA¹* allele was also observed in *Rickettsia* from *H. pruni*, and *gltA²*, in *Rickettsia* from *I. asiaticum* and *M. cerasi*. The *gltA¹* allele was identical to the *Rickettsia* DNA from *A. pisum* strain PAR (the USA) registered in GenBank as FJ666756 (see Fig. 2). The *gltA²* allele has been discovered for the first time. The newly obtained *Rickettsia* DNA sequences differed significantly from bacterial DNA in *Sitobion miscanthi* aphids (HQ645973) genetically close to *R. bellii* (see Fig. 2). The evolutionary divergence value between the *R. bellii* group and *Rickettsia* group in the present paper amounted to 8.2 %, which greatly exceeded the divergence between, for example, the *R. typhi* and *R. prowazekii* species (2 %).

Bacteria of the genus *Wolbachia*. Within the genus *Wolbachia*, there are 16 phylogenetic supergroups (Glowska et al., 2016). *Wolbachia* from *Aphis pomi* #1 (MT302366) and #3 (MT302367) were clustered with supergroup M bacteria, and *Wolbachia* from *Chaitophorus tremulae* (MT302365) and *A. pomi* #2 (MT302368), with supergroup B (Fig. 3). The DNA differences in the *ftsZ* gene sequence of *Wolbachia* from *A. pomi* #2 (MT302368) and #1 (MT302366) amounted to 14.3 % (67 of 466 bp). At the same time, the *ftsZ* gene allele

of *Wolbachia* from *A. pomi* #2 (MT302368) had only three different substitutions (0.6 %, 3 from 469 bp) if compared to the *Wolbachia* DNA from the Aspen leaf aphid *Chaitophorus tremulae* (MT302365).

The sample of *A. pomi* infected with group B *Wolbachia* was collected from cotoneaster, and the samples of *A. pomi* infected with group M *Wolbachia*, from cotoneaster and apple trees (see Table 1). The distance between the collection sites, where *A. pomi* were collected from the same plant species but infected with different *Wolbachia* strains, was over 20 km. At the same time, the distance between the collection sites, where *A. pomi* were collected from different plant species but infected with the same *Wolbachia* strain, did not exceed 4 km (see Table 1).

Bacteria of the genus *Spiroplasma*. *Spiroplasma* observed in *A. pisum* were clustered with the bacteria encountered in *A. pisum* in Japan (AB048263), Britain (JX943566, JX943567), and *A. craccivora* from the USA (KF362032) (Fig. 4). Variation of 16S rRNA genes of *Spiroplasma* in aphids from *A. pisum* collected in geographically separated sites amounted to 0.3–0.6 % (5–8 nucleotide substitutions per 974 bp). All of them are included in the *Spiroplasma ixodetis* clade. The latter also had the symbionts of other insects, such as lady beetles *Anisosticta novemdecimpunctata* (AM087471)
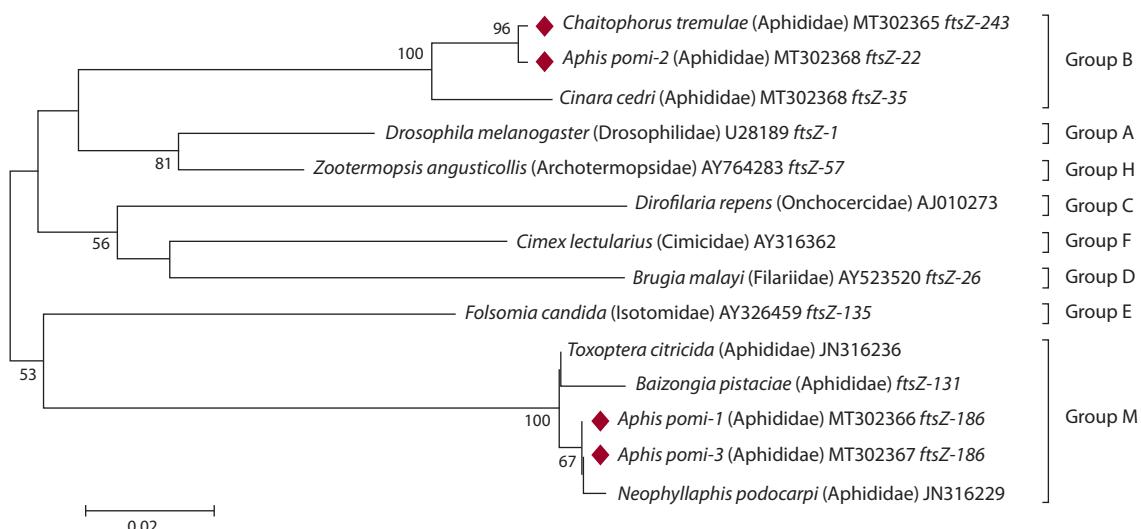
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Fig. 3. Phylogenetic reconstruction of *Wolbachia* diversity according to the analysis of 466 bp of the *ftsZ* gene.

Wolbachia supergroups are shown on the right. Bacteria are indicated by host species. The bacterial isolates obtained in this study are highlighted with diamonds.
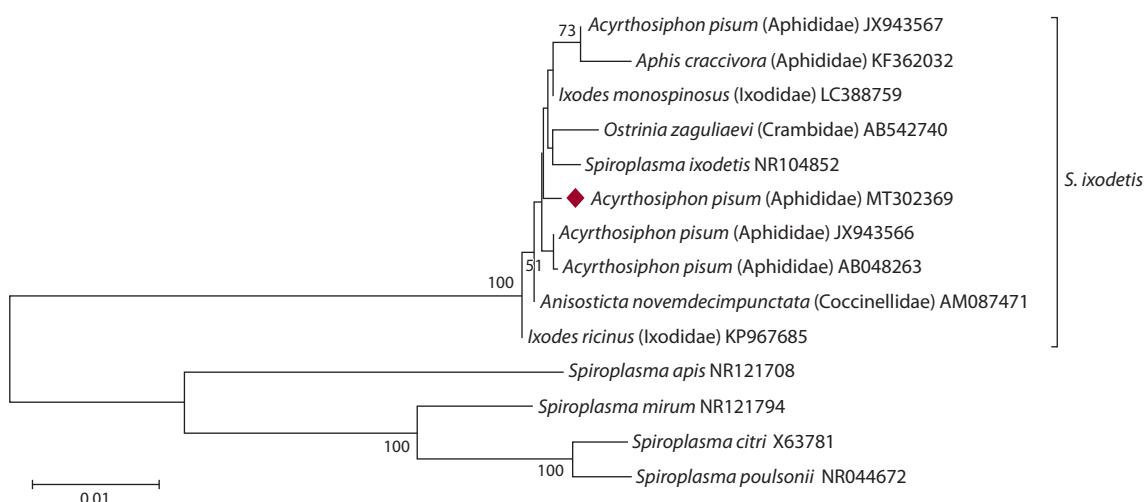

Fig. 4. Phylogenetic reconstruction of *Spiroplasma* diversity according to the analysis of 974 bp of the 16S rRNA gene.

Insect bacteria are indicated by host species. The bacterial isolate obtained in this study is highlighted with a diamond.

and the moth *Ostrinia zaguliaevi* (AB542740). Nucleotide variation within this clade did not exceed 0.6 %. However, nucleotide variation between the clades of the genus *Spiroplasma* reached 10–16 %.

Discussion

The study has demonstrated that at least 21 aphid species were represented in the 32 samples investigated. Most aphid species studied (18 of 21) belong to the family Aphididae, while the families Anoeciidae, Callaphididae, and Chaitophoridae are each represented by one species. Two species were not confined to their plant hosts, whereas 19 aphid species were encountered on particular plant species.

For the first time, *Rickettsia* infection has been observed in *I. asiaticum*, *M. cerasi*, *H. pruni*, *E. tiliae*, and *C. tremulae*, and *Wolbachia* infection, in *A. pomi* and *C. tremulae*. As opposed

to other studies (see Zytynska, Weisser, 2016), aphid species collected in Moscow in the present research were mostly infected by *Rickettsia* rather than *Wolbachia*. The specimens of the Aspen leaf aphid *C. tremulae* infected with *Rickettsia* and *Wolbachia* have different mtDNA haplotypes, although it has yet to be studied in a larger number of samples whether cytoplasmic components are inherited together in this particular aphid species. *Rickettsia* and *Spiroplasma* coinfection was recorded in half of the specimens of the green pea aphid *A. pisum*. Many authors have already noted the presence of *Rickettsia* and *Spiroplasma* among the *A. pisum* facultative symbionts in Europe (Nyabuga et al., 2010; Ferrari et al., 2012; Gauthier et al., 2015), the USA (Russell et al., 2013; Smith et al., 2015), and Japan (Tsuchida et al., 2002). Among 28 *A. pisum* specimens in the sample from Zvenigorod, infection rates were 61 % (*Rickettsia*) and 86 % (*Spiroplasma*),

which is several times as large as the previous figures, 8 and 3 % in 119 isofemale lines from 81 populations in Japan (Tsuchida et al., 2002), 48 and 9 % among 318 specimens in the USA (Russell et al., 2013), 4 and 22 % in 30 lines (Nyabuga et al., 2010), 23 and 27 % in samples collected from eight legume species from England and Germany (Ferrari et al., 2012). It is possible that these high bacterial infection rates only existed for a short period, since both *Spiroplasma* and *Rickettsia* reduce the life span of aphids and inhibit their fertility (Simon et al., 2007, 2011; Mathé-Hubert et al., 2019). According to our observations, prolonged laboratory cultivation of *A. pisum* previously resulted in a loss of *Spiroplasma* symbiotic bacteria.

Aphid *Rickettsia* in our collections form a separate cluster. It was shown earlier that *Rickettsia* from *A. pisum* were attributed to the *R. bellii* group based on a comparison of four bacterial genes (Weinert et al., 2009). Other bacterial genes in different aphid species are to be studied to find out whether they form a separate species within the genus *Rickettsia.* The bacterial group *R. bellii* is the basal group of *Rickettsia* formed before the pathogenic spotted fever group and murine typhus group (Stothard et al., 1994). Four genetically different alleles of the *Rickettsia gltA* gene were discovered in the six aphid species studied, two of them encountered in *A. pisum*. One allele (MT302359) was observed in specimens from three aphid genera, another one (MT302358), in two genera, and the two remaining species had unique allelic variants of symbionts (MT302363-64). Thus, we may assume that different aphid species were infected with *Rickettsia* independently.

Spiroplasma discovered in *A. pisum* based on DNA analysis of the 16S rRNA gene is clustered with the bacteria previously observed in *A. pisum* and *A. craccivora*. It has been shown that *Spiroplasma* in *A. pisum* cause male offspring deaths at early larval stages, i. e. androcide or male-killing (Simon et al., 2011), and are genetically close to the *Spiroplasma* that caused androcide in aphid predators, i. e. lady beetles (Tinsley, Majerus, 2006) and moths (Tabata et al., 2011). All these bacteria are a part of the *Spiroplasma ixodetis* group. *S. ixodetis* is an endosymbiont described in ticks, but it is also common in other arthropods. Phylogenetic studies have shown that *S. ixodetis* strains are subjected to multiple horizontal transfers between ticks and other arthropods including aphids (Binetruy et al., 2019).

The present study has discovered *Wolbachia* infection in the apple aphid *A. pomi* for the first time. *Wolbachia* was found in 100 % of *A. pomi* specimens in our collections, even though this symbiont had not been observed in this aphid species from Greece earlier (Augustinos et al., 2011). The apple aphid *A. pomi* from three collection sites in Moscow (#1, 2, and 3 in Table 1) were infected with two *Wolbachia* strains, one attributed to supergroup B and another, to supergroup M (see Fig. 3), which had been described as prevalent in the aphid species of Spain, Portugal, Greece, Israel, and Iran (Augustinos et al., 2011), China (Wang et al., 2014), and the Azores (Moreira et al., 2019). Since the data shows the group's low genetic variation, one might assume that it emerged quite recently and spread rapidly across aphid populations (Wang et al., 2014).

The results allow us to assume that infection of *A. pomi* with a specific *Wolbachia* strain is not linked to the plant host species, but rather to its habitat. The noteworthy fact of discovering allelic variants of the *Wolbachia ftsZ* gene with only slight differences in various aphid species (*A. pomi* and *C. tremulae*) may imply the possibility of bacterial gene exchange during horizontal transfers of *Wolbachia* among insects, for example, through parasites or plants. This hypothesis is corroborated by the discovery of closely related alleles of *Wolbachia* genes in other unrelated insects (Ilinsky, Kosterin, 2017; Shaikevich et al., 2019). Symbiont DNA diversity is persuasive evidence of maternal inheritance and horizontal transfer being the key distribution mechanisms of facultative bacteria in aphids.

Conclusions

While laboratory research results provide an increasingly better understanding of the role of aphid symbionts, data on symbiont distribution in aphid populations in natural ecosystems are still insufficient. The present paper provides the first report on the genetic diversity of bacterial endosymbionts in previously understudied aphid species. Aphid population screening in Moscow and the Moscow Region made it possible to newly identify *Rickettsia* infection genetically different from infection with *R. bellii*, to which aphid symbionts are typically attributed, in six aphid species (Weinert et al., 2009). Whether these bacteria should be considered a new species is to be decided by studying a larger number of *Rickettsia* alleles. An apple aphid *A. pomi* infection with two *Wolbachia* strains has been discovered for the first time, one being in the supergroup B strain, which is genetically close to *Wolbachia* from Aspen leaf aphids. The second *Wolbachia* strain from *A. pomi* belongs to supergroup M. Regardless of the strain, 100 % of *A. pomi* specimens in the present study were infected with *Wolbachia*, and we thus may assume that there is a selection mechanism of infected specimens in place, which involves the reproductive manipulations discovered in *Spiroplasma* from *A. pisum* (Simon et al., 2011). However, most aphid generations are asexual, and if symbiont infections are maintained in natural aphid populations by reproductive effects, then this reproductive phenotype has to be of great value for the insect host. The obvious advantage of androcide is that it prevents inbreeding in the aphid population (Simon et al., 2011). As for now, it is unclear whether these advantages outweigh the possible side effects of symbiont infection during the asexual phase of the lifecycle. The positive effect, i. e. resistance against natural enemies (parasitoid wasps, pathogenic fungi, and heat stress), seems to be the key driver of an increasing incidence of *Wolbachia*, *Spiroplasma*, and *Rickettsia* facultative endosymbionts in aphid populations.

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