https://doi.org/10.17816/ecogen17437-45

ECOLOGICAL GENETICS OF *ADALIA* BEETLES: VARIABILITY AND SYMBIOTIC BACTERIA IN EUROPEAN POPULATIONS OF THE TEN-SPOT LADYBIRD BEETLE *ADALIA DECEMPUNCTATA*

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Cite this article as: Shaikevich EV, Zakharov IA, Honek A. Ecological genetics of *Adalia* beetles: variability and symbiotic bacteria in european populations of the ten-spot ladybird beetle *Adalia decempunctata*. *Ecological genetics*. 2019;17(4):37-45. https://doi.org/10.17816/ecogen17437-45.

Received: *04.06.2019* Revised: *29.10.2019* Accepted: *17.12.2019*

❀ **Background.** *Adalia decempunctata* L. (Coleoptera: Coccinellidae) — ten-spot ladybird beetle, widespread morphologically variable Palearctic species. **Materials and methods.** DNA polymorphism and infection with *Wolbachia*, *Spiroplasma* and *Rickettsia* symbiotic bacteria were investigated. **Results.** Eight different haplotypes of the mitochondrial *COI* gene, seven of which were previously unknown, were found in 92 *A. decempunctata* individuals from nine European collection places: Prague, Rome, Florence, Hamburg, Paris, Stockholm, Moscow, Feodosia and Yalta. *A. decempunctata* is less variable in mtDNA compared to *A. bipunctata*. Symbiotic bacteria *Wolbachia* and *Spiroplasma* were not detected. Only *Rickettsia* infection was found in *A. decempunctata* specimens, gathered in Stockholm and Feodosia. *Rickettsia* from *A. decempunctata* from Feodosia and Stockholm differ by 0.5% in *gltA* gene. *Rickettsia* from *A. decempunctata* from Feodosia is clustered with *Rickettsia* from *A. bipunctata* and *Coccinella* sp. based on the analysis of the *gltA* gene. **Conclusion:** Three of the eight mtDNA haplotypes are present in the *A. decempunctata* gene pool from geographically distant habitats. A small amount of nucleotide substitutions between *Rickettsia* from *A. decempunctata* and *A. bipunctata* suggests a single origin of the symbiont in the ladybirds of the genus *Adalia*, the results do not exclude subsequent horizontal transfers between individuals of both species.

❀ **Keywords:** *Adalia* ladybirds; DNA variability; endosymbiotic bacteria.

ЭКОЛОГИЧЕСКАЯ ГЕНЕТИКА ЖУКОВ РОДА *ADALIA*: ИЗМЕНЧИВОСТЬ И СИМБИОТИЧЕСКИЕ БАКТЕРИИ В ЕВРОПЕЙСКИХ ПОПУЛЯЦИЯХ ДЕСЯТИТОЧЕЧНОЙ БОЖЬЕЙ КОРОВКИ *ADALIA DECEMPUNCTATA*

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Для цитирования: Шайкевич Е.В., Захаров И.А., Хонек А. Экологическая генетика жуков рода *Adalia*: изменчивость и симбиотические бактерии в европейских популяциях десятиточечной божьей коровки *Adalia decempunctata* // Экологическая генетика. — 2019. — Т. 17. — № 4. — С. 37—45. https://doi.org/10.17816/ecogen17437-45.

Поступила: *04.06.2019* Одобрена: *29.10.2019* Принята: *17.12.2019*

❀ **Цель.** Задачей настоящей работы было изучить изменчивость ДНК десятиточечных божьих коровок *Adalia decempunctata* L. (Coleoptera: Coccinellidae) из девяти городов Европы и филогенетические связи их симбиотических бактерий. **Методы.** Исследовали полиморфизм гена *COI* митохондриальной ДНК и зараженность *Wolbachia*, *Spiroplasma* и *Rickettsia* методом ПЦР и секвенированием. **Результаты.** Восемь гаплотипов гена *COI* мтДНК, семь из которых до этого не были известны, обнаружены у 92 особей *A. decempunctata* из девяти мест сбора в Европейской части ареала, а именно: из Праги, Рима, Флоренции, Гамбурга, Парижа, Стокгольма, Москвы, Феодосии и Ялты. *A. decempunctata* менее изменчивы по мтДНК по сравнению с двуточечной *A. bipunctata*. Симбиотические бактерии *Wolbachia* и *Spiroplasma* в изученных *A. decempunctata* не выявлены. Зараженность *Rickettsia* обнаружена у *A. decempunctata* в Стокгольме и Феодосии. Проведено сравнение симбионтов *A. decempunctata*, *A. bipunctata* и семиточечной *Coccinella* sp. ДНК бактерии *Rickettsia* из *A. decempunctata* из Феодосии и Стокгольма по гену *gltA* различаются на 0,5 % и один из вариантов идентичен симбионту *A. bipunctata* и *Coccinella* sp. **Выводы.** Три гаплотипа мтДНК присутствуют в генофонде *A. decempunctata*, собранных в географически далеких местах обитания. Количество нуклеотидных замен между *Rickettsia* из *A. decempunctata* и *A. bipunctata* позволяет предполагать единое происхождение симбионта у божьих коровок рода *Adalia*, полученные результаты не исключают последующих горизонтальных переносов *Rickettsia* между особями обоих видов.

❀ **Ключевые слова:** божьи коровки *Adalia*; полиморфизм ДНК; эндосимбиотические бактерии.

INTRODUCTION

Adalia decempunctata (Linnaeus, 1758), is a ladybird with ten spots on the elytra. It is a widespread Palearctic species and occurs in Europe from Scandinavia to Italy and from Portugal to the Urals. The easternmost record for this species is Yekaterinburg in Russia, with no reliable distribution records east of the Urals.

Adalia ladybirds, including *A. decempuncta*, are among the most morphologically variable Coccinellidae [1–3]. Previous studies on a highly variable species closely related to *A. decempuncta*, (*A. bipunctata* [Linnaeus 1758]), revealed significant polymorphism within this species not only in morphology, but also in mitochondrial DNA [4–7]. In *A. bipunctata*, 18 mitochondrial haplotypes were found [5, 6]. The mitochondrial diversity in *A. decempuncta* has not yet been studied.

More than 60% of insect species are infected with symbiotic bacteria, which often impair host reproduction [8]. In ladybirds, symbionts cause androcide, which is the death of male offspring, and accordingly, a shift in the sex-ratio of offspring toward females [9]. Coccinellidae ladybirds are especially susceptible to infection with symbiotic bacteria; 13 out of the 30 European species studied to date were found to be infected with bacterial symbionts of *Wolbachia*, *Spiroplasma*, and *Rickettsia* [9]. Bacteria are transmitted to offspring via transovarial transmission, along with the mother's cytoplasm, which leads to the joint inheritance and spread of mitochondrial DNA and symbiont. Previous studies on the two-spot ladybird, *A. bipunctata*, found an association between certain mtDNA haplotypes and infection status with either *Rickettsia* or *Spiroplasma* [4, 6]. *Rickettsia* infection has also been documented in the ten-sot ladybird, *A. decempunctata*, in Germany [10], Sweden [11], and Great Britain [12]. However, neither *Wolbachia* nor *Spiroplasma* infection have been found to date in this species [10–12].

The objective of this work was to: 1) characterize the genetic diversity and uncover phylogeographic patterns in *A. decempunctata* populations sampled at nine European cities; 2) determine the frequency of bacterial infection, and assess the diversity and phylogenetic relationships between symbiotic bacteria taxa; and 3) compare both the genetic and morphological diversity between *A. decempunctata* and a closely related species, *A. bipunctata*.

MATERIALS AND METHODS

Ladybirds were collected in Moscow, Prague, Paris, Florence, Rome, and Hamburg in 2012 and 2015, and in Feodosia and Yalta in 2017. Additionally, mtDNA and *Rickettsia* diversity were examined in *A. decempunctata* from Sweden, where infection with bacterial symbionts had previously been verified [11]. Beetles were identified to species using morphological characters, according to the pattern of the elytra and pronotum [2]. Beetles were tested for infection with symbiotic bacteria (n = 199) and partial *COI* was sequenced for 92 individuals. Two individuals of *Coccinella* sp., living sympatrically with *A. decempunctata* in the town of Kem (Karelia, Russia), were also examined using mtDNA and bacterial DNA markers. Bacterial symbionts were sequenced from specimens of the two-spot ladybird *A. bipunctata*, collected in 2015 from Buryatia and Karelia in Russia. Further individuals of *A. bipunctata* (84 individuals, collected June 2009, St. Petersburg), were available from a previous study [6, 7] and were used in comparisons of color polymorphism and mtDNA variability with *A. decempunctata* from Prague (n = 116).

DNA was isolated from dry adult insects or those preserved in 70%–90% ethanol using the DIAtom™ DNA Prep Kit (Isogen, Moscow). Infection with bacterial symbionts was assessed by PCR using specific primers for three bacterial symbionts: the *Spiroplasma* 16S rRNA small subunit gene [13], the *Rickettsia gltA* gene [10], and the *Wolbachia wsp* gene [14]. The polymorphic region of the cytochrome oxydase subunit I gene (*COI*) of mtDNA was amplified using the primers C1j-1951 and C1N-2618 [4]. The second internal transcribed spacer (ITS2) of the rRNA gene ribosomal cluster was amplified with the primers 5.8S and 28S [15]. PCR products were isolated from agarose gels using the Clean up kit (Eurogen, Russia). DNA fragments were sequenced on an ABI 310 sequencer using the forward and reverse primers and the ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequences were deposited in GenBank under the accession numbers KJ645081-KJ645085 and MK932842-MK932845 (mitochondrial haplotypes), KJ645086-KJ645094 (ITS2), and MK932846-MK932850 (*Rickettsia gltA*).

Data analysis was performed using MEGA6 [16] and DnaSP v5 [17]. A maximum likelihood dendrogram for the *Rickettsia gltA* sequences was constructed using the Tamura-Nei model and 1000 bootstrap iterations in the MEGA6 program [16]. A mtDNA haplotype network was constructed in NETWORK ver. 4.6.1.6 [18]. Sequence divergence between mitochondrial haplotypes was calculated as the average number of nucleotide substitutions per site between two sequences [16]. Following Zhivotovsky, intra-population diversity was determined using the indicator μ (average number of morphs) [19]. When analyzing the occurrence of symbionts in the samples, confidence intervals (%) were calculated using samples of ten or more individuals using the Clopper–Pearson method, as in [20–22].

RESULTS

DNA polymorphism of *A. decempunctata*

92 *A. decempunctata* from across the nine collection sites were successfully sequenced for *COI* (616 bp) (Table 1). Eight mitochondrial haplotypes were found, which differed at nine variable sites. All mutations were related to A-G or C-T transitions and were synonymous.

Three mitochondrial haplotypes were rather common and were detected at two or more collection sites. The haplotype H1 occurred in all nine populations and in 74% (n = 68) of individuals (Table 1). In the haplotype network, this particular variant is ancestral (root) for all other mitochondrial types of *A. decempunctata* (Fig. 1). All haplotypes were interconnected by one or two sequential mutations (Fig. 1). Two hypothetical mitochondrial haplotypes, between H6 and H2 and between H1 and H8, did not occur in the studied samples. The evolutionary sequence divergence between mtDNA haplotypes did not exceed 0.8%, in agreement with the estimated level of intraspecific difference.

The genetic diversity within *A. decempunctata* based on *COI* is summarized in Table 2. The haplotypic diversity was highest in individuals from Prague and Florence. At three sampling sites: Paris, Feodosia, and Yalta, the number of individuals studied was low, and only H1 haplotype was found. The results of the Tajima's D and Fu's Fs tests were not statistically significant (Table 2) and indicated that the detected mutations are neutral in nature. Our results demonstrate that, in general, *A. decempunctata* in Europe retain a rather high level of haplotype diversity with a low level of nucleotide variability (Table 2). The ITS2 region sequenced was 900 bps in length and was identical in all individuals studied.

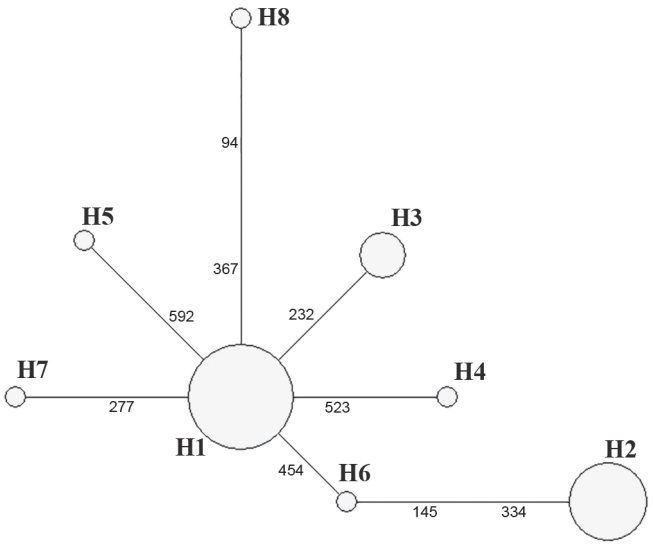

Fig. 1. Intraspecific polymorphism of mtDNA haplotypes of *A. decempunctata*. Eight variable haplotypes are represented on the network in proportion to their occurrence in the population

For analysis of mtDNA of *A. decempunctata*, a sequence similar to the most variable part of the *COI* gene in ladybirds of close species *A. bipunctata* was used [4]. When comparing mtDNA variability between *A. decem-*

Table 1

Place and year of collection of investigated *A. decempunctata*, the number of individuals with different mitochondrial haplotypes

Local population	Collection place	Year	Number of analysed individuals	H1	H2	H3	H4	H5	H6	H7	H8
Czech Republic, Prague	Ruzyne, Crop Research Institute (50°09' N 14°30' E)	2012	29	19	7	2	1	0	0	0	0
France, Paris	Near the metro station Cite (48°51' N 2°20' E)	2012	3	3	0	0	0	0	0	0	0
Russia, Moscow	"Neskuchny sad" park (55°43' N 37°35' E)	2015	10	8	1	0	0	0	1	0	0
Italy, Rome	Monte Pincio (41°91' N 12°48' N)	2015	20	18	0	0	0	0	0	1	1
Italy, Florence	Parco dell Anconella (43°76' N 11°30' E)	2015	9	3	5	0	0	1	0	0	0
Germany, Hamburg	Danziger Strasse (53°56' N 10°01' E)	2015	6	5	1	0	0	0	0	0	0
Sweden, Stockholm	Kungens Kurva Kurva (59°16' N 17°54' E)	2001	5	2	0	3	0	0	0	0	0
Feodosia (Crimea)	Embankment (45°01'44.3" N 35°22'38.7" E)	2017	5	5	0	0	0	0	0	0	0
Yalta (Crimea)	Embankment (44°29'14.7" N 34°09'37.9" E)	2017	5	5	0	0	0	0	0	0	0
Total			92	68	14	5	1	1	1	1	1

Table 2

Genetic diversity in *A. decempunctata* populations based on analysis of the COI gene

Local population	H	Hd	K	Pi	Tajima's D	Fu's Fs statistic
Czech Republic, Prague	4	0.52463	1.3399	0.00218	0.40983	1.970
France, Paris	1	0	0	0	–	–
Russia, Moscow	3	0.37778	0.75556	0.00123	−1.03446	−0.046
Italy, Rome	3	0.19474	0.3	0.00049	−1.72331	−1.143
Italy, Florence	3	0.63889	1.88889	0.00308	1.15206	1.658
Germany, Hamburg	2	0.33333	1	0.00163	−1.23311	1.609
Sweden, Stockholm	2	0.6	0.6	0.00098	1.22474	0.626
Feodosia (Crimea)	1	0	0	0	–	–
Yalta (Crimea)	1	0	0	0	–	–
Total	8	0.43168	1.01027	0.00164	−1.08219	−2.116

Note. H – number of haplotypes, Hd – haplotype diversity, K – average number of nucleotide differences, Pi – nucleotide diversity (PiJC), Tajima's D and Fu's Fs statistics – neutrality tests (not significant, $p > 0.05$).

Table 3

***A. decempunctata* and *A. bipunctata* intrapopulation diversity**

Species, local population	N	The average number of morphs (drawing on the elytra), μ	The average number of mtDNA haplotypes, μ	Intrapopulation diversity of mtDNA, Hd
A. decempunctata, Prague	116	2.850 ± 0.055	3.362 ± 0.408	0.556 ± 0.086
A.bipunctata, Saint Petersburg	84	3.010 ± 0.127	9.290 ± 1.007	0.749 ± 0.079

punctata and *A. bipunctata*, the latter was found to have higher diversity (Table 3).

Variability of color pattern (morphological polymorphism)

Three morphs were found in the local population of *A. decempunctata* from Prague ($n = 116$ individuals), which was the most densely sampled. These morphs were: bimaculata, decempustulata, and typica; they differed in elytra patterns, and had abundances of 23, 74, and 44 individuals, respectively. Among the populations of *A. bipunctata* included in our study, that of St. Petersburg was distinguished by having a very high number of melanic forms [6]. Due to the study, we could compare the levels of intraspecific variability of mtDNA and morphological characters (color variability) in two closely related species – *A. decempunctata* and *A. bipunctata*. *A. bipunctata* was found to be more variable in mtDNA diversity and have the same diversity in color patterns (Table 3).

The symbionts of *A. decempunctata* and other ladybirds

Potential infection with the bacterial symbionts *Rickettsia*, *Wolbachia*, or *Spiroplasma*, was assessed in *A. decempunctata* from Prague ($n = 116$), Moscow ($n = 24$), Rome ($n = 20$), Florence ($n = 10$), Hamburg ($n = 6$), Paris ($n = 3$), Feodosia ($n = 12$), and Yalta ($n = 8$). Out of the 199 individuals tested, only one individual (from Feodosia) was found to be infected with *Rickettsia*. Our analysis was supplemented by infection status data for 18 individuals of *A. decempunctata* from Stockholm (Table 4). Neither infection with *Wolbachia* nor *Spiroplasma* was detected in any individual. Amplification of the mtDNA *COI* locus was used as a DNA quality control; *A. decempunctata* and *A. bipunctata* individuals infected with bacterial symbionts were used as positive controls. The confidence intervals established in this study enabled the assessment of the level of infection. Apart from cases where *Rickettsia* was detected, the confidence interval for

Table 4

Local population, number of tested *A. decempunctata*, the number of individuals, infected with *Wolbachia*, *Spiroplasma* and *Rickettsia*

Local population	N	Number of cases (% infection)			95% confidence interval *,**
		Wolbachia	*Spiroplasma*	*Rickettsia*	
Czech Republic, Prague	116	0	0	0	0–3
France, Paris	3	0	0	0	–
Russia, Moscow	24	0	0	0	0–14
Italy, Rome	20	0	0	0	0–17
Italy, Florence	10	0	0	0	0–31
Germany, Hamburg	6	0	0	0	–
Sweden, Stockholm [10]	18	0	0	4 (22)	0–19*; 6–48**
Feodosia (Crimea)	12	0	0	1 (8)	0–26*; 0,2–38**
Yalta (Crimea)	8	0	0	0	–
Total	204	0	0	5	0,8–5

Note. *CI is calculated for samples ≥ 10; ** CI is specified for *Rickettsia*.

each infection was the same (Table 4). The low number of individuals sampled in each collection precludes a definite statement on the level of bacterial infection in the *A. decempunctata* population as a whole. The CI(95%) for the sample from Prague, where 116 individuals were tested, was 0–3. The infection with symbiotic bacteria could be lower than 3%, and we did not detect this within our sampling.

To study the genetic diversity of bacterial symbionts, the citrate synthase gene (*gltA*) of *Rickettsia* from *A. decempunctata* from Feodosia and Stockholm was sequenced. In addition, *gltA* was sequenced from individuals of *A. bipunctata fasciatopunctata* from Ulan-Ude (Buryatia), and *A. bipunctata* and *Coccinella sp.* from Kem (Karelia). The DNA sequences obtained were compared with those available for ladybirds in GenBank. *Rickettsia* from *A. decempunctata* from Feodosia and Stockholm differ by two nucleotide substitutions. Those from Feodosia cluster with other *Rickettsia* sequences from *A. bipunctata*, *A. b. fasciatopunctata*, and *Coccinella sp.* (Fig. 2). Those from Stockholm were identical to sequences from Germany and Great Britain (Fig. 2).

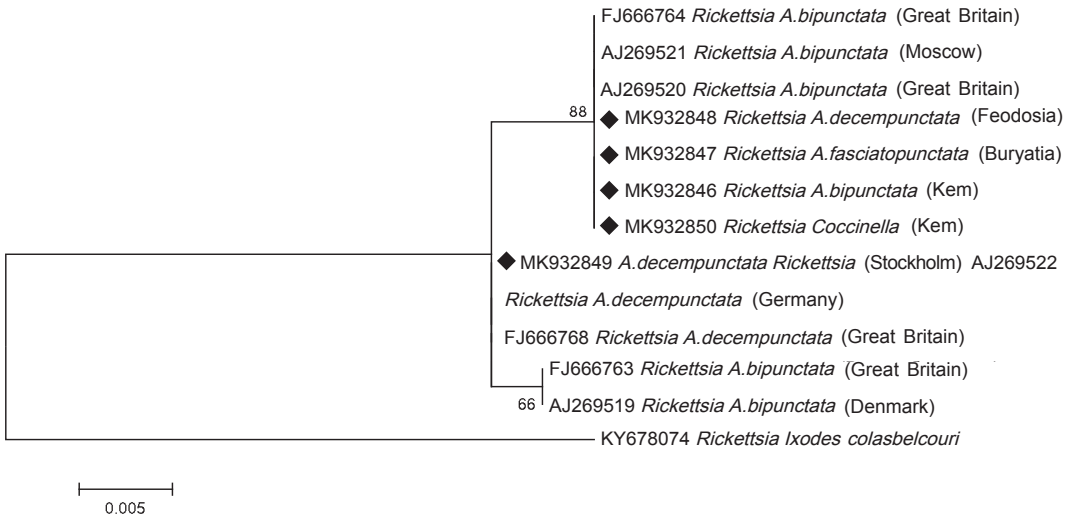

Fig. 2. Phylogenetic tree of *Rickettsia* based on *gltA* gene sequences. Hosts of the intracellular symbiotic bacteria *Rickettsia* and the places of their collection are indicated. The sequences obtained in this work are marked with black diamonds. Other sequences are selected from GenBank for comparison, registration numbers are given. *Rickettsia* from *Ixodes colasbelcouri* was used as an outgroup

The *gltA* sequences for *A. bipunctata* form two distinct clusters on the dendrogram (Fig. 2). Namely, a clade of *A. bipunctata Rickettsia* (FJ666763, AJ269519) and a second clade of *A. bipunctata Rickettsia* which is one substitution different from *A. decempunctata Rickettsia* (FJ666768, AJ269522).

DISCUSSION

We conducted a large-scale study on the variability of nuclear and mtDNA in 92 individuals of *A. decempunctata* across nine collection sites in the Palearctic and found evidence for *Rickettsia* infection in individuals from Stockholm and Feodosia.

Five mitochondrial haplotypes were recovered from individuals collected in Prague where sampling intensity was highest. At other collection sites, the number of mtDNA haplotypes found depended on the number of individuals studied (Table 1). For example, in Rome, 20 ladybirds were collected which had three mtDNA haplotypes; in Paris, only three ladybirds were collected and shared the same haplotype (H1). This mitochondrial variant (H1) was found at all sample sites (Table 1). Previously published *COI* sequences of *A. decempunctata* from Germany (AJ312061) and Great Britain (DQ155924, DQ155760) also belong to the mtDNA haplotype designated by us as H1. We found haplotype H2 in Prague and Florence, and also in Moscow and Hamburg. Type H3 was found in Prague and Stockholm. Overall, eight mitochondrial haplotypes were detected in this study, seven of which were previously unknown. Six of the eight mtDNA haplotypes differ in one substitution (out of 616 bp). Three of the eight haplotypes have a geographically widespread occurrence in this species. A quarter of the studied individuals in the Prague population and 40% of ladybirds from Florence have a unique H2 haplotype, which differs from the other haplotypes by three nucleotide substitutions (0.49%). Using the mutation rate for mtDNA in Drosophila ($6.2 \cdot 10^{-8}$) [23], and a generation time of 1 or 1.5 per year, implies a divergence time of 55–83 thousand years between H1 and H2. The mtDNA diversity in *A. decempunctata* predates the last glaciation which covered a significant part of the species range in Europe. The finding of a single nuclear (ITS2) sequence indicates the absence of barriers to cross-breeding and exchange of genetic information between individuals. The comparisons of intraspecific DNA variability and color variation in the two closely related species, *A. decempunctata* and *A. bipunctata*, showed that the latter had higher mtDNA variation, but a similar level of morphological variation.

Infection of ladybirds of the Coccinellidae family with bacterial symbionts is intensively studied, often due to the significance of these insects as predators of pests in agriculture [9, 24, 25]. In the invasive species *Harmonia axyridis* in Russia, *Spiroplasma* infection was detected in the native populations [26, 27]. In the two-spot ladybird *A. bipunctata*, infection with symbiotic bacteria of three genera was found in the European and Asian parts of the distribution [7, 10]. *Rickettsia* was found in *A. decempunctata* in England and Germany [10]; while *Wolbachia* and *Spiroplasma* were never reported.

Although 199 individuals of *A. decempunctata* from eight European cities were screened for the presence of symbiotic bacteria of three genera, *Rickettsia*, *Wolbachia*, and *Spiroplasma*, only individuals from Feodosia were found to have the bacterium *Rickettsia*. In 2001, we detected *A. decempunctata* specimens infected with *Rickettsia* in Stockholm with a 23% infection rate [11]. In the current study, some of the sample sizes per site are low. Therefore it is possible that infections with symbiotic bacteria went undetected. The infection rate in Stockholm in 2001 was high, and the calculation of the confidence interval showed that it could reach 48%. Based on our sampling in Prague, the infection rate does not exceed 3% if it is present in a given local population. It is known that in ladybirds, only part of the population is typically infected with symbiotic bacteria. Additionally, bacteria are often lost in a population [9] if there are no selective factors contributing to their spread. *Spiroplasma* infection was found to decrease the viability of *A. bipunctata* larvae [24]; in the same work, no similar effect was detected with *Rickettsia* and *Wolbachia* infections. In ladybirds, symbiotic bacteria disrupt reproduction, resulting in no male offspring, which leads to a shift toward females in the gender ratio. Under certain conditions, this gives the population some advantages, as the remaining larvae eat eggs that have stopped developing. However, in favorable living conditions, uninfected females leave more offspring than infected ones [24]. Imperfect maternal inheritance combined with the meager benefits of androcide and the absence of other benefits for infected females can induce an intense selection against individuals infected with symbiotic bacteria.

We detected *Rickettsia* infection in individuals of *A. decempunctata* collected in Stockholm and Feodosia, which are at the northern and southern limits of the distributional range for this species. The results of this study are the first report of the presence of *Rickettsia* in the southern part of the species range. Previously, infection was found only in the north, in the UK, Germany [10], and Sweden [11]. We investigated the variability of the *Rickettsia* citrate synthase (*gltA*) gene, since this part of the bacterial genome has been used in other studies and it was shown to be more informative for bacterial phylogenetic analysis than *16S* rRNA [10]. The differences revealed in the *gltA* gene of *Rickettsia* in *A. decempunctata* from Stockholm and Feodosia may have a different origin. First, the same mutations can arise

by independent or unrelated mutations in a given gene, although the probability of this is low considering the low number of mutations detected. Second, in Feodosia, horizontal transfer of bacteria from the sympatric species of *A. bipunctata* is possible. This has already been documented between *A. bipunctata* and *A. decempunctata* in Denmark [10]. This hypothesis of horizontal transfer is also supported by the fact that three different strains of *Rickettsia* were found in laboratory lines of *A. bipunctata*, one of which clusters with *Rickettsia* from *A. decempunctata* based on a comparison of the *atpA*, *coxA*, *gltA*, and *16S* rRNA genes [28]. Our data, together with those obtained from other ladybird species, do not support the idea of strong coevolution of the parasite and the host. Studying more DNA sequences of the *Adalia* symbionts and other Coccinellidae species collected in geographically remote locations will help clarify this issue in the future.

Ladybirds with seven spots on the elytra, similar in morphology to *Coccinella septempunctata* (Coccinellidae), were found at our collection sites. Species identification using barcoding showed that the ladybird individual with seven spots was not previously recorded. The closest species, *Coccinella magnifica* (KU916547) from Germany, is distinguished by four nucleotide substitutions. Therefore, until further information is received, we designate this individual *Coccinella* sp. (MK932845). Interestingly, in the course of this study, we first discovered the *Rickettsia* bacterium infection in *Coccinella* sp. Previously, only *Wolbachia* was reported in beetles of this genus [9, 24, 25]. The sequences of the *gltA* gene of *Rickettsia* in *Coccinella* sp. were identical to those of *A. bipunctata* (Fig. 2). In addition, individuals of *A. bipunctata* and *Coccinella* sp. were collected at the same site in the city of Kem (Karelia), which suggests both the possible horizontal transfer of bacterial symbionts between different species of coccinellids, and/or the contamination of *Coccinella* sp after eating eggs of *A. bipunctata* infected with *Rickettsia*.

Since the *A. decempunctata* specimens collected in Prague, Moscow, Yalta, Rome, Florence, Hamburg, and Paris were not infected with the symbiotic bacteria *Rickettsia*, *Spiroplasma*, and *Wolbachia*, we could not establish any relation between the mtDNA haplotype and infection with the symbiotic bacterium, as was the case with *A. bipunctata*. In Stockholm, the H1 haplotype was detected in one individual infected with *Rickettsia* and in uninfected individuals, while the H3 haplotype was found in three *Rickettsia* infected individuals. However, ladybirds from Prague with the H3 haplotype of mtDNA were not infected. In Feodosia, both individuals infected with *Rickettsia* and uninfected ones shared the H1 haplotype. Due to low sample sizes, however, a relationship between mtDNA haplotype with *Rickettsia* infection cannot be ruled out. The low number of nucleotide substitutions in *Rickettsia* sequences between *A. decempunctata* and *A. bipunctata* suggests a common origin of the symbiont in ladybirds of the genus *Adalia* but does not exclude subsequent horizontal transfer events between individuals of both species.

FUNDING

The current study was funded by RFBR under the research project No. 19-04-00739; the collection of material was partially performed by I.A. Zakharov according to the state order No. 011220190002. A. Honek was supported from grant no. 17-06763S of the Czech Science Foundation

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