Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Identification of *Wolbachia* Strains in Two Sibling Species of *Neoseiulus* Predatory Mites and Their Prey

Julia M. MALYSH*[1], Svetlana M. MALYSH[2], Olga V. TRAPEZNIKOVA[3], Sergey A. TIMOFEEV[4] Natalia A. BELYAKOVA[5], Yuri S. TOKAREV[6]

[1,2,3,4,5,6]All-Russian Institute of Plant Protection, St. Petersburg, Russia

[1]https://orcid.org/0000-0002-7353-421X, [2]https://orcid.org/0000-0002-4749-6221, [3]https://orcid.org/0000-0003-1353-467X
[4]https://orcid.org/0000-0001-6664-3971, [5]https://orcid.org/0000-0002-9192-5871, [6]https://orcid.org/0000-0003-0524-0778

*Corresponding author e-mail: malyshjm@mail.ru

Article Info

Received: 19.10.2025
Accepted: 11.12.2025
Online published: 19.01.2026
DOI: 10.29133/yyutbd.1805326

Keywords

Acaridae,
Bacterial endosymbiont,
Housekeeping genes,
Multilocus phylogeny,
Phytoseiidae

Abstract: *Wolbachia* screening in mites is necessary for understanding of how their biological functions can be affected, including development of approaches to induce parthenogenesis, making the predator's cultures more effective and stable. Here we studied *Wolbachia* infection in two sibling species of *Neoseiulus* predatory mites (one thelytokous and another bisexual) as well as their feed mites to test two working hypotheses: 1) a thelytokous mite *Neoseiulus agrestis* harbors *Wolbachia*, unlike its bisexual sibling species *Neoseiulus neoagrestis* and 2) feed mites are not the source of *Wolbachia* detection in *Neoseiulus*. To test these hypotheses, we performed PCR screening and multilocus sequence typing. It showed *Wolbachia* infection in *N. agrestis,* but not *N. neoagrestis*. Since the former is a thelytokous species, and the latter is not, *Wolbachia* might contribute to this peculiarity. The *Wolbachia* isolate from *N. agrestis* belongs to the supergroup B, being similar to the strains from lepidopteran insects as well as Syrphidae (Diptera). *Wolbachia* infection status of the thelytokous species *N. agrestis* is shown for the first time. As for the feed mites, *Wolbachia* was not detected in *Carpoglyphus lactis* and *Thyreophagus entomophagus,* but occurred in *Tyrophagus putrescentiae*. That bacterial strain formed a basal branch in relation to the supergroup B and demonstrated only a partial genetic identity to the Czech isolate from *T. putrescentiae*. Thus, *Wolbachia* from the predatory and feed mites are genetically different. The new *Wolbachia* sequences are deposited to GenBank serving as an important source of molecular data for comparative studies of *Wolbachia* parasites.

To Cite: Malysh, J M, Malysh, S M, Trapeznikova, O V, Timofeev, S A, Belyakova, N A, Tokarev, Y S, 2026. Identification of *Wolbachia* Strains in Two Sibling Species of *Neoseiulus* Predatory Mites and Their Prey. *Yuzuncu Yil University Journal of Agricultural Sciences*, (1): 1805326. DOI: https://doi.org/10.29133/yyutbd.1805326

1. Introduction

A range of predatory mites of the family Phytoseiidae (Parasitiformes: Mesostigmata) are widely applied in commercial biological control of insect and mite pests of agriculture under greenhouse and field conditions (Knapp et al., 2018). Noteworthy, among the top ten arthropod biocontrol agents, as many as three species are Phytoseiidae belonging to the genera of *Amblyseius, Neoseiulus,* and *Phytoseiulus*. In this article, we studied *Wolbachia* infection in two sibling species of predatory mites, *Neoseiulus agrestis* (Karg) and *Neoseiulus neoagrestis* Khaustov et al., as well as their feed mites from

the order of Sarcoptiformes: *Thyreophagus entomophagus* (Laboulbéne and Robin), *Carpoglyphus lactis* (L.), and *Tyrophagus putrescentiae* (Schrank).

Neoseiulus agrestis, known to be thelytokous (Moradi et al., 2023a; Fu et al., 2025), is spread across Europe, Asia, North America and South Africa (Tuovinen and Lindqvist, 2014; Kazemi et al., 2022; Farazmand et al., 2023). It is polyphagous, dwells in the soil cover (Szabo and Penzes, 2013), and is found as single specimens on herbaceous plants (Kolodochka and Bondarev, 2014), including flowers (Tuovinen and Lindqvist, 2014). Although knowledge about the potential of *N. agrestis* as a biological control agent is limited (Popov and Belyakova, 2022; Moradi et al., 2023a), it is applied against a range of pests, such as thrips and spider mites, similarly to the predatory mites *Neoseiulus barkeri* Hughes and *Neoseiulus cucumeris* Oudemans, which lead the same lifestyle (Çobanoğlu and Güldali, 2017; Sampson et al., 2021).

A closely related species *N. neoagrestis* differs morphologically from *N. agrestis* by the presence of gland gd2 pores. Males are present among individuals of *N. neoagrestis*, unlike *N. agrestis* (Khaustov et al., 2022), so that thelytoky is among differential diagnostic characters of the latter. *Neoseiulus neoagrestis* was reported in litter and ground vegetation on the Black Sea coast of the Krasnodar Area in Sochi. Khaustov et al. (2022) assumed that the area of this new species of predatory mites is not limited to Russia since they revealed GenBank entries of Canadian origin of high similarity to *N. neoagrestis*.

Tyrophagus putrescentiae is a cosmopolitan synanthropic polyphagous mite species of the family Acaridae. This species is characterized by sexual reproduction (Sánchez-Ramos and Castañera, 2000). It has become known as a widespread household allergenic agent contaminating food and house dust (Yu et al., 2020; Zhou et al., 2023). In addition, *T. putrescentiae* is an important pest of stored foods and sowing seeds. It disseminates toxigenic fungi, contributing to the contamination of food and feed with mycotoxins (Malik et al., 2018). Being undemanding and reproducing rapidly, it is able to displace cultures of other mites in a short period of time. For example, after 25 days of co-cultivation, *T. putrescentiae* outnumbered *C. lactis* by 118 times (Krasavina and Trapeznikova, 2020). Nevertheless, despite the high harmfulness and wide range of preferred feed types of *T. putrescentiae*, it is exploited as a feed mite in rearing of predatory mites of the genus of *Neoseiulus* (Pekas et al., 2017; Su et al., 2019; Gu et al., 2020; Krasavina and Trapeznikova, 2020; Ashraf et al., 2023; Moradi et al., 2023a and 2023b).

Thyreophagus entomophagus is another cosmopolitan mite species of the family Acaridae. It occurs in beehives and acts as a pest of stored goods, such as bee products, rye, and wheat bran (Chmielewski, 1990). In spite of cases of misidentification of this species, but the heteromorphic deutonymphal stage was proven to be absent from the commercial culture of *T. entomophagus*. Therefore, the species is able of asexual reproduction, which is a beneficial trait for mass cultivation (Klimov et al., 2025). The species is widely used for mass breeding of predatory mites due to its favorable characteristics, such as ease of consumption by predators, as well as lower allergenicity and less harmfulness than *T. putrescentiae* (Klimov et al., 2025).

Carpoglyphus lactis is a cosmopolitan mite species of the family Carpoglyphidae, characterized by sexual reproduction (Bakr et al., 2021). It is a pest of stored goods, such as dried fruit, honey and dairy products (Chmielewski, 2002). Similarly to the aforementioned species, it is widely utilized as a feed source for the mass rearing of predatory mites (Momen et al., 2020). In the laboratory, *C. lactis* is able to grow only in certain types of bran, and requires additions of dried apple flour or apple pollen (Krasavina and Trapeznikova, 2020).

The bacterial endosymbiont *Wolbachia* (Hertig) (Rickettsiales: Ehrlichiaceae) performs a variety of roles in its arthropod hosts, from inconspicuous presence to direct control of physiology and vital processes disruption (Moran and Baumann, 2000). Among its important possible effects, the host sex ratio bias plays a vital role, performed via thelytokous parthenogenesis, feminization, androcide and cytoplasmic incompatibility (Burdina and Gruntenko, 2022). *Wolbachia* has been studied in sufficient detail in arthropods of medical importance – insects (Shaikevich et al., 2019; Khoo et al., 2020; Laidoudi et al., 2020; Minwuyelet et al., 2023) and ticks (Duron et al., 2017). Other studies are focused on *Wolbachia* in agricultural (Tokarev et al., 2018; Muro et al., 2023), horticultural (Bykov et al., 2021), and forest pests (Bykov et al., 2020; Gagalova et al., 2022). Research of *Wolbachia* was also performed in insects which serve as biological control agents: coccinellids (Shaikevich et al., 2021 and 2023;

Romanov and Zakharov, 2023), ichneumonids (Klopfstein et al., 2018), and braconids (Bagheri et al., 2019; Kryukova et al., 2023; Amini et al., 2024; Utkuzova et al., 2025).

Meanwhile, the information about *Wolbachia* in phytoseiid mites is rather scarce, as no comprehensive studies have been conducted. The research aimed at detection of this endosymbiont was mainly performed using solitary information units (gene loci), inapplicable for comparison. For example, based upon *16S* ribosomal DNA sequencing, *Wolbachia* was found in *Neoseiulus paspalivorus* (De Leon) (Famah Sourassou et al., 2014) and *Metaseiulus (Galendromus) occidentalis* (Nesbitt) (Johanowicz and Hoy, 1996). Alternatively, *wsp* gene was sequenced for an endosymbiont from *Amblyseius swirskii* (Athias-Henriot) (Pournajafi et al., 2023). Based solely upon positive signals obtained using polymerase chain reaction (PCR) for the *ftsZ* gene, it was supposed that *Wolbachia* is present in *Phytoseiulus persimilis* Athias-Henriot, *M. occidentalis*, *N. barkeri*, and *Neoseiulus bibens* (Blommers) (Breeuwer and Jacobs, 1996). In some studies, *Wolbachia* was found both in the predatory phytoseiid mites and their prey, for example, *M. occidentalis* and *Tetranychus urticae* Koch (Acari: Tetranychidae) (Hoy and Jeyaprakash, 2005), as well as *N. cucumeris* and *T. putrescentiae* (Pekas et al., 2017). It is important to note that comprehensive studies of *Wolbachia* have been conducted in acariform mites, including *T. putrescentiae* (Erban et al., 2021; Klimov et al., 2024; Hubert et al., 2025).

The search for *Wolbachia* in mites is of particular interest not only because this endosymbiont may cause thelytoky in its hosts, but also due to the possibility of artificial infection, leading to formation of parthenogenetic mites. Such predator lines may be more effective and stable as biological control agents (Andrianov, 2022).

The taxonomy of *Wolbachia* has its difficulties due to low informativeness or absence of morphological data. Therefore, taxonomy and diagnosis are based solely on the results of molecular phylogenetics. The slow rate of evolution of the *16S rRNA* gene (Werren et al., 1995) and the extensive recombination of the *wsp* gene (Baldo et al., 2005) have posed challenges to the diagnostics of *Wolbachia* strains and respective phylogenetic studies. Therefore, the multi-locus sequence typing (MLST) system utilizing conserved housekeeping genes was established as the standard for classifying *Wolbachia*. The MLST approach allows discrimination of strains exploiting their sequence type (ST) (equivalent to a haplotype) on the basis of a combination of alleles (i.e., allelic profile) of housekeeping genes (five ubiquitous genes coding for glutamyl-tRNA amidotransferase, subunit B (*gatB*); cytochrome c-oxidase, subunit I (*coxA*); conserved hypothetical protein (*hcpA*), cell division protein (*ftsZ*), and fructose bisphosphate aldolase (*fbpA*)) (Baldo et al., 2006b). The main evolutionary lineages of *Wolbachia* are called supergroups, indicated by capital Latin letters (Zhou et al., 1998), which replace the species nomenclature of bacteria of the genus *Wolbachia*. To establish the boundaries between these supergroups, a set of molecular characters presented in the publicly available Genbank and PubMLST databases is used.

The purpose of this work was to determine the infection status of inherited bacterial endosymbionts in two closely related species of predatory mites *N. agrestis* and *N. neoagrestis*, as well as their prey mites followed by determination of the phylogenetic position of the endosymbionts.

The main hypotheses of this research were:

1. The thelytokous mite *N. agrestis* harbors *Wolbachia*, unlike its sibling species *N. neoagrestis*, which is bisexual.

2. Feed mites are not the source of *Wolbachia* detection in *Neoseiulus.*

2. Materials and Methods

2.1. Sampling of mites

Live cultures of the predatory mites *N. agrestis* and *N. neoagrestis* (collected in Russia: Altai Area 50°01' N 88°36' E, and Krasnodar Area 43°33' N, 39°55' E (Khaustov et al., 2022)) alongside with their prey, *T. putrescentiae* and *T. entomophagus*, respectively, were kindly provided by the staff of Tyumen State University. In St. Petersburg, *N. agrestis* was switched to feeding on *C. lactis* and *T. entomophagus*, while *N. neoagrestis* continued to feed on *T. entomophagus*. However, *T. putrescentiae*, as a “weed” and not a prey mite, penetrated the cultures of both predatory mite species. To exclude the possibility of contamination between the sibling species, *N. neoagrestis* was kept at the Institute of Applied Entomology INAPPEN and *N. agrestis* – at the All-Russian Institute of Plant Protection, the

facilities separated by a distance of 30 km. Predatory and prey mites were bred according to the method described by Krasavina and Trapeznikova (2022).

Predatory and feed mites were separated from the breeding substrate by sifting it through a 1 mm sieve. Mites were collected with a soft thin brush and placed in 1.5 ml Eppendorf tubes with 70% ethanol. For each species of predatory mites two replicates of 30-50 specimens per tube were prepared. As for predatory mites infected with *Wolbachia*, two more replicates of 10 and 20 specimens per tube were prepared. For each species of the prey mites, as many as three tubes of 10 specimens were prepared.

2.2. Molecular genetic procedures

Before DNA extraction, ethanol was removed from the test tubes containing mite specimens. Genomic DNA extraction was carried out by homogenizing the mite specimens using plastic pestles. Incubation was carried out for 3 hours at 65 °C in a lysis buffer containing 2% CTAB and 0.2% beta-mercaptoethanol. Extracted DNA was washed/precipitated sequentially with a mixture of chloroform-isoamyl alcohol, chloroform, isopropanol and 70% ethanol (modified from Sambrook et al., 1989).

Amplification of the target loci of genomic DNA of mites and their endosymbionts was carried out by PCR in Tertsik (DNA-Technology) and BIS M (BIS-N) amplifiers using a ready-made commercial mixture DreamTaq Green PCR Master Mix 2X (Thermo Fisher Scientific). Aliquots of genomic DNA (6 µl) and locus-specific primers (1 µl each) were added to the mixture (total volume 20 µl). We used the following cycling conditions: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72°C for 1 min, and a final elongation step of 72 °C for 5 min (Malysh et al., 2020).

For direct sequencing, PCR products were separated by 1% agarose gel electrophoresis. Amplicons of the expected size were purified by sorption on silicon oxide particles (Vogelstein and Gillespie, 1979; Malferrari et al., 2002). The DNA concentration after purification from the gel was determined by 2% agarose gel electrophoresis in the presence of a MassRuler DNA weight standard (Thermo Fisher Scientific). Samples with DNA concentration of 10-20 ng μl^{-1} were Sanger-sequenced at Evrogen (Russia).

2.3. Genotyping of mites

To confirm the species identification of the mites, PCR, sequencing, and phylogenetic reconstructions were carried out. To obtain the amplicons of ribosomal DNA (rDNA), the primer pairs ITS1 : ITS4, ITS5 : ITS4 (White et al., 1990), and ITSSF : ITSSR (Tixier et al., 2012) were exploited. This allowed sequencing the small subunit ribosomal RNA gene (partial sequence), internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and large subunit ribosomal RNA gene (partial sequence). The reads were combined to acquire a longer sequence. The primer pairs LCOHCOF : LCOHCOR (Tixier et al., 2012; Vicente dos Santos and Tixier, 2017) and LCO1490 : HCO2198 (Folmer et al., 1994) were used for cytochrome *c* oxidase subunit I (COX1) gene. The aforementioned primer pairs were used for identification of closely related species of the predatory mites (*N. agrestis*, *N. neoagrestis*). As for the *Wolbachia*-infected feed mite (*T. putrescentiae*), only LCO1490 : HCO2198 primers were exploited.

2.4. *Wolbachia* screening

To identify intracellular *Wolbachia* bacteria, the surface protein *wsp* primers (wsp81f : wsp691r) (Braig et al., 1998) were utilized. For a more detailed genotyping, additional markers from the standard set of MLST: *gatB*, *coxA*, *hcpA*, *ftsZ* and *fbpA* (Baldo et al., 2006b) were used.

To screen for *Wolbachia*, primers for *16S rRNA* gene were employed: WspecF and WspecR (Werren and Windsor, 2000) with a short-amplified fragment (~400 bp). To obtain a longer region (~1 500 bp), 16SA1 and 16SB1 primers (Fukatsu and Nikoh, 1998) were used. Primers for the *gltA* gene were also used (Casiraghi et al., 2005).

To obtain better quality sequences, several amplicons were cloned in pAL-TA vector (Evrogen). The resulting plasmids (6 clones of *coxA*, 5 clones of *gatB*, and 7 clones of *hcpA*), purified with Plasmid DNA diaGene Kit (DIAM, Russia), were sequenced using the standard M13f primer. Sequence alignment, analysis of nucleotide sequence variations and preparation of files for subsequent studies were performed in the BioEdit 7.0.8.0 application (Hall, 1999). Chimera test for *16S rRNA* gene

sequences was performed using Mothur version 14.8.2 (Schloss et al., 2009) and high-quality ribosomal RNA databases SILVA (https://www.arb-silva.de).

2.5. Phylogenetic analysis

Pools of rDNA and COX1 gene sequences were used separately for phylogenetic reconstructions with different sets of mite species utilizing standard GTR+I+G model in MrBayes 3.1.2.

The similarity of the *Wolbachia* sequences was checked in Genbank on the NCBI server (www.ncbi.nlm.nih.gov) using the built-in BLAST utility and PubMLST database (http://pubmlst.org/wolbachia). The latter source includes only *wsp* and the five MLST genes. Whole genome shotgun sequencing projects were used to extract the loci of interest for comparative analysis of *Wolbachia* genes from Czech *T. putrescentiae* ##TSA: GIJY01 and MAG: JAUEMM01 from GenBank.

Wolbachia profile was determined using PubMLST (Jolley et al., 2018) based upon the routinely utilized housekeeping genes. For phylogenetic studies, the sequences of these genes were employed with the addition of citrate synthase (*gltA*) (Werren et al., 1995; Casiraghi et al., 2005; Baldo et al., 2006a and 2006b). Representatives of the supergroups K, N, O, P, Q, S, U, and W were not included in the phylogenetic analysis due to the lack of whole-genome sequences or some of MLST genes in the GenBank database. Phylogenetic reconstructions were obtained using alignment of 5 MLST loci by Bayesian Inference in MrBayes version 3.1.2 and Maximum Likelihood Mega11 (utilizing standard GTR+I+G model in both applications) and then visualized in Dendroscope version 3.7.2.

3. Results

3.1. Detection of *Wolbachia* and confirmation of mite species identification

Using the specific molecular probes, *Wolbachia* was detected in the thelytokous predatory mite *N. agrestis* and its prey mite *T. putrescentiae*. Meanwhile, *Wolbachia* was not detected in the bisexual predatory mite *N. neoagrestis* and in the prey mites *C. lactis* and *T. entomophagus*.

Sequencing the diagnostic loci of the sibling mite species resulted in clear and unambiguous rDNA and COX1 chromatograms (Figure 1). The rDNA sequence, 626 b.p. long, displayed 3 nucleotide substitutions, corresponding to 99.52% sequence similarity. The COX1 was more variable, showing 81.79% similarity (118 nucleotide changes per 648 b.p.). Noteworthily, the peaks at the polymorphic sites on the chromatograms were clean, containing no additional overlapping minor peaks, thus ruling out a possibility of cross-contamination of the DNA samples (Figure 1).

The species identification of predatory mites, as well as *Wolbachia*-infected feed mite, was confirmed. For the *N. agrestis* sample, there was 100% identity to the Genbank entries ##OK490283-OK490286 and OK491216. As for *N. neoagrestis,* there was 99.7-100% similarity to the Genbank entries ##OK490280-OK490282 and MZ577376-MZ577378. Concerning *T. putrescentiae,* there was sequence similarity of 99.9% to ##MT571336 and KY986246-KY986250. Phylogenetic reconstructions using rDNA (Figure 2) and COX1 (Figure 3) genes confirmed the species identity and separate phylogenetic position of the mites.

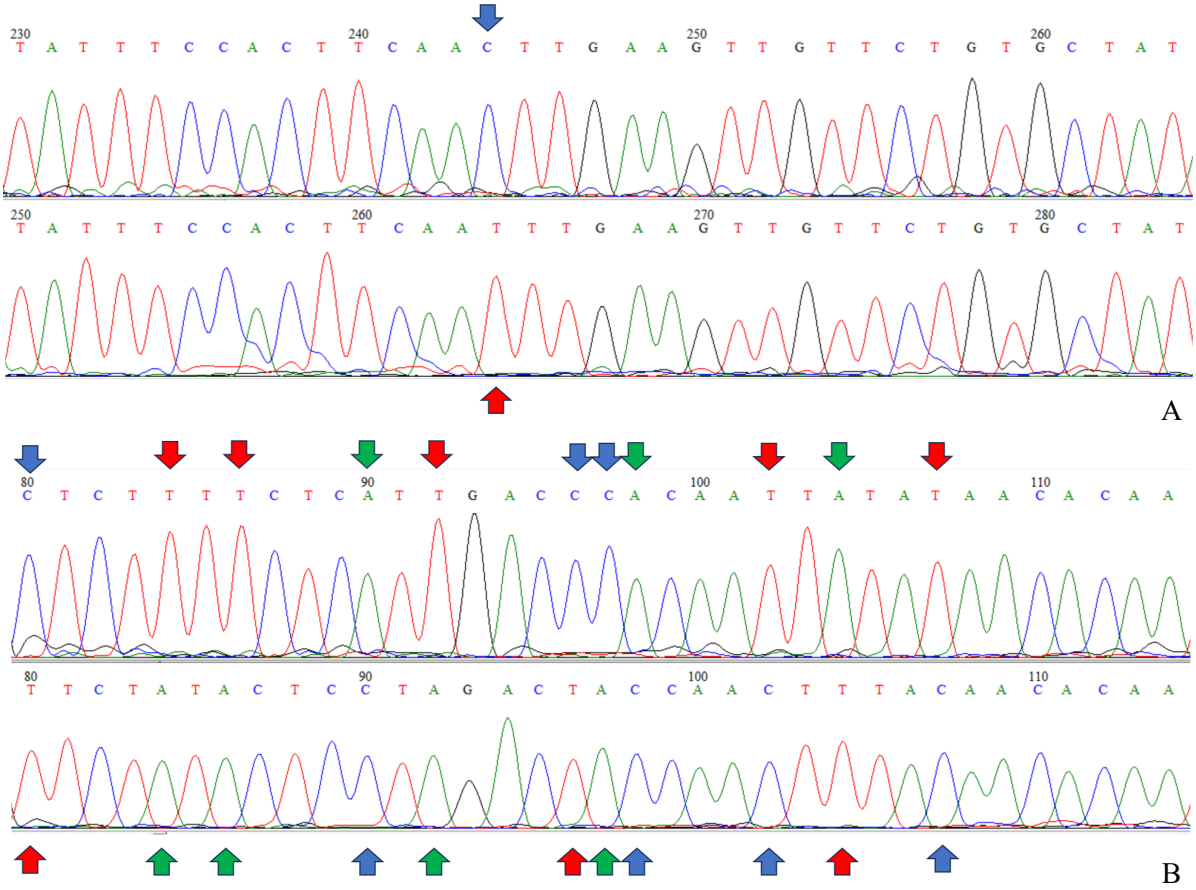
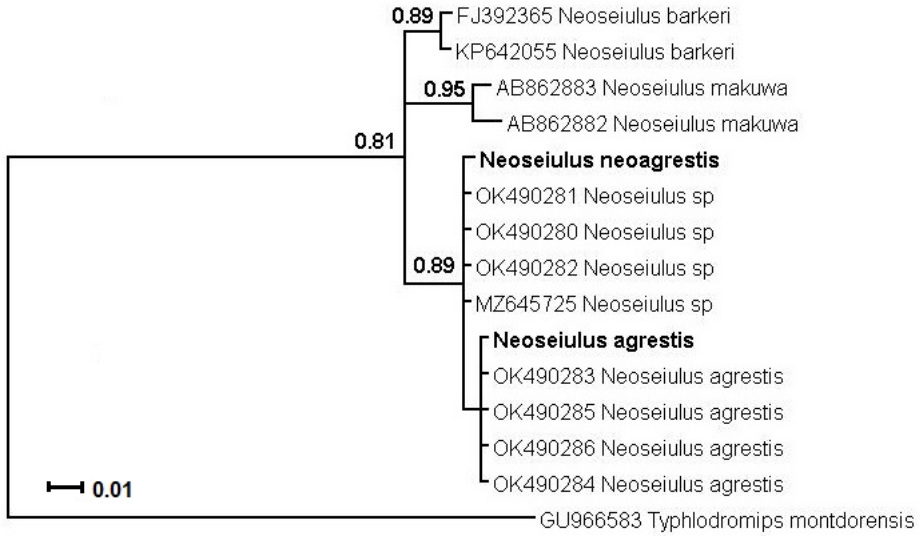
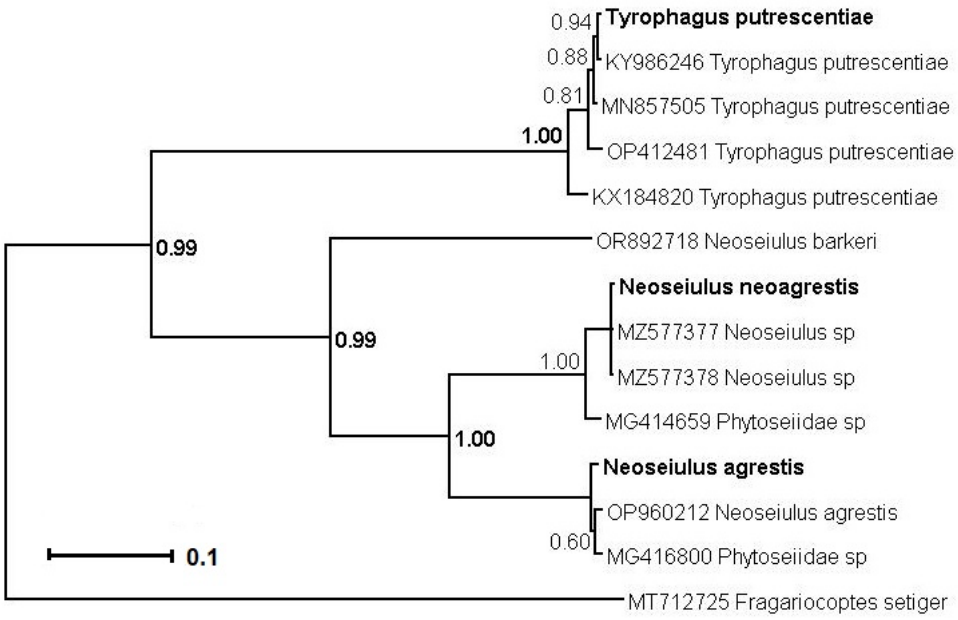


Figure 1. Portions of chromatograms of ribosomal DNA (A) and cytochrome *c* oxidase subunit I (B) loci demonstrating clean peaks at polymorphic sites (indicated by color arrows) in the sibling species of *Neoseiulus agrestis* (upper lane) and *Neoseiulus neoagrestis* (lower lane).



Numbers at the splits indicate branch support as posterior probability for Bayesian Inference. The scale bar is 0.01 expected changes per site. *Typhlodromips montdorensis* (Schicha) (Mesostigmata: Phytoseiidae) was used as the outgroup.

Figure 2. Phylogenetic tree of *Neoseiulus* species using ribosomal DNA (sequence length 640 nucleotides) in MrBayes 3.1.2.



Numbers at the splits indicate branch support as posterior probability for Bayesian Inference. The scale bar is 0.1 expected changes per site. *Fragariocoptes setiger* (Nalepa) (Trombidiformes: Phytoptidae) was used as the outgroup.

Figure 3. Phylogenetic tree of mite species using a cytochrome *c* oxidase subunit I gene (sequence length 650 nucleotides) in MrBayes 3.1.2.

Thus, the first working hypothesis that the thelytokous mite *N. agrestis* harbors *Wolbachia*, unlike its sibling species *N. neoagrestis*, which is bisexual, was confirmed.

3.2. *Wolbachia* gene sequence comparison

For the positive samples, the *16S rRNA*, *wsp*, *gltA* and five MLST (*gatB*, *fbpA*, *coxA*, *ftsZ*, and *hcpA*) gene amplicons of *Wolbachia* were produced and sequenced. The sequences have been deposited in GenBank under the accession numbers listed in Table 1.

Table 1. Identified *Wolbachia* gene sequences in the hosts *Neoseiulus agrestis* (Acari: Mesostigmata) and *Tyrophagus putrescentiae* (Acari: Sarcoptiformes)

***Wolbachia* gene**	***Neoseiulus agrestis***		***Tyrophagus putrescentiae***	
	Accession number GenBank	**Sequence length, bp**	**Accession number GenBank**	**Sequence length, bp**
gltA	PV296118	983	PV340590	954
16S rRNA	PV156482	1 377	PV156483	1 371
wsp	PV296119	558	PV340587	520
fbpA	PV296121	478	PV340588	440
ftsZ	PV296122	510	PV340589	461
gatB	PV296123	463	PV340592	339
hcpA	PV296124	493	PV340593	448
coxA	PV296120	467	PV340591	432

To demonstrate the diversity of hosts in which similar genotypes were found in Genbank and PubMLST databases, we built charts with colored sections for each locus of the *Wolbachia* isolates from mites (Figure 4).

Numerous colors, corresponding to different arthropod host orders, and sequence similarity (close to 100% in each of the eight loci analyzed) clearly indicates that the ST of the *Wolbachia* endosymbiont of *N. agrestis* is not unique, being most widespread in Lepidoptera (Figure 4A). As for the Russian isolate from *T. putrescentiae,* the charts are less variegated (Figure 4B), which means that this ST is presented in a lesser amount of the host orders. For example, these variants of *gltA* and *coxA* were previously known only for Lepidoptera, while *16S rRNA* is characteristic of *T. putrescentiae* only.

Two strains TSA: GIJY01 and MAG: JAUEMM01 were identical to each other at seven loci, while *wsp* was not detected. Interestingly, none of these sequence variants was found in lepidopteran hosts, being predominant in Diptera (*gltA, fbpA*, and *coxA*) or Arachnida (other loci).

Thus, *Wolbachia* from *N. agrestis* (Figure 4A) differed remarkedly from *Wolbachia* isolates ex *T. putrescentiae* of Russian origin (Figure 4B) at all 8 loci studied, and *T. putrescentiae* of Czech origin (Figure 4C) at all 7 available loci. Meanwhile, when the two *T. putrescentiae* isolates of *Wolbachia* were compared between each other, only 2 loci (*16S rRNA* and *ftsZ*) displayed substantial similarity (Figure 4B vs 4C).

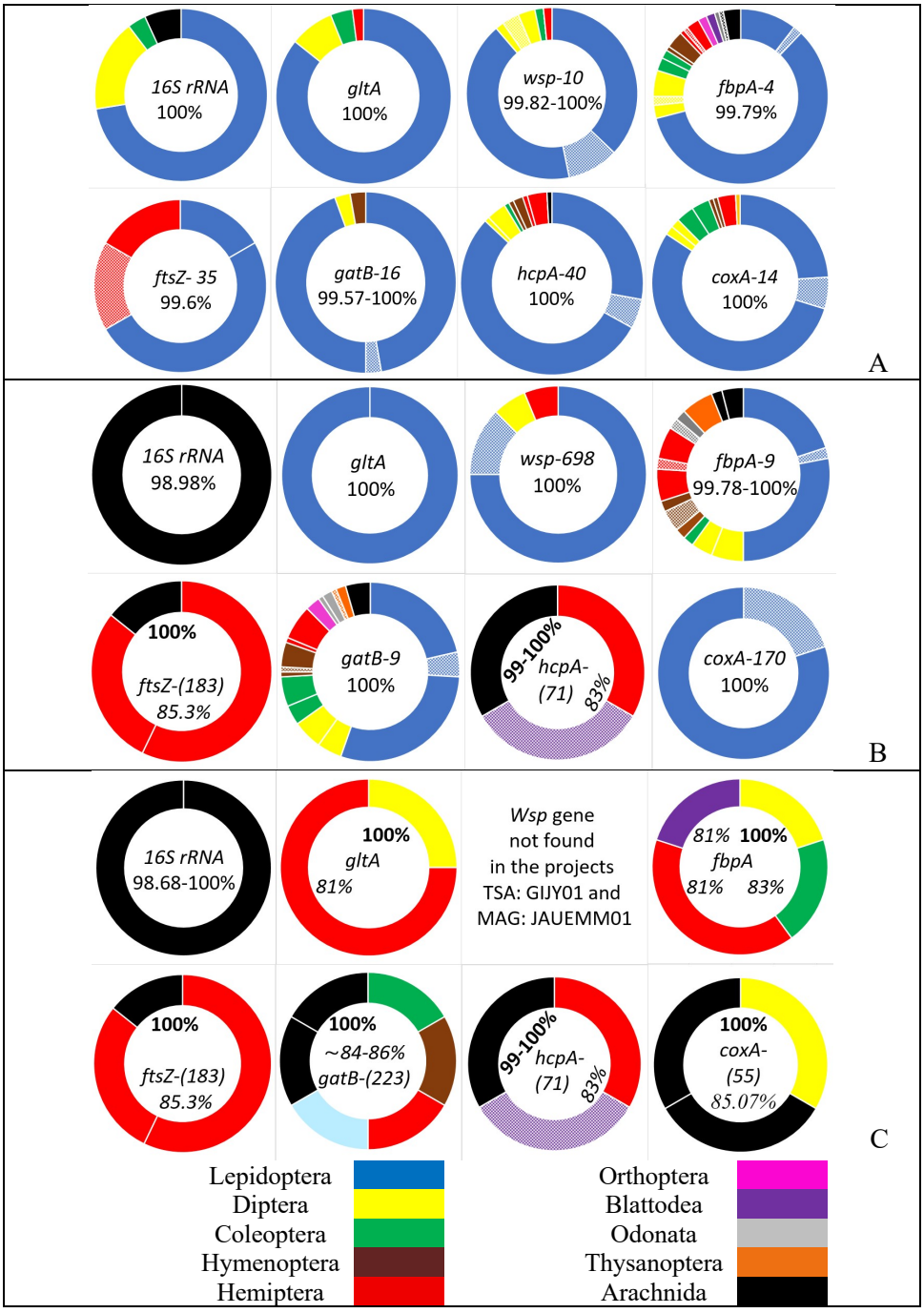


Figure 4. Similarity of each locus of examined *Wolbachia* genotypes from *Neoseiulus agrestis* (A), *Tyrophagus putrescentiae,* Russia (B), and *T. putrescentiae*, Czech Republic (C).

The charts represent distribution of most similar gene sequences, maximal similarity is indicated within the chart, while the colored sectors represent closest endosymbiont genotypes from respective host orders in Genbank or PubMLST. When the information for a given host order is sourced from the two databases, the left part and right parts of each section reflect the proportion of sequences presented in Genbank and MLST, respectively, while the intermediate hatched zone displays records found in both databases.

3.3. *Wolbachia* MLST profiles comparison

We obtained the complete MLST profiles of *Wolbachia* isolates from the predatory mite *N. agrestis* and its feed mite *T. putrescentiae*. They differed at all the loci examined (Table 2). The MLST profile of *Wolbachia* from *N. agrestis* almost completely matched the ST 150 (clonal complex STC-41), except for the *ftsZ* locus, which deviated from *ftsZ-35* and *ftsZ-36* by 2 and 9 nucleotides, respectively. In addition, the *fbpA* locus differed from *fbpA-4* by 1 nucleotide.

As for the MLST profile of *Wolbachia* from *T. putrescentiae,* no identical STs in the PubMLST database were located. Nevertheless, the *gatB-9*, *coxA-170*, *fbpA-9*, and *wsp-698* loci were not unique, unlike the *ftsZ* and *hcpA* loci. Thus, the *ftsZ* locus differed from *ftsZ-183* by 63 nucleotides, and *hcpA* from *hcpA-71* by 73 nucleotides.

Table 2. MLST profiles of *Wolbachia* isolates from the present study (in bold) and their closest relatives

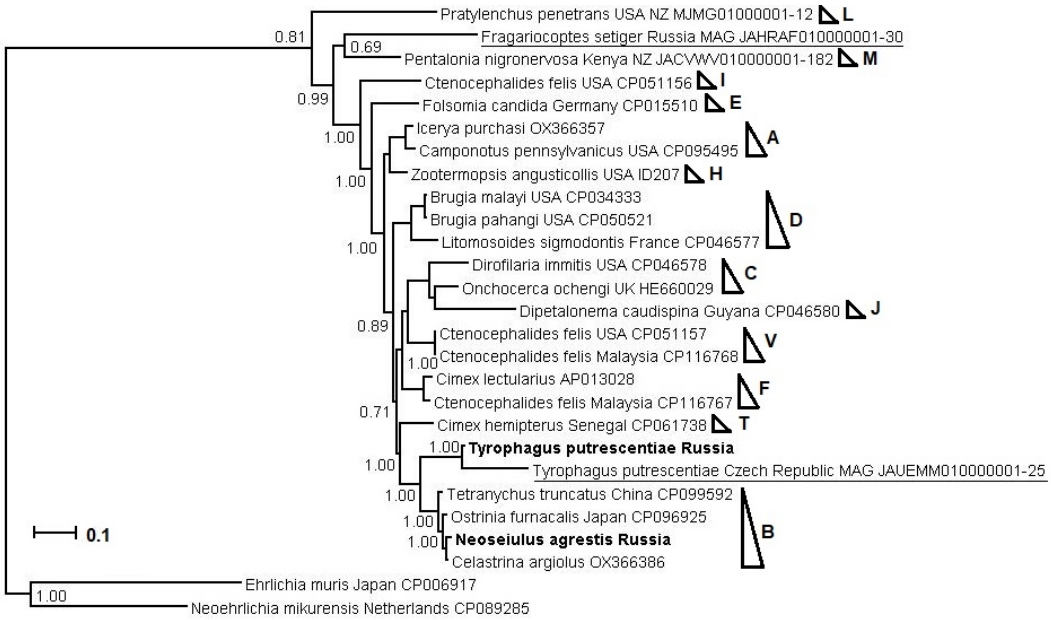
Host species	Sequence type (ST)	Loci					Supergroup
		gatB	coxA	hcpA	ftsZ	fbpA	
Neoseiulus agrestis		**16**	**14**	**40**	**35***	**4***	
Celastrina argiolus	150	16	14	40	36	4	B
Tyrophagus putrescentiae		**9**	**170**	71**	*183***	**9**	
Dendrolimus superans	353	9	170	159	22	9	B
***Tyrophagus putrescentiae* (Czech Republic)**		223**	55**	71**	*183***	333**	
Pentalonia nigronervosa	402	218	202	219	183	344	M

* alleles with 1 or 2 nucleotide substitutions.
** alleles with several dozen substitutions.
The loci of supergroup B for *Wolbachia* from *Neoseiulus agrestis* and *Tyrophagus putrescentiae* are highlighted in bold.

Based upon MLST profile comparison, it can be concluded that *Wolbachia* from *N. agrestis* belongs to the supergroup B. Yet, the precise allocation of the endosymbiont from *T. putrescentiae* cannot be so easily established, as three loci are typical of the supergroup B, while the two other loci are quite different from those present in PubMLST. To resolve this question, we relied on a phylogenetic reconstruction.

3.4. *Wolbachia* phylogenetic analysis

The *Wolbachia* isolate from *N. agrestis* clustered with isolates of the supergroup B, and the isolate of *Wolbachia* from *T. putrecentiae* formed a basal branch in relation to supergroup B (Figure 5). The tree topology is the same when using both Bayesian Inference (MrBayes version 3.1.2) and Maximum Likelihood (Mega 11).



Numbers at the splits indicate branch support as posterior probability for Bayesian Inference. Capital Latin letters in bold font indicate supergroups of *Wolbachia*. The scale bar is 0.1 expected changes per site. Alphaproteobacteria *Ehrlichia muris* Wen et al. and *Neoehrlichia mikurensis* Wass et al. (homotypic synonym: *Candidatus* Neoehrlichia mikurensis Kawahara et al.), belonging to order Rickettsiales, families Ehrlichiaceae and Anaplasmataceae, respectively, were used as the outgroup.

Figure 5. Phylogenetic tree of *Wolbachia* isolates from 27 host species using 5 MLST loci (concatenated sequence length 2 474 nucleotides) in MrBayes 3.1.2.

Wolbachia isolates of predatory mites *N. agrestis* and their prey *T. putrescentiae* turned out to be different. When explored at individual loci, the similarity between isolates ranged from 81.0 to 99.7%, while comparison of the concatenated sequences showed 423 nucleotide substitutions per 4 978 bp, corresponding to the 91.5% sequence similarity.

Thus, the second working hypothesis is supported using comparison of each locus pair, as well as their concatenated sequences and MLST profiles, and phylogenetic reconstructions of *Wolbachia* from the predatory mite and its feed mite.

4. Discussion

Thelytoky is not very common among phytoseiid mites. In addition to *N. agrestis*, obligate thelytoky is described only in *Amblyseius guatemalensis* (*A. elongatus*) (Chant), *Amblyseius parasundi* Blommers, and *Neoseiulus (Amblyseius) aurescens* (Athias-Henriot) (Kennett, 1958; Blommers, 1974; Gaponyuk, 1989). Some other species may express thelytoky which is not obligate. Those are *Amblyseius herbicolus* (*A. deleoni*) Chant, *Typhlodromips sessor* (De Leon), *Neoseiulus (Amblyseius) salish* (Chant and Hansell), *Typhlodromus transvaalensis* (Nesbitt), *Transeius* (*Amblyseius) herbarius* (Wainstein), *Phytoseius intermedius* (Evans and McFarlane), and *N. tunus* (De Leon) (Wysoki, 1973; Sciarappa and Swift, 1977; Wysoki and Bolland, 1983; Kolodochka, 1984; Azevedo et al., 2016; Cavalcante et al., 2017; Zhang and Zhang, 2021; Toyoshima, 2021). Among the aforementioned species, infection with *Wolbachia* has not been documented before.

Anyway, the current study is the first report of *Wolbachia* infection in *N. agrestis*. The *Wolbachia* genotype from this host species displays only slight differences as compared to the endosymbiont isolates infecting butterflies and moths of various families, as well as the hoverfly, *Eupeodes latifasciatus* (Macquart). *Neoseiulus agrestis* is polyphagous and, besides arthropod prey, may feed on pollen just like the other mites of this genus (Tsolakis et al., 2019). The horizontal exchange by the endosymbionts may therefore occur between different arthropod hosts in the flowers, which makes it expectable to find the same (or nearly the same) genotypes of *Wolbachia* in mites and butterflies. Besides, some phytoseiid mites may feed on lepidopteran insect eggs (Amano and Chant, 1986), which in turn may serve as a source of horizontally transferred endosymbionts from moths to mites via

predation. Moreover, some insects, while feeding by pollen and nectar at the adult stage, may be larval predators of aphids, thrips, and spider mites. These include the larvae of syrphid flies, such as *E. latifasciatus* (Pekas et al., 2020), and it can be therefore said that they share a common ecological niche with the predatory mites. Minor genetic differences revealed by MLST may indicate that the horizontal transfer occurred once in their evolutionary history, and the mutations started to accumulate since then. A negligible amount of these mutations suggests that this transfer occurred relatively recently.

An important outcome of this research is the demonstration of the endosymbiotic bacterium presence in the thelytokous mite, but not in its sibling species, bisexual *N. neoagrestis.* And because *Wolbachia* is among the factors influencing arthropod physiology, including reproductive strategies, it is important to screen other thelytokous and bisexual mite species for this endosymbiont. In spite of deficiency of practical studies in this field, the idea of *Wolbachia* influence on sex determination in its mite hosts has been broadcasted in scientific literature (Cavalcante et al., 2017; Andrianov, 2022; Zhang et al., 2025; Fu et al., 2025). If more examples of *Wolbachia* presence in thelytokous, but not bisexual relatives will be found, this would serve as a more profound confirmation of this hypothesis.

Regarding *Wolbachia* from *T. putrescentiae*, it is important to note that Klimov et al. (2024) insisted on attribution of their isolate from *T. putrescentiae* to the supergroup Q, but showed relationships of their isolate to the "type" isolate (ex *Torotrogla*) of that clade in a supplementary file only (with rather low bootstrap support values). In the main figure 4 they only displayed isolates ex *Fragariocoptes* and *Thyrophagus* without inclusion of *Torotrogla*. The figure legends are obscure, not indicating real diversity of the loci utilized. As far as we can judge, Glowska et al. (2015) could provide just two loci (*16S rRNA+coxA*) for one isolate, two other loci for another isolate (*coxA+groEL*), and variable sets of three loci (either *16S rRNA+coxA+gltA* or *coxA+gltA+groEL*) for the two other strains. Meanwhile, Klimov et al. (2024) based their supergroup allocation on what they called “four datasets: genomic, five standard phylogenetic loci (Glowska et al., 2015), five multilocus sequence typing (MLST) loci (Baldo et al., 2006), and 16S rRNA”. However, it is known since long that *16S rRNA* is not suitable for taxonomy purposes in *Wolbachia* (Werren et al., 1995) while the statement “MLST as in Glowska et al. (2015)” doesn’t correspond to the limited set of markers provided by the latter research.

We therefore cannot consider it as a well-supported resolution so as to validate the supergroup Q. This rather looks like an unsuccessful attempt to classify *Wolbachia* from different mite species into a single "mite-specific" supergroup.

Both our study and that of Klimov et al. (2024) showed that different approaches result in divergent grouping of certain isolates from mites. Hence, the question of their phylogenetic position remains unresolved and requires a unified set-up of characters, exploited in *Wolbachia* systematics. MLST-based phylogeny according to Baldo et al. (2006b) clearly demonstrates that the endosymbionts from Acariformes is not a single group, as *Wolbachia* from *Fragariocoptes setiger* (Nalepa) (Trombidiformes: Phytoptidae) belongs to the supergroup M, while that from *T. putrescentiae* is close to the supergroup B (Figure 5).

Conclusion

Our results show that *Wolbachia* is present in the thelytokous predatory mite species *N. agrestis* and is absent in the congeneric bisexual species *N. neoagrestis*, thus supporting the first hypothesis. Since this endosymbiont is responsible for reproduction and sex determination in different arthropods, this is quite possible that it is *Wolbachia* that defines sexual reproduction type in two sibling *Neoseiulus* species. This phenomenon requires further detailed examination to facilitate our understanding the role of endosymbiotic bacteria in reproductive success and speciation of phytoseiid mites.

The second working hypothesis that the feed mites are not the source of *Wolbachia* infection of *Neoseiulus* is confirmed by detailed molecular genetic investigation. The genotypes of *Wolbachia* from the predatory mites and their prey are not related, so that the respective endosymbionts were not shared through trophic interactions of their hosts. Meanwhile, identical or highly similar genotypes of *Wolbachia* are present in diverse arthropods, suggesting evolutionary host shift via horizontal transfer.

Ethical Statement

Ethical approval is not required for this study as it does not involve human or animal research.

Conflicts of Interest

The authors declare that there is no conflict of interest to disclose.

Artificial Intelligence Declaration

The authors declare that no generative artificial intelligence tools were used at any stage of the preparation of this manuscript, including the writing, editing, or refinement of the text, or the creation of any images, figures, graphics, tables, or related titles.

Funding Statement

The research is supported by Russian Science Foundation, project # 20-66-47010, and the State Assignment, project # FGEU-2025-0002.

Author Contribution

Study conception and design: NAB, OVT, JMM; methodology: JMM, OVT, SAT; investigation: OVT; SAT, SMM, JMM; analysis and interpretation of results: JMM, YST; visualization: JMM, YST; draft manuscript preparation: JMM, YST; review and editing: JMM, YST, SMM; funding acquisition: NAB. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

The authors are indebted to Alexander A. Khaustov (Tyumen State University, Institute of Environmental and Agricultural Biology (X-BIO), Russia) for providing the predatory mites *N. agrestis* and *N. neoagrestis* as a part of the collaboration on this project and Ken Smith (Santo Domingo, Dominican Republic) for English grammar and style correction of the manuscript. Yury Yu. Ilinsky (Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences) is gratefully acknowledged for criticism given on an early draft of the manuscript.

References

Andrianov, B. V. (2022). Bacterial symbionts and pathogens in populations of Phytoseiidae mites (Phytoseiidae, Mesostigmata). *Biology Bulletin Reviews, 12*(1), S78–84. https://doi.org/10.1134/S2079086422070027

Amano, H., & Chant, D. A. (1986). Laboratory studies on the feeding habits, reproduction and development of three phytoseiid species, *Typhlodromus pomi*, *Phytoseius macropilis* and *Amblyseius finlandicus* (Acari: Phytoseiidae), occurring on abandoned apple trees in Ontario, Canada. *Experimental and Applied Acarology*, *2*(4), 299–313.

Amini, S., Fathipour, Y., Hoffmann, A., & Mehrabadi, M. (2024). *Wolbachia* affect female mate preference and offspring fitness in a parasitoid wasp. *Pest Management Science, 80*(10), 5432–5439. https://doi.org/10.1002/ps.8272

Ashraf, S. E., Asmaa, R. A., & Eman, H. W. (2023). Developmental and life table of *Neoseiulus californicus* (Acari: Phytoseiidae) fed on three astigmatid mites and *Tetranychus urticae* (Acari: Tetranychidae). *Egyptian Journal of Plant Protection Research Institute, 6*(1), 64–73.

Azevedo, L. H., Castilho, R. D. C., & De Moraes, G. J. (2016). Suitability of the litchi erineum mite, *Aceria litchii* (Keifer), as prey for the mite *Phytoseius intermedius* Evans & MacFarlane (Acari: Eriophyidae, Phytoseiidae). *Systematic and Applied Acarology, 21*(3), 270–278. https://doi.org/10.11158/saa.21.3.2

Bagheri, Z., Talebi, A. A., Asgari, S., & Mehrabadi, M. (2019). *Wolbachia* induce cytoplasmic incompatibility and affect mate preference in *Habrobracon hebetor* to increase the chance of its transmission to the next generation. *Journal of Invertebrate Pathology, 163*, 1–7. https://doi.org/10.1016/j.jip.2019.02.005

Bakr, A., Rezk, H. A., Abd El-Hamid, M. M., & Osman, S. I. (2021). Studying the biology of *Carpoglyphus lactis* (L.) reared on dried apricots and its control using plant oil extracts. *Alexandria Science Exchange Journal, 42*(2), 407–411. https://doi.org/10.21608/asejaiqjsae.2021.171640

Baldo, L., Bordenstein, S., Wernegreen, J. J., & Werren, J. H. (2006a). Widespread recombination throughout *Wolbachia* genomes. *Molecular Biology and Evolution, 23*(2), 437–449. https://doi.org/10.1093/molbev/msj049

Baldo, L., Dunning Hotopp, J. C., Jolley, K. A., Bordenstein, S. R., Biber, S. A., Choudhury, R. R., Hayashi, C., Maiden, M. C., Tettelin, H., & Werren, J. H. (2006b). Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Applied and Environmental Microbiology, 72*(11), 7098–7110. https://doi.org/10.1128/AEM.00731-06

Baldo, L., Lo, N., & Werren, J. H. (2005). Mosaic nature of the *Wolbachia* surface protein. *Journal of Bacteriology, 187*(15), 5406–5418. https://doi.org/10.1128/jb.187.15.5406-5418.2005

Blommers, L. (1974). Species of the genus *Amblyseius* Berlese, 1914, from Tamatave, east Madagascar (Acarina: Phytoseiidae). *Bulletin Zoologisch Museum, 3*(19), 143–148.

Braig, H. R., Zhou, W., Dobson, S. L., & O'Neill, S. L. (1998). Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *Journal of Bacteriology, 180*(9), 2373–2378. https://doi.org/10.1128/JB.180.9.2373-2378.1998

Breeuwer, J. A., & Jacobs, G. (1996). *Wolbachia*: intracellular manipulators of mite reproduction. *Experimental and Applied Acarology, 20*, 421–434. https://doi.org/10.1007/BF00053306

Burdina, E. V., & Gruntenko, N. E. (2022). Physiological aspects of *Wolbachia pipientis–Drosophila melanogaster* relationship. *Journal of Evolutionary Biochemistry and Physiology, 58*(2), 303–317. https://doi.org/10.1134/s0022093022020016

Bykov, R., Kerchev, I., Demenkova, M., Ryabinin, A., & Ilinsky, Y. (2020). Sex-specific *Wolbachia* infection patterns in populations of *Polygraphus proximus* Blandford (Coleoptera; Curculionidae: Scolytinae). *Insects, 11*(8), 547. https://doi.org/10.3390/insects11080547

Bykov, R., Yurlova, G., Demenkova, M., & Ilinsky, Y. (2021). Is *Aporia crataegi* unsuitable host of *Wolbachia* symbionts? *Plant Protection News, 104*(1), 53–60. https://doi.org/10.31993/2308-6459-2021-104-1-14945

Casiraghi, M., Bordenstein, S. R., Baldo, L., Lo, N., Beninati, T., Wernegreen, J. J., Werren, J. H., & Bandi, C. (2005). Phylogeny of *Wolbachia pipientis* based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F super-group, and evidence for further diversity in the *Wolbachia* tree. *Microbiology, 151*(12), 4015–4022. https://doi.org/10.1099/mic.0.28313-0

Cavalcante, A. C., Demite, P. R., Amaral, F. S., Lofego, A. C., & De Moraes, G. J. (2017). Complementary description of *Neoseiulus tunus* (De Leon) (Acari: Mesostigmata: Phytoseiidae) and observation on its reproductive strategy. *Acarologia, 57*(3), 591–599. https://doi.org/10.24349/acarologia/20174178

Chmielewski, W. (1990). Bio-ecology and population development of *Thyreophagus entomophagus* - an acarid mite found in beehives. *Pszczelnicze Zeszyty Naukowe. 34*, 31–42.

Chmielewski, W. (2002). Acaro-entomological contaminations of propolis. *Journal of Apicultural Science, 46*, 17–23.

Çobanoğlu, S., & Güldali, B. (2017). Plant parasitic and predatory mites (Acari: Tetranychidae, Phytoseiidae) and population density fluctuation of two-spotted spider mite (*Tetranychus urticae* Koch) on strawberry in the Mersin province of Turkey. *Journal of Zoological Sciences, 5*(2), 57–67.

Duron, O., Binetruy, F., Noël, V., Cremaschi, J., McCoy, K. D., Arnathau, C., Plantard, O., Goolsby, J., Pérez de León, A. A., Heylen, D. J., & Van Oosten, A. R. (2017). Evolutionary changes in symbiont community structure in ticks. *Molecular ecology, 26*(11), 2905–2921. https://doi.org/10.1111/mec.14094

Erban, T., Klimov, P. B., Harant, K., Talacko, P., Nesvorna, M., & Hubert, J. (2021). Label-free proteomic analysis reveals differentially expressed *Wolbachia* proteins in *Tyrophagus putrescentiae*: Mite allergens and markers reflecting population-related proteome differences. *Journal of Proteomics, 249*, 104356. https://doi.org/10.1016/j.jprot.2021.104356

Famah Sourassou, N., Hanna, R., Breeuwer, J. A., Negloh, K., de Moraes, G. J., & Sabelis, M. W. (2014). The endosymbionts *Wolbachia* and *Cardinium* and their effects in three populations of the predatory mite *Neoseiulus paspalivorus*. *Experimental and Applied Acarology, 64*(2), 207–221. https://doi.org/10.1007/s10493-014-9820-0

Farazmand, A., Jalaeian, M., Kamali, H., Saboori, A., Tixier, M. S., & Kreiter, S. (2023). Phytoseiidae (Acari: Mesostigmata) in four provinces of Iran. *Persian Journal of Acarology, 12*(1), 21–58. https://doi.org/10.22073/pja.v12i1.77425

Folmer, O., Hoeh, W. R., Black, M. B., & Vrijenhoek, R. C. (1994). Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology, 3*(5), 294–299.

Fu, X. T., Zeng, K. K., Huo, Z. J., Chang, J., & Meng, R. X. (2025). Demographic and predatory parameters of a thelytokous phytoseiid mite as a biocontrol agent for spider mites. *Pest Management Science, 81*(8), 4540–4547. https://doi.org/10.1002/ps.8811

Fukatsu, T., & Nikoh, N. (1998). Two intracellular symbiotic bacteria from the mulberry psyllid *Anomoneura mori* (Insecta, Homoptera). *Applied and Environmental Microbiology, 64*(10), 3599–3606. https://doi.org/10.1128/AEM.64.10.3599-3606.1998

Gagalova, K. K., Whitehill, J. G., Culibrk, L., Lin, D., Lévesque-Tremblay, V., Keeling, C. I., Coombe, L., Yuen, M. M., Birol, I., Bohlmann, J. & Jones, S. J. (2022). The genome of the forest insect pest *Pissodes strobi* reveals genome expansion and evidence of a *Wolbachia* endosymbiont. *G3 Genes Genomes Genetics, 12*(4), jkac038. https://doi.org/10.1093/g3journal/jkac038

Gaponyuk, I. L. (1989). Discovery of thelytoky in the predatory mite *Amblyseius aurescens* (Parasitiformes, Phytoseiidae). *Vestnik Zoologii, 4*, 82.

Glowska, E., Dragun-Damian, A., Dabert, M., & Gerth, M. (2015). New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). *Infection, Genetics and Evolution, 30*, 140–146. https://doi.org/10.1016/j.meegid.2014.12.019

Gu, X. Y., Li, G. Y., & Zhang, Z. Q. (2020). Indirect effects in predator-prey interaction: development and predation rates by immature *Neoseiulus cucumeris* increased by odour from its prey (*Tyrophagus putrescentiae*). *Systematic and Applied Acarology, 25*(7), 1247–1256. https://doi.org/10.11158/saa.25.7.7

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hoy, M. A., & Jeyaprakash, A. (2005). Microbial diversity in the predatory mite *Metaseiulus occidentalis* (Acari: Phytoseiidae) and its prey, *Tetranychus urticae* (Acari: Tetranychidae). *Biological Control, 32*(3), 427–441. https://doi.org/10.1016/j.biocontrol.2004.12.012

Hubert, J., Glowska-Patyniak, E., Dowd, S.E., & Klimov, P.B. (2025). *Cardinium* disrupts *Wolbachia*–host dynamics in the domestic mite *Tyrophagus putrescentiae*: evidence from manipulative experiments. *MSystems, 10*(5), e01769-24. https://doi.org/10.1128/msystems.01769-24

Johanowicz, D. L., & Hoy, M. A. (1996). *Wolbachia* in a predator–prey system: 16S ribosomal DNA analysis of two phytoseiids (Acari: Phytoseiidae) and their prey (Acari: Tetranychidae). *Annals of the Entomological Society of America, 89*(3), 435–441. https://doi.org/10.1093/aesa/89.3.435

Jolley, K. A., Bray, J. E., & Maiden, M. C. J. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Research*, *3*, 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Kazemi, S., Mohammad-Doustaresharaf, M., & Döker, I. (2022). An annotated checklist of the Iranian Phytoseiidae (Acari: Mesostigmata), with an updated key to the species. *Systematic and Applied Acarology, 27*(4), 697–748. https://doi.org/10.11158/saa.27.4.6

Kennett, C. E. (1958) Some predacious mites of the subfamilies Phytoseiinae and Aceosejinae (Acarina: Phytoseiidae, Aceosejidae) from Central California with descriptions of new species. *Annals of the Entomological Society of America, 51*(5), 471–479. https://doi.org/10.1093/aesa/51.5.471

Khaustov, V. A., Döker, I., Joharchi, O., Kazakov, D. V., Khaustov, A. A., Moradi, M., Fang, X-D., & Klimov, P. (2022). A new, broadly distributed species of predacious mites, *Neoseiulus neoagrestis* sp. nov. (Acari: Phytoseiidae) discovered through GenBank data mining and extensive morphological analyses. *Systematic and Applied Acarology, 27*(10), 2038–2061. https://doi.org/10.11158/saa.27.10.14

Khoo, J. J., Kurtti, T. J., Husin, N. A., Beliavskaia, A., Lim, F. S., Zulkifli, M. M. S., Al-Khafaji, A. M., Hartley, C., Darby, A. C., Hughes, G. L. et al. (2020). Isolation and propagation of laboratory strains and a novel flea-derived field strain of *Wolbachia* in tick cell lines. *Microorganisms, 8*, 988. https://doi.org/10.3390/microorganisms8070988

Klimov, P. B., Hubert, J., Erban, T., Perotti, M. A., Braig, H. R., Flynt, A., He, Q., & Cui, Y. (2024). Genomic and metagenomic analyses of the domestic mite *Tyrophagus putrescentiae* identify it as a widespread environmental contaminant and a host of a basal, mite-specific *Wolbachia* lineage (supergroup Q). *International Journal for Parasitology, 54*(13), 661–674. https://doi.org/10.1016/j.ijpara.2024.07.001

Klimov, P. B., Kolesnikov, V. B., Khaustov, A. A., Khaustov, V. A., Merckx, J., Duarte, M. V. A., Vangansbeke, D., Geudens, I., & Pepato, A. (2025). Typification of the economically important species *Thyreophagus entomophagus* (Acari: Astigmata: Acaridae) used for the industrial production of predatory mites: the designation of a neotype with detailed morphological and DNA sequence data. *Animals, 15*(3), 357. https://doi.org/10.3390/ani15030357

Klopfstein, S., van Der Schyff, G., Tierney, S., & Austin, A. D. (2018). *Wolbachia* infections in Australian ichneumonid parasitoid wasps (Hymenoptera: Ichneumonidae): evidence for adherence to the global equilibrium hypothesis. *Biological Journal of the Linnean Society, 123*(3), 518–534. https://doi.org/10.1093/biolinnean/blx157

Knapp, M., van Houten, Y., van Baal, E., & Groot, T. (2018). Use of predatory mites in commercial biocontrol: current status and future prospects. *Acarologia, 58*, 72–82. https://doi.org/10.24349/acarologia/20184275

Kolodochka, L. A. (1984). Analysis of some ecological characteristics of parthenogenetic and bisexual species of phytoseiid mites. *Vestnik Zoologii, 5*, 47–53 (in Russian).

Kolodochka, L. A., & Bondarev, V. Yu. (2014). To the species composition of plant-dwelling phytoseiid mites (Parasitiformes, Phytoseiidae) of the Lugansk region of Ukraine. *Ukrainian Entomological Journal,* 2, 52–56 (in Russian).

Krasavina, L., & Trapeznikova, O. (2020). Assessment of different species of fodder mites for mass rearing of the predatory mites *Amblyseius swirskii* and *Neoseiulus cucumeris* (Mesostigmata, Phytoseiidae) under laboratory conditions. *Plant Protection News, 103*(3), 177–181. https://doi.org/10.31993/2308-6459-2020-103-3-13943 (in Russian).

Krasavina, L., & Trapeznikova, O. (2022). Improvement of breeding methods of predatory mites *Neoseiulus cucumeris* and *Transeius montdorensis* for biological plant protection. *Plant Protection News, 105*(2), 87–93. https://doi.org/10.31993/2308-6459-2022-105-2-15269 (in Russian).

Kryukova, N. A., Kryukov, V. Y., Polenogova, O. V., Chertkova, E. A., Tyurin, M. V., Rotskaya, U. N., Alikina, T., Kabilov, M. R., & Glupov, V. V. (2023). The endosymbiotic bacterium *Wolbachia* (Rickettsiales) alters larval metabolism of the parasitoid *Habrobracon hebetor* (Hymenoptera: Braconidae). *Archives of Insect Biochemistry and Physiology, 114*(4), e22053. https://doi.org/10.1002/arch.22053

Laidoudi, Y., Levasseur, A., Medkour, H., Maaloum, M., Ben Khedher, M., Sambou, M., Bassene, H., Davoust, B., Fenollar, F., Raoult, D., & Mediannikov, O. (2020). An earliest endosymbiont, *Wolbachia massiliensis* sp. nov., strain PL13 from the bed bug (*Cimex hemipterus*), type strain of a new supergroup T. *International Journal of Molecular Sciences, 21*(21), 8064. https://doi.org/10.3390/ijms21218064

Malferrari, G., Monferini, E., DeBlasio, P., Diaferia, G., Saltini, G., Del Vecchio, E., Rossi-Bernardi, L., & Biunno, I. (2002). High-quality genomic DNA from human whole blood and mononuclear cells. *BioTechniques, 33*(6), 1228–1230. https://doi.org/10.2144/02336bm09

Malik, A., Gulati, R., Duhan, K., & Poonia, A. (2018). *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) as a pest of grains: A review. *Journal of Entomology and Zoology Studies, 6*(2543), e2550.

Malysh, J., Malysh, S., Kireeva, D., Kononchuk, A., & Demenkova, M. (2020). Detection of *Wolbachia* in larvae of *Loxostege sticticalis* (Pyraloidea: Crambidae) in European and Asian parts of Russia. *Plant Protection News, 103*(1), 49–52. https://doi.org/10.31993/2308-6459-2020-103-1-49-52

Minwuyelet, A., Petronio, G. P., Yewhalaw, D., Sciarretta, A., Magnifico, I., Nicolosi, D., Di Marco, R., & Atenafu, G. (2023). Symbiotic *Wolbachia* in mosquitoes and its role in reducing the transmission of mosquito-borne diseases: updates and prospects. *Frontiers in Microbiology, 14*, 1267832. https://doi.org/10.3389/fmicb.2023.1267832

Momen, F., Fahim, S., & Barghout, M. (2020). Mass production of predatory mites and their efficacy for controlling pests. In N. El-Wakeil, M. Saleh & M. Abu-hashim (Eds.), *Cottage industry of biocontrol agents and their applications: Practical aspects to deal biologically with pests and stresses facing strategic crops* (pp. 157–200). Cham: Springer International Publishing.

Moradi, M., Misharova, Y. V., Snigirev, V. V., Döker, I., Joharchi, O., & Khaustov, V. A. (2023a). Life history of *Neoseiulus agrestis* (Karg) (Acari: Phytoseiidae) fed on the storage mite, *Thyreophagus* sp. (Acari: Acaridae) at different temperatures. *Acarologia, 63*(3), 817–825. https://doi.org/10.24349/6x1q-8mmr

Moradi, M., Joharchi, O., Döker, I., Khaustov, V. A., Salavatulin, V. M., Popov, D. A., & Belyakova, N. A. (2023b). Effects of temperature on life table parameters of a newly described phytoseiid predator, *Neoseiulus neoagrestis* (Acari: Phytoseiidae) fed on *Tyrophagus putrescentiae* (Acari: Acaridae). *Acarologia, 63*(1), 31–40. https://doi.org/10.24349/n3ej-nn6s

Moran, N. A., & Baumann, P. (2000). Bacterial endosymbionts in animals. *Current opinion in microbiology, 3*(3), 270–275. https://doi.org/10.1016/S1369-5274(00)00088-6

Muro, T., Hikida, H., Fujii, T., Kiuchi, T., & Katsuma, S. (2023). Two complete genomes of male-killing *Wolbachia* infecting *Ostrinia* moth species illuminate their evolutionary dynamics and association with hosts. *Microbial Ecology, 86*(3), 1740–1754. https://doi.org/10.1007/s00248-023-02198-7

Pekas, A., De Craecker, I., Boonen, S., Wäckers, F. L., & Moerkens, R. (2020). One stone; two birds: concurrent pest control and pollination services provided by aphidophagous hoverflies. *Biological Control*, *149*, 104328. https://doi.org/10.1016/j.biocontrol.2020.104328

Pekas, A., Palevsky, E., Sumner, J. C., Perotti, M. A., Nesvorna, M., & Hubert, J. (2017). Comparison of bacterial microbiota of the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) and its factitious prey *Tyrophagus putrescentiae* (Acari: Acaridae). *Scientific Reports, 7*(1), 1–12. https://doi.org/10.1038/s41598-017-00046-6

Popov, D., & Belyakova, N. (2022). World experience in the production and use of phytoseiid mites. *Plant Protection News, 105*(2), 68–86. https://doi.org/10.31993/2308-6459-2022-105-2-15282 (in Russian).

Pournajafi, H., Khanjani, M., & Soltani, J. (2023). Sex-ratio deviation and sex-determining bacteria detected by multiplex PCR in the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae). *Persian Journal of Acarology, 12*(2), 337–344. https://doi.org/10.22073/pja.v12i2.77489

Romanov, D. A., & Zakharov, I. A. (2023). Distribution of symbiotic bacteria *Spiroplasma*, *Rickettsia*, *Wolbachia* in populations of *Adalia bipunctata* (Linnaeus, 1758). *Symbiosis, 9*, 11–13 https://doi.org/10.1007/s13199-023-00942-8

Sambrook, J., Fritsch, E., & Maniatis, T. (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.

Sampson, C., Bennison, J., & Kirk, W. D. J. (2021). Overwintering of the western flower thrips in outdoor strawberry crops. *Journal of Pest Science, 94*, 143–152. https://doi.org/10.1007/s10340-019-01163-z

Sánchez-Ramos, I., & Castañera, P. (2000). Acaricidal activity of natural monoterpenes on *Tyrophagus putrescentiae* (Schrank), a mite of stored food. *Journal of Stored Products Research, 37*(1), 93–101. https://doi.org/10.1016/S0022-474X(00)00012-6

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., & Sahl, J. W. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Sciarappa, W. J., & Swift, F. C. (1977). Biological studies of *Typhlodromips sessor* (Acarina: Phytoseiidae). *Annals of the Entomological Society of America, 70*(2), 285–288. https://doi.org/10.1093/aesa/70.2.285

Shaikevich, E., Bogacheva, A., Rakova, V., Ganushkina, L., & Ilinsky, Y. (2019). *Wolbachia* symbionts in mosquitoes: Intra- and intersupergroup recombinations, horizontal transmission and evolution. *Molecular phylogenetics and evolution, 134*, 24–34. https://doi.org/10.1016/j.ympev.2019.01.020

Shaikevich, E. V., Gorbacheva, A. A., & Romanov, D.A. (2023). Ectoparasitic mites: Vectors of bacterial symbionts among insects. *Biology Bulletin of the Russian Academy of Sciences, 50*, 338–347. https://doi.org/10.1134/S1062359023700231

Shaikevich, E. V., Romanov, D. A., & Zakharov, I. A. (2021). The diversity of *Wolbachia* and its effects on host reproduction in a single *Adalia bipunctata* (Coleoptera: Coccinellidae) population. *Symbiosis, 85*(2), 249–257. https://doi.org/10.1007/s13199-021-00808-x

Su, J., Dong, F., Liu, S. M., Lu, Y. H., & Zhang, J. P. (2019). Productivity of *Neoseiulus bicaudus* (Acari: Phytoseiidae) reared on natural prey, alternative prey, and artificial diet. *Journal of Economic Entomology, 112*(6), 2604–2613. https://doi.org/10.1093/jee/toz202

Szabo, A., & Penzes, B. (2013). A new method for the release of *Amblyseius andersoni* (Acari: Phytoseiidae) in young apple orchards. *European Journal of Entomology, 110*(3), 477–482. https://doi.org/10.14411/eje.2013.063

Tixier, M. S., Okassa, M., & Kreiter, S. (2012). An integrative morphological and molecular diagnostics for *Typhlodromus pyri* (Acari: Phytoseiidae). *Zoologica Scripta, 41*, 68–78. https://doi.org/10.1111/j.1463-6409.2011.00504.x

Tokarev, Y. S., Yudina, M. A., Malysh, J. M., Bykov, R. A., Frolov, A. N., Grushevaya, I. V., & Ilinsky, Y. Y. (2018). Prevalence rates of the endosymbiotic bacterium of the *Wolbachia* genus in natural populations of *Ostrinia nubilalis* and *Ostrinia scapulalis* (Lepidoptera: Pyraloidea: Crambidae) in Southwestern Russia. *Russian Journal of Genetics: Applied Research, 8*, 172–177. https://doi.org/10.1134/S2079059718020119

Toyoshima, S. (2021). A tool to survey predatory phytoseiid species against Thrips. *Japanese Journal of Applied Entomology and Zoology, 65*(4), 173–180. https://doi.org/10.1303/jjaez.2021.173

Tsolakis, H., Sinacori, M., Ragusa, E., & Lombardo, A. (2019). Biological parameters of *Neoseiulus longilaterus* (Athias-Henriot) (Parasitiformes, Phytoseiidae) fed on prey and pollen in laboratory conditions. *Systematic and Applied Acarology, 24*(9), 1757–1768. https://doi.org/10.11158/saa.24.9.12

Tuovinen, T., & Lindqvist, I. (2014). Effect of introductions of a predator complex on spider mites and thrips in a tunnel and an open field of pesticide-free everbearer strawberry. *Journal of Berry Research, 4*(4), 203–216. https://doi.org/10.3233/JBR-140081

Utkuzova, A. M., Chertkova, E. A., Kryukova, N. A., Malysh, J. M., & Tokarev, Y. S. (2025). “Hostbusters”: The bacterial endosymbiont *Wolbachia* of the parasitoid wasp *Habrobracon hebetor* improves its ability to parasitize lepidopteran hosts. *Insects, 16*(5), 464. https://doi.org/10.3390/insects16050464

Vicente dos Santos, V., & Tixier, M. S. (2017). Which molecular markers for assessing which taxonomic level? The case study of the mite family Phytoseiidae (Acari: Mesostigmata). *Cladistics, 33*(3), 251–267. https://doi.org/10.1111/cla.12166

Vogelstein, B., & Gillespie, D. (1979). Preparative and analytical purification of DNA from agarose. *Proceedings of the National Academy of Sciences USA, 76*(2), 615–619. https://doi.org/10.1073/pnas.76.2.615

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

Werren, J. H., Zhang, W., & Guo, L. R. (1995). Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proceedings of the Royal Society B: Biological Sciences, 261*, 55–63. https://doi.org/10.1098/rspb.1995.0117

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315–322). Academic Press, New York, https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wysoki, M. (1973). Further studies on karyotypes and sex determination of phytoseiid mites (Acarina: Mesostigmata). *Genetica, 44*, 139–145. https://doi.org/10.1007/BF00155967

Wysoki, M., & Bolland, H. R. (1983). Chromosome studies of phytoseiid mites (Acari: Gamasida). *International Journal of Acarology, 9*(2), 91–94. https://doi.org/10.1080/01647958308683319

Yu, C-H., Tsai, J-J., Lin, Y-H., Yu, S-J., & Liao, E-C. (2020). Identification the cross-reactive or species-specific allergens of *Tyrophagus putrescentiae* and development molecular diagnostic kits for allergic diseases. *Diagnostics, 10*, 665. https://doi.org/10.3390/diagnostics10090665

Zhang, K., Cao, J., Li, X., & Zhang, Z-Q. (2025). Investigating interspecific mating in the thelytokous predatory mite *Amblyseius herbicolus* (Chant) (Acari: Phytoseiidae), with comparative observations from three sexually reproducing phytoseiid species. *Experimental and Applied Acarology, 95*(8). https://doi.org/10.1007/s10493-025-01034-6

Zhang, K., & Zhang, Z. Q. (2021). The dried fruit mite *Carpoglyphus lactis* (Acari: Carpoglyphidae) is a suitable alternative prey for *Amblyseius herbicolus* (Acari: Phytoseiidae). *Systematic and Applied Acarology, 26*(11), 2167–2176. https://doi.org/10.11158/saa.26.11.15

Zhou, W. G., Rousset, F., & O'Neill, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. *Proceedings of the Royal Society B: Biological Sciences, 265*, 509–515. https://doi.org/10.1098/rspb.1998.0324

Zhou, Y., Klimov, P. B., Gu, X., Yu, Z., Cui, X., Li, Q., Pan, R., Yuan, C., Cai, F., & Cui, Y. (2023). Chromosome-level genomic assembly and allergome inference reveal novel allergens in *Tyrophagus putrescentiae*. *Allergy, 78*(6), 1691–1695. https://doi.org/10.1111/all.15656