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Article

New host detection of the parasitic mite, *Erythraeus pistacicus* (Trombidiformes: Erythraeidae) from Iran and indication of possible infection with bacterial symbionts

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ABSTRACT
During a survey of gall-inducing aphids on pistachio trees in Razavi-Khorasan Province, larvae of a parasitic mite were collected inside the galls of *Forda hirsuta* (Aphididae). The mite was identified as *Erythraeus* (*Erythraeus*) *pistacicus* Haitlinger, Mehrnejad & Šundić based on morphological, and molecular data. This is the first record of this mite from the host aphid, *F. hirsuta*, and also the third record of the occurrence of the mite in Iran. The survey of possible infections with bacterial symbionts in natural populations of *E.* (*E.*) *pistacicus* from Mashhad and Feizabad localities in northeastern Iran revealed the presence of three bacterial symbionts, *Wolbachia*, *Planomicrobium*, and *Cardinium* at different infection rates (55%, 30%, and 10% respectively), while *Arsenophonus*, *Rickettsia*, and *Spiroplasma* were not detected. Investigating bacterial symbionts in predatory insects/mites provide a valuable framework for better understanding the complex interactions between symbionts and their hosts and will lead to developing more efficacious biocontrol strategies particularly, those seeking to decrease reliance on chemical pesticides.

KEYWORDS: *Cardinium*, gall aphids, microbial symbionts, pistachio aphids, *Planomicrobium*, *Wolbachia*.

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INTRODUCTION

In arthropods, the presence of inherited bacterial symbionts is widespread and encompasses a wide range of diversity (Zhang *et al*. 2017; Zhu *et al*. 2018). Evidence suggests that arthropods commonly host endosymbiotic bacteria, and recent research highlights the significance of these bacterial communities residing within arthropods (Sparagano and De Luna 2008; De Luna *et al*. 2009). These symbionts that are referred to as maternal agents, affect the ecology, evolution, and reproductive biology of their hosts (Pina *et al*. 2020).

These internal microbial symbionts can play a significant role in numerous aspects of their host's ecology (Zélé *et al*. 2020; Konecka 2022). Bacterial symbionts show the potency to expand the biochemical abilities of their host, thereby facilitating their adaptation to novel ecological niches (Akman *et al*. 2002; Zientz *et al*. 2004). Among the identified symbionts of arthropods, *Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophonus*, and *Spiroplasma* are the most common bacteria (Duron *et al*. 2008; Weinert *et al*. 2015; Zélé *et al*. 2018). *Wolbachia* is distributed across various arthropod groups and is known to infect many species (Werren *et al*. 2008; Zhang *et al*. 2017). The infection frequency of this symbiotic bacteria in the host species is estimated to be approximately 52% (Weinert *et al*.

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2015; Konecka 2022). However, studies have shown variations in the infection rates of individuals within species populations (Zug and Hammerstein 2012; Konecka 2022). Cytoplasmic incompatibility, initially described in insects and mites, is the most widespread consequence of *Wolbachia* and *Cardinium* infections (Zhu *et al*. 2012; Sourassou *et al*. 2014).

The aforementioned symbionts are also recognized to manipulate their arthropod host's reproduction (Giorgini *et al*. 2009). They can induce deleterious phenotypes such as cytoplasmic incompatibility, induction of parthenogenesis, feminization, and male killing (Chaisiri *et al*. 2015). New assessments of bacterial contamination in arthropods have revealed that up to 5% of arthropods are contaminated with *Arsenophonus*, 5–10% with *Spiroplasma*, 13% with *Cardinium*, and 24% with *Rickettsia* (Mathé-Hubert *et al*. 2019; Pina *et al*. 2020).

Predatory mites, similar to other arthropod species, are closely associated with their symbiotic bacteria, which can have a variable critical effect on fitness parameters of the host (Pekas *et al*. 2017). The Erythraeidae family comprises 57 genera and over 850 species (Beron 2017). The larvae of this family typically parasitize other arthropods, while nymphs and adults are free-living predators targeting small insects (Xu *et al*. 2022). The subgenus *Erythraeus* (*Erythraeus*) Latreille consists of 46 species that have been described based on larval characteristics (Šundić *et al*. 2015). Of these, 10 species have been reported from Iran: *E*. (*E*.) *akbariani* Haitlinger & Saboori, 1996; *E*. (*E*.) *sabrinae* Haitlinger & Saboori, 1996; *E*. (*E*.) *shojaii* Saboori & Babolmorad, 2000; *E*. (*E*.) *garmsaricus* Saboori, Goldarazena & Khajeali, 2004; *E*. (*E*.) *hypertrichotus* Saboori, Goldarazena & Khajeali, 2004; *E*. (*E*.) *mirabi* Khanjani, Ueckermann & Ul-Hassan, 2007; *E*. (*E*.) *adanaensis* Saboori & Cobanoglu, 2010 (Azimi *et al*. 2010); *E*. (*E*.) *chrysoperlae* Khanjani, Mirmoayedi, Fayaz & Sharifian, 2012; *E*. (*E*.) *populi* Khanjani, Mirmoayedi, Faayaz & Sharifian, 2012; and *E*. (*E*.) *pistacicus* Haitlinger, Mehrnejad & Šundić, 2016 (Haitlinger *et al*. 2016).

Arthropod pests have posed the main problem for pistachio growers during the recent decades in Iran. Among them, the common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hem.: Psyllidae) is indigenous to Iran and currently represents the most serious pest throughout pistachio-producing regions in the country (Mehrnejad 2019). Additionally, the wrinkling aphid of pistachio leaf, *Forda hirsuta* Mordvilko (Hem.: Aphididae), is one of the pests of pistachio trees. This aphid causes the shrinkage, thickening, and changing the color of the pistachio leaves. Since the aphid feeds on the edges of leaves, the thick and rolled upward wrinkles are formed, causing a change in the green color to red. Therefore, its economic damages are not limited to direct sap feeding but also include the twisting of pistachio leaves and the decrease in photosynthesis efficiency (Sadre Mohammadi *et al*. 2007).

The mite *E*. (*E*.) *pistacicus* is one of the natural enemies of some important pistachio pests, such as the common pistachio psylla, *A. pistaciae*, and the mirid, *Farsiana pistaciae* Linnavuori (Hem.: Miridae) (Haitlinger and Mehrnejad 2017), and the galling aphid, *F. hirsuta* (this study). This mite exhibits parasitic behavior during its larval stage and functions as a predator during its nymphal and adult stages.

In this study, larval samples of this mite were frequently collected from the aphid *F. hirsuta*, commonly found on cultivated pistachio trees. Considering the importance of applying bio-control agents in integrated pest management programs, achievement of detailed knowledge on these agents, especially the factors that affect their efficiency are necessary.

This work aims to: a) report a novel association between the mite, *E*. (*E*.) *pistacicus* and the wrinkling aphid of pistachio leaf, *F. hirsuta* and b) to contribute to the knowledge of bacterial symbionts associated with the larval stage of the Parasitengona mites.

MATERIAL AND METHODS

Sampling

During a survey of gall-inducing aphids on pistachio trees in Razavi-Khorasan Province

(northeastern Iran), larvae of a parasitic mite were collected from galls of *F. hirsuta* on the cultivated pistachio trees (Table 1). From April to October 2022, mite samples were collected on a weekly basis and preserved in 97% ethanol for subsequent morphological and molecular studies. Out of 229 collected mites, 200 were selected for molecular analysis and 24 for morphological examination via microscopic slide preparation.

Table 1. Collected mite populations of *Erythraeus* (*Erythraeus*) *pistacicus* from Mashhad and Feizabad, Northeast of Iran.

Locality	Population code	Presence of the mite	Number of mites collected	Longitude and latitude
Fariman	FR	-	0	35° 42' 28" N, 59° 51' 10" E
Feizabad	FZ	+	107	35° 43' 42" N, 61° 04' 29" E
Kalat	KL	-	0	36° 35' 36" N, 60° 22' 38" E
Mashhad	MS	+	122	36° 12' 42" N, 59° 54' 12" E
Neyshabour	NS	-	0	36° 12' 51" N, 58° 47' 46" E
Sarakhs	SR	-	0	36° 31' 23" N, 61° 03' 40" E

Morphological identification the mite

The mite samples were first clarified using lactic acid for one week at room temperature, and then slide-mounted in Heinz's medium (Krantz and Walter 2009). Determining the identity of the collected mite predator was done by Dr. Javad Noei (Department of Plant Protection, Faculty of Agriculture, University of Birjand, Birjand, Iran). The voucher specimens are deposited in the Insect Museum of Ferdowsi University of Mashhad, Mashhad, Iran.

Molecular identification of the mite (DNA extraction and barcoding)

The Tissue Genomic DNA Extraction mini-Kit (Yekta Taghiz Azma, Tehran, Iran) was used to extract total DNA from 10 individual mites of each population, following the manufacturer's instruction. Prior to extracting DNA from the parasitic mites, they were starved for 12 hours to allow voiding of the gut content to reduce the likelihood of false positive results due to symbiotic associates of undigested prey within the digestive tract. For DNA extraction, mites were subjected to mechanical grinding utilizing a plastic pestle. The quantity and quality of the extracted DNA were assessed using a Thermo NanoDrop 1000 (Thermo Scientific, Finland) and confirmed by visualization on a 1% agarose gel. The DNA samples were then stored at −20 °C until PCR processing.

To perform molecular identification, the primer pair of LCO1490 (5′-GGTCA ACAAA TCATA AAGAT ATTGG-3′) and HCO2198 (5′-TAAAC TTCAG GGTGA CCAAA AAATCA-3′) were used to amplify the barcode region of *COI* gene (Folmer *et al*. 1994). The PCR cycling conditions consisted of an initial denaturation step at 94 °C for 3 minutes, followed by 35 cycles: 30 seconds at 94 °C, 40 seconds at 52 °C, and 30 seconds at 72 °C. Finally, there was a 10-minute extension step at 72 °C. The PCR product was sequenced by Macrogen Corporation (Seoul, South Korea). The forward and reverse sequences were then assembled using Geneious version 2019. To assess sequence similarities, the BLAST program of the GenBank database was applied.

Surveying the bacterial DNA

In order to screen the mite probable symbiont bacteria, specific primers were used to amplify the targeted regions of the bacterial genes according to the references depicted in Table 2. However, it should be noted that the presence of bacteria identified through DNA sequencing does not determine whether these bacteria are inherent symbionts of the mite or if they are merely ingested bacteria acquired from other host materials. PCR reactions were carried out in a total volume of 25 µl, consisting of 12.5 µl buffer mix and 1 µl each of forward and reverse primers (10 pmol μl^{-1}). The

PCR was conducted in an Eppendorf thermocycler (Biometra, Germany) following the conditions outlined in Table 3. A negative control with no DNA was included in each PCR run. Amplification products were analyzed by electrophoresis on 1% agarose gels. The PCR products were sequenced by Macrogen (Seoul, South Korea), and their homology were ascertained via NCBI database.

Table 2. PCR primers used to identify the bacterial symbionts in *Erythraeus* (*Erythraeus*) *pistacicus* populations from Mashhad and Feizabad, Northeast of Iran.

Target symbiont	Primer name	Primersequence (5′-3′)	Product size	Reference
Arsenophonus	Ars23S-1 Ars23S-2	CGTTTGATGAATTCATAGTCAAA GGTCCTCCAGTTAGTGTTACCCAAC	550 bp	Thao and Baumann (2004)
Cardinium	Car-SPF Car-SPR	CGGCTTATTAAGTCAGTTGTGAAATCCTAG TCCTTCCTCCCGCTTACACG	544 bp	Nakamura *et al*. (2009)
Planomicrobium	63F-CG TKSSspR	GCCTAATACATGCAAGTCGAACGG TAGCCGTGGCTTTCTGGTAA	450 bp	Mateos *et al*. (2006)
Rickettsia	16SA1 Rick16SR	AGAGTTTGATCMTGGCTC CATCCATCAGCGATAAATCTT	200 bp	Fukatsu and Nikoh (1998)
Spiroplasma	63F-CG TKSSspR	GCCTAATACATGCAAGTCGAACGG TAGCCGTGGCTTTCTGGTAA	450 bp	Fukatsu and Nikoh (2000)
Wolbachia	81-F 691-R	TGGTCCAATAAGTGAAGAAAC AAAAATTAAACGCTACTCCA	600 bp	Zhou *et al*. (1998)

Table 3. PCR conditions to detect bacterial symbionts in *Erythraeus* (*Erythraeus*) *pistacicus*.

Symbiont	Primer name	No. cycle	Denaturation		Annealing		Elongation	
			°C	S	°C	S	°C	S
Arsenophonus	Ars23S-1/Ars23S-2	35	95	30	60	30	72	45
Cardinium	Car-SPF/Car-SPR	35	95	30	57	30	72	60
Planomicrobium	63F-CG/TKSSspR	35	95	30	60	30	72	60
Rickettsia	16SA1/Rick16SR	35	94	60	60	60	72	120
Spiroplasma	63F-CG/TKSSspR	35	95	30	60	30	72	60
Wolbachia	81-F/691-R	35	94	40	57	40	72	60

Phylogenetic analyses

The obtained sequences along with the representative sequences of symbiotic bacteria retrieved from GenBank were aligned using ClustalW in MEGA X (Kumar *et al*. 2018). The phylogenetic tree was generated with Neighbor joining method (NJ) with 1000 bootstrap replicates using MEGA X software. The haplotypes of the symbionts were calculated using DnaSP v6 software (Rozas *et al*. 2017). The PopART software (Leigh and Bryant 2015) was used to build median joining (MJ) network.

Data availability

All new sequences have been deposited in GenBank under the accession number of OR946448 for *Wolbachia*, OR916427 for *Cardinium* and OR916428 for *Planomicrobium*. The COI sequence of *E.* (*E.*) *pistacicus* was deposited in GenBank under the accession number PP266980.

RESULTS

Morphological and molecular identification of the Parasitengona mite

From April to October 2022, various regions of Razavi-Khorasan province, especially Faizabad and Mashhad, were sampled, and it was found that *F. hirsuta* was the most common galling aphid species on pistachio trees in northeastern Iran. Notably, from mid of April to July, larvae of the parasitic mite, *E.* (*E.*) *pistacicus*, were detected within the galls, actively feeding on fundatrix aphids (Fig. 1). This discovery marks the first recorded instance of *F. hirsuta* serving as a host for this parasitic mite species. The peak activity of mites coincided from mid-May to early July. Approximately 7% of *F. hirsuta* galls were occupied by the mite, with an average of 1–3 mites found within each gall. Typically, the mite was observed attached to the abdominal part of the aphid, where it engaged in feeding activities.

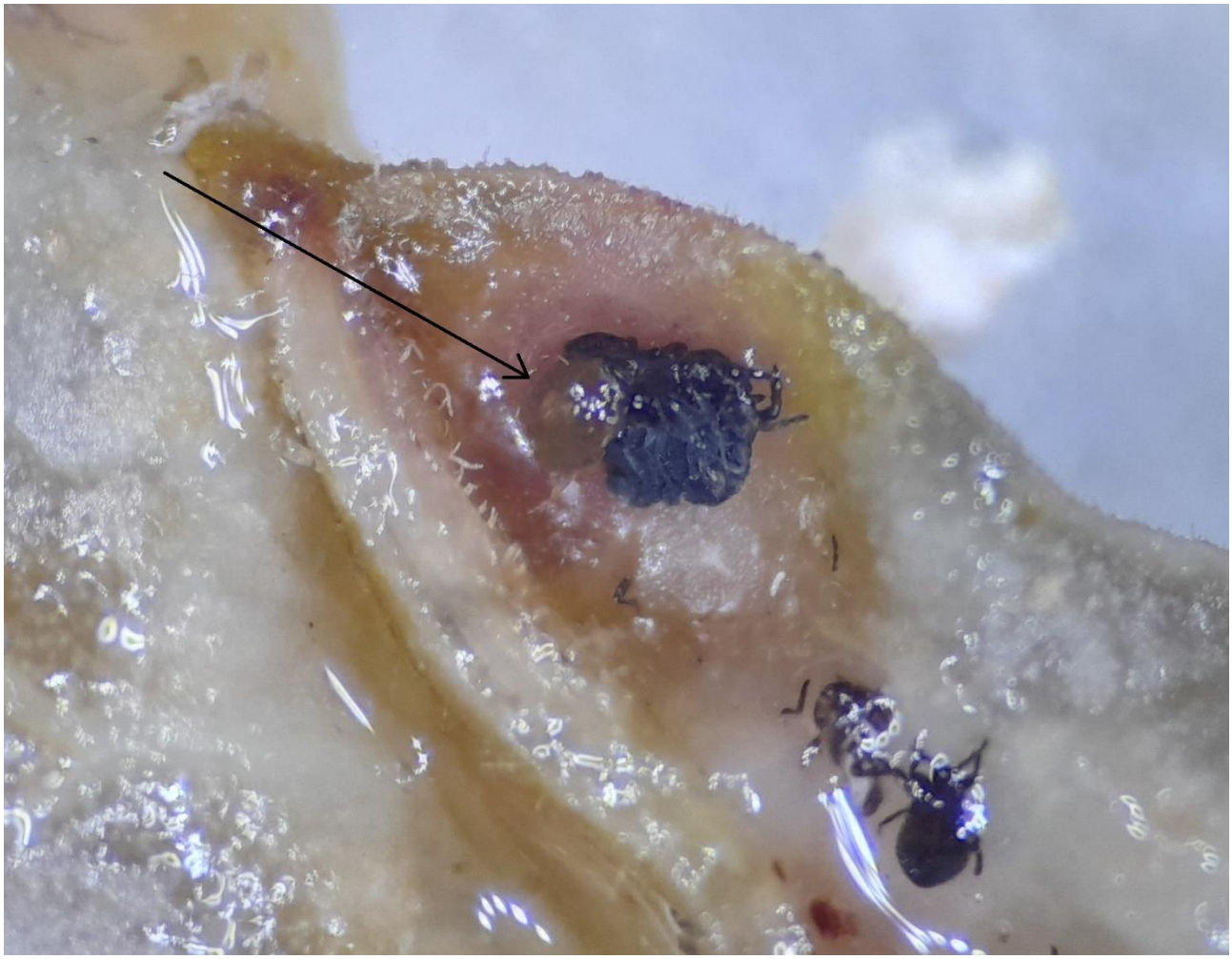

Figure 1. *Erythraeus* (*Erythraeus*) *pistacicus* Haitlinger, Mehrnejad & Šundić, 2016 larva (Black arrow) inside the gall, feeding on the aphid, *Forda hirsuta* Mordvilko, 1928, on pistachio trees. June 2022, Mashhad, Northeast of Iran.

In terms of morphological identification, our specimens were similar to the description of the mite by Haitlinger *et al*. (2016). Although, the formula of the legs in the text does not match the figures (for example, the formula of tibia of the second leg in the text is 2φ, 2ζ, 15n, while the eupatidia (ζ) is only on the tarsus, but in original paper it is mentioned on tibia. Despite this, on their drawings (Fig. 7) eupathidia are shown on their correct position. Therefore, some differences in the following features should be considered: fPp = 0-B-B-BBB$_2$-6NωζCp; Leg I: Ta – 1ω, 1ε, 2ζ, 1Cp,

26n; Ti – 2φ, 1κ, 15n; Ge – 1σ, 1κ, 8n; TFe – 5n; BFe – 3n; Tr – 1n; Cx – 1. Leg II: Ta – 1ω, 2ζ, 1Cp, 22–23n; Ti – 2φ, 15n; Ge – 8n, 1κ; TFe – 5n; BFe – 3n; Tr – 1n; Cx – 1. Leg III: Ta – 1ζ, 24n; Ti – 1φ, 15n; Ge – 8n; TFe – 5n; BFe – 3n; Tr– 1n; Cx – 1 (Noei, Personal communication).

We amplified approximately 604 bp of the COI gene from the parasitic mite. A BLAST search in the GenBank database showed 92.38% similarity with *Erythraeus garmsaricus* (OQ052990). A COI Neighbor-joining tree of the present study and the Genbank sequence data is presented in Figure 2. The NJ tree grouped our sequence with those of Erythraeidae family from Genbank with high bootstrap value (78). The tree confirmed morphological and nblast results about species determination as well.

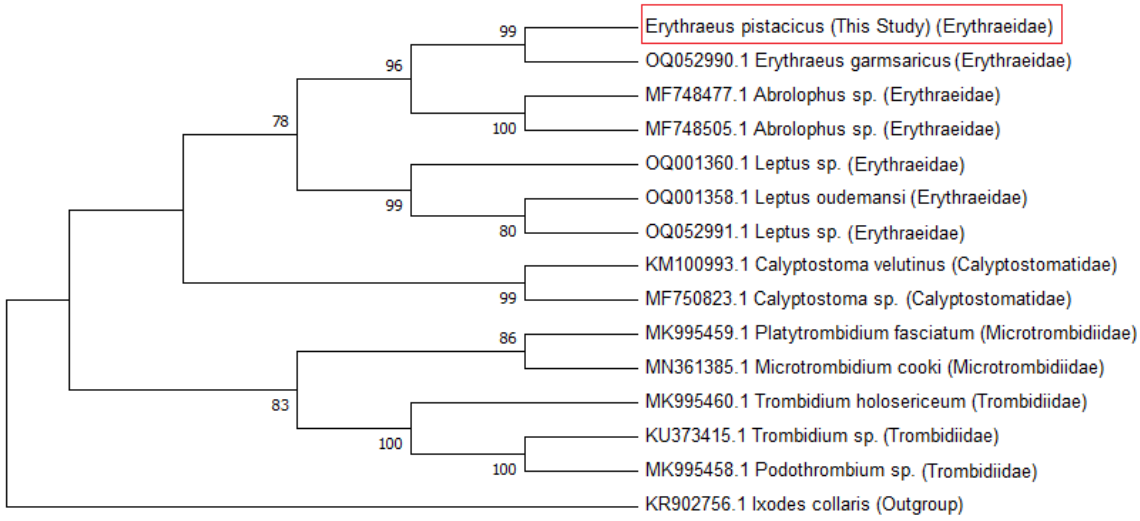

Figure 2. Neighbor-joining tree of COI sequences of the Erythraeidae mites of the present study and the Genbank sequence data. Numbers above/below nodes represent bootstrap values.

Detection of bacterial DNA

The DNA of two secondary endosymbionts including *Wolbachia* sp. (Alphaproteobacteria) and *Cardinium* sp. (Bacteroidota (syn.: Bacteroidetes)) and a probable gut fauna symbiont, *Planomicrobium* sp. (formerly *Planococcus* sp.) (Bacillota) were detected in the populations of *E.* (*E.*) *pistacicus* examined in the current study (Table 4).

Table 4. Prevalence of bacterial symbionts in two *Erythraeus* (*Erythraeus*) *pistacicus* populations from Mashhad and Feizabad, northeast of Iran.

Bacterial symbionts	Population code	
	Feizabad	Mashhad
Arsenophonus	–	–
Cardinium	+	–
Planomicrobium	+	+
Rickettsia	–	–
Spiroplasma	–	–
Wolbachia	+	+

The Mashhad and Feizabad populations were PCR-positive for the *Wolbachia* surface protein (*wsp*) gene. The phylogenetic tree based on *wsp* gene for *Wolbachia* symbiont (Fig. 3) indicated that

the symbiont identified in the current study did not form a monophyletic group with other *wsp* gene sequences for *Wolbachia* present in Genbank. In other words, there was not a clear differentiation with high bootstrap support between the sequence of this study and other GenBank sequences (Fig. 3). The *Wolbachia* sequence of our study was relatively closely related to the ones of a *Tetranychus* spp. and a lycaenid species, as depicted in Fig. 3.

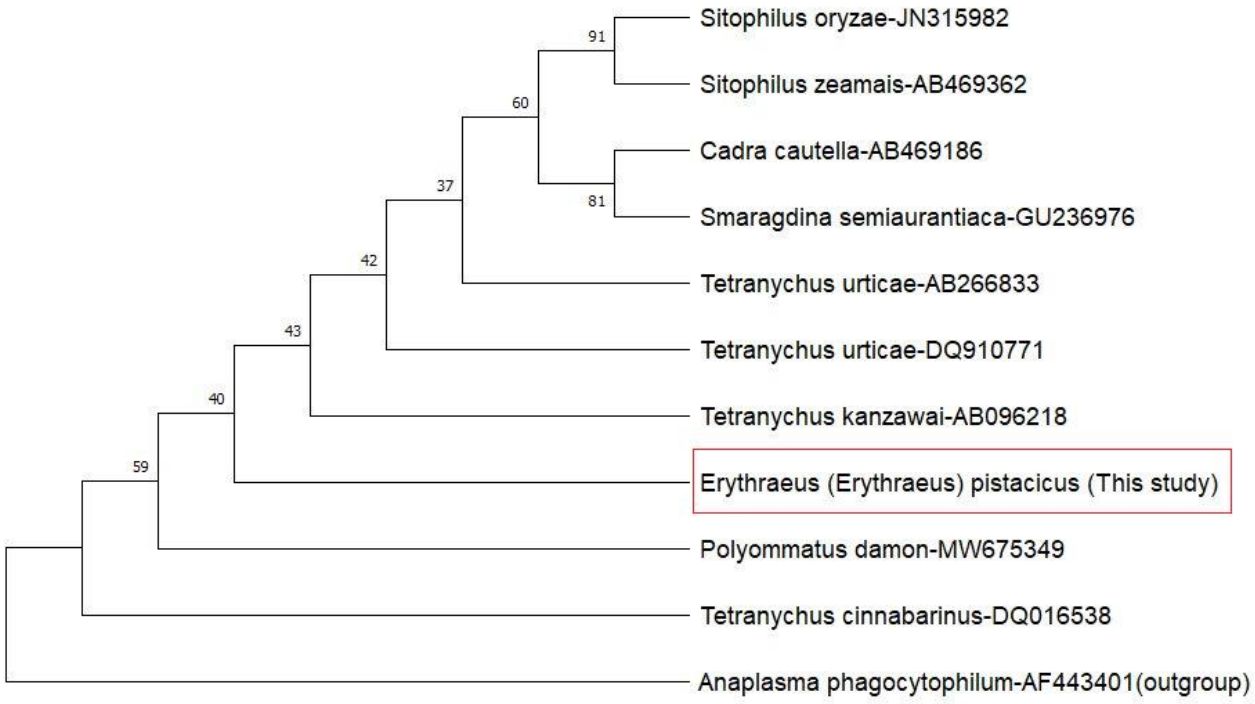

Figure 3. Phylogenetic tree based on *wsp* sequences of *Wolbachia*, constructed by a neighbor-joining procedure. *Wolbachia* strains are depicted by the host name. The accession numbers are shown after the host name. Numbers on the nodes indicate bootstrap percent confidence values.

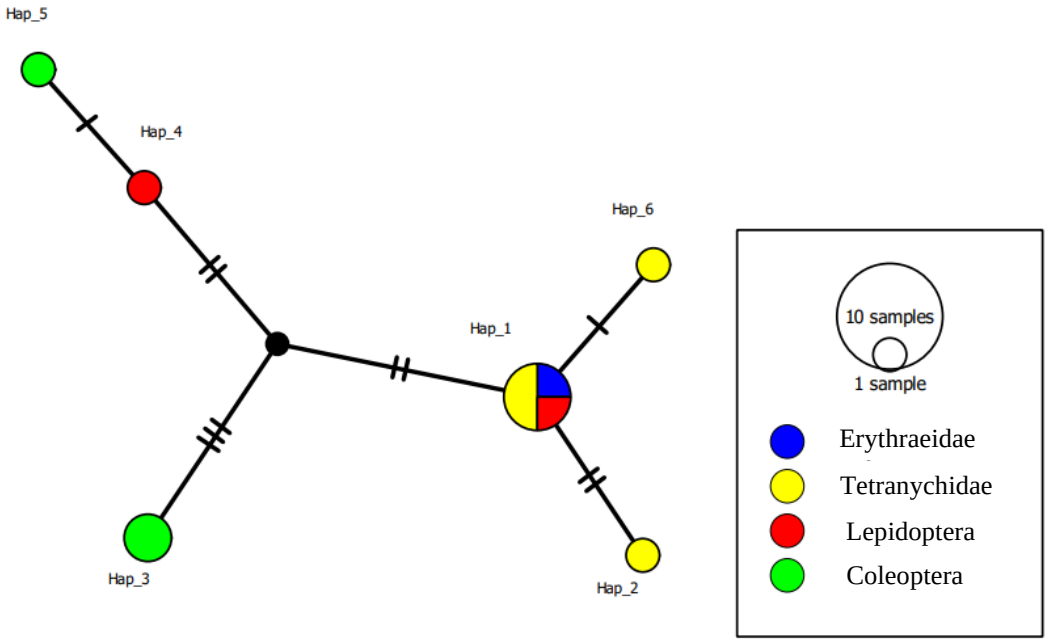

Figure 4. Haplotype network of *Wolbachia* endosymbionts based on *wsp* gene.

Primer pairs targeting a highly conserved region of 16S rDNA in *Cardinium* genus amplified a 455 bp fragment and were independently analyzed with other similar sequences related to different mites. According to tree inference (Fig. 5), the sequence of *Cardinium* sp. endosymbiont of *E.* (*E.*) *pistacicus* has been found to be associated with predatory mites of pest storage (*Cheyletus eruditus* (Schrank)) and two genera of honeybee parasitic mites (*Carpoglyphus* and *Aeroglyphus*).

The 16S rDNA fragment contained 43 variable sites that delineated 18 haplotypes (Fig. 6). The most common haplotype (haplotype 1) is shared in three specimens and composed of specimens related to three mite families namely Erythraeidae (this study), Cheyletidae and Carpoglyphidae. Haplotypes 5, 7, 14 and 16, each is composed of 2 specimens. Thirteen haplotypes are unique, composed of only one specimen. The haplotype network topology mirrors the topology of the phylogenetic tree (Figs. 5–6).

The presence of *Planomicrobium* sp. was confirmed in both studied populations using the primer pairs related to the gram-positive bacteria. As no data was available in GenBank on the isolates of *Planomicrobium* sp. derived from insect sources, the phylogenetic tree was drawn based on the 16S rDNA sequences of the bacterial reference strains (Fig. 7). The tree indicated that the *Planomicrobium* sequence of this study clustered with environmental *Planomicrobium* sp. (MK422467). The haplotype network has the same topology with the NJ tree that *Planomicrobium* sequence of the current study forms a unique haplotype (Figs. 7–8).

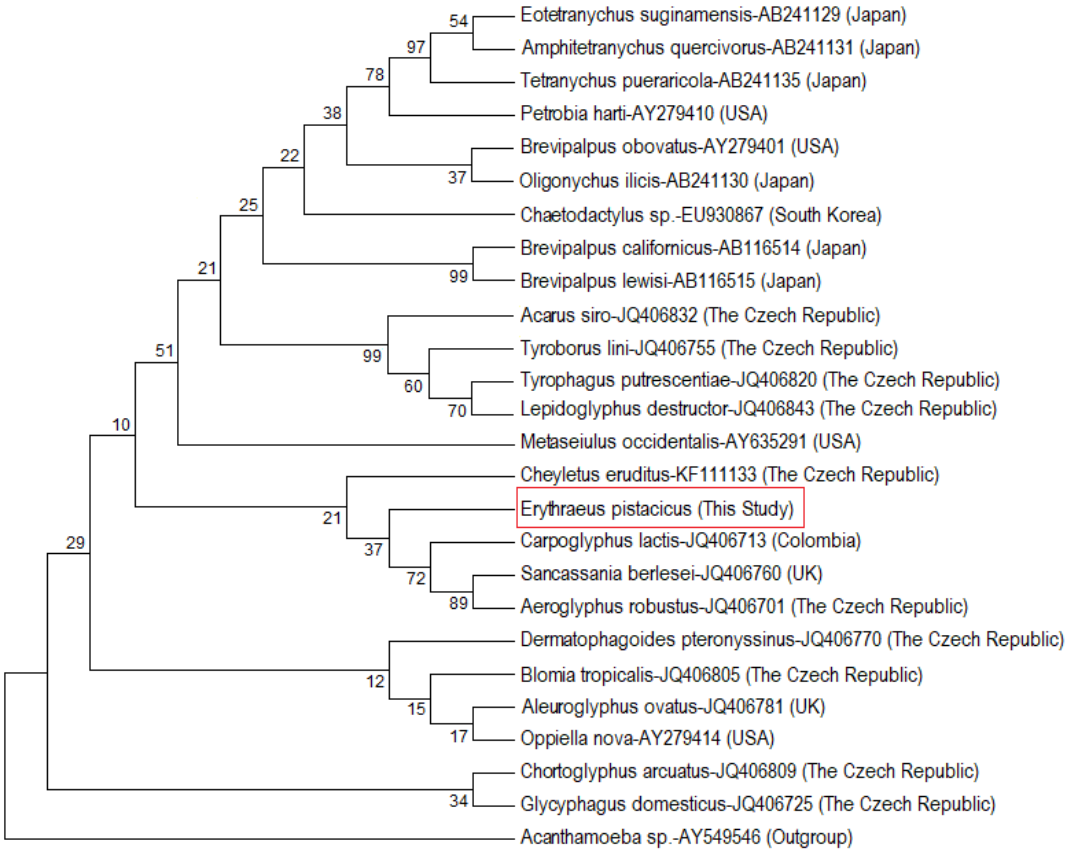

Figure 5. Phylogenetic tree based on 16S rRNA sequences of *Cardinium*, constructed by a neighbor-joining procedure. *Cardinium* strains are depicted by the host name. The accession numbers are shown after the host name. Numbers on the nodes indicate bootstrap percent confidence values.

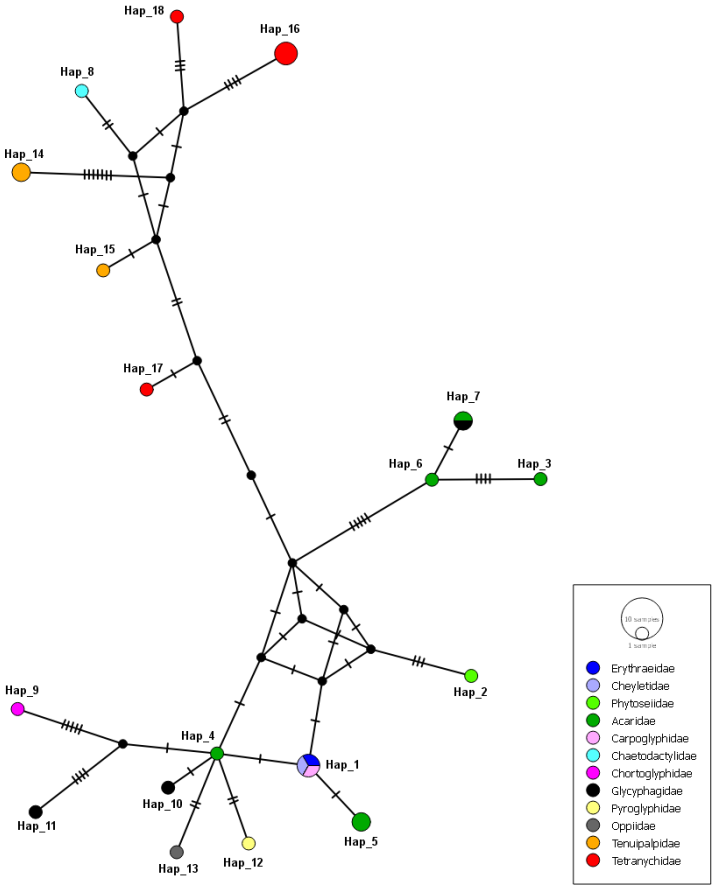

Figure 6. Haplotype network of *Cardinium* endosymbionts based on 16S rDNA sequences.

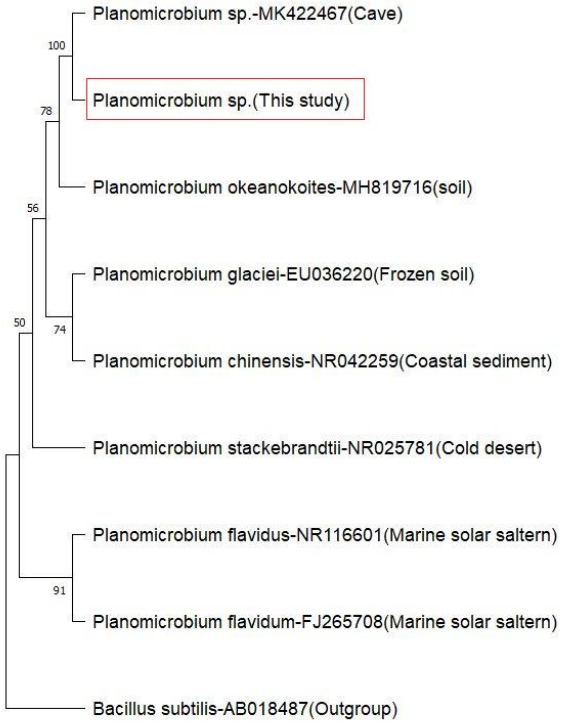

Figure 7. Phylogenetic relationship of *Planomicrobium* symbiont identified from *Erythraeus* (*Erythraeus*) *pistacicus* with related sequences retrieved from GenBank. The tree was constructed using neighbor-joining procedure. The sequence obtained from *E.* (*E.*) *pistacicus* in this study is in red box. Sequence from *Bacillus subtilis* was used as an outgroup.

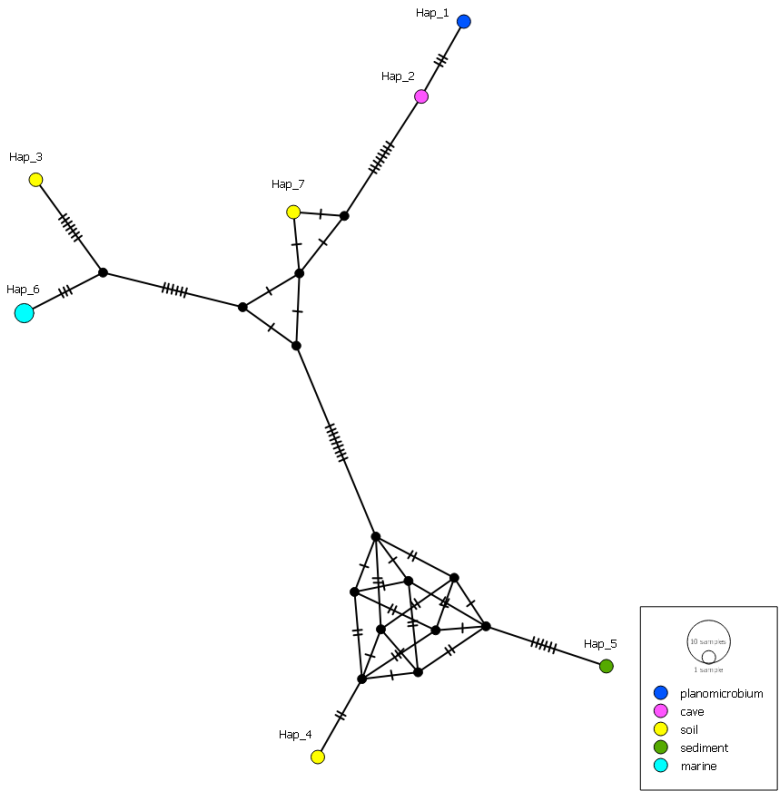

Figure 8. Haplotype network of *Planomicrobium* spp. based on 16S rDNA sequences.

Although all samples were screened for the presence of *Arsenophonus*, *Rickettsia*, and *Spiroplasma* genera, no evidence of these endosymbionts was detected in the studied populations.

DISCUSSION

In the current study, for the first time, *F. hirsuta* is documented as a new host for the parasitic mite, *E*. (*E*.) *pistacicus*. This erythraeid mite species was found within the galls of *F. hirsuta* on cultivated pistachio trees. The aforementioned parasitic mite was previously recorded feeding on other hosts including *A. pistaciae* (Psyllidae) and *Farsiana pistaciae* (Miridae) (Haitlinger and Mehrnejad 2017) on wild pistachio trees in mountainous areas.

The microbial diversity of various populations of *E*. (*E*.) *pistacicus* was also examined in our study by 16S rRNA sequencing. The bacterial symbionts may have a notable effect on the practical use of a predator in biocontrol programs of pests (Machtelinckx *et al*. 2009). The occurrence and role of endosymbiont bacteria are well studied in some groups of biocontrol insects such as hymenopterous parasitoids and coccinellid predators (Floate *et al*. 2006). Nonetheless, relatively few studies have focused on the endosymbiotic bacteria of predatory mites, despite their significant role in biocontrol of various insect pests (Sourassou *et al*. 2014; Pekas *et al*. 2017).

This study confirms the presence of DNA of *Wolbachia* endosymbiont in all the examined populations of *E*. (*E*.) *pistacicus* with different infection rates. The average infection rate for *Wolbachia* was 55% obtained in 11 out of 20 tested individuals. The highest infection rate was recorded in Mashhad population (MS) by 70%. The Feizabad population (FZ) exhibited the infection rate of 40%. The characterized endosymbiont *Cardinium* sp. was only present in a few *E*. (*E*.) *pistacicus* individuals in the Feizabad population with an infection rate of 20%. The third genus,

Planomicrobium sp., was also recognized in both evaluated populations (FZ: 20% and MS: 40%) (Table 5).

Table 5. Detection frequencies of symbionts in two populations of *Erythraeus* (*Erythraeus*) *pistacicus* from Mashhad and Feizabad, Northeast of Iran.

Population code	Symbionts (N_f/N_t)					
	Arsenophonus	***Cardinium***	***Planomicrobium***	***Rickettsia***	***Spiroplasma***	***Wolbachia***
FZ	0/10	2/10	2/10	0/10	0/10	7/10
MS	0/10	0/10	4/10	0/10	0/10	4/10

N_f: The number of infected individuals; N_t: The total number of individuals sampled.

The bacteria *Wolbachia*, *Cardinium* and *Planomicrobium* were detected in two out of six studied populations of *E.* (*E.*) *pistacicus*. Despite detecting some endosymbionts in the parasitic mite, it should be noted that ensuring that gut bacteria belong specifically to the parasitic mite and not its host is a complex task. Their intricate ecological interactions and unique biological features might contribute to this complexity. Parasitengona mites harbor diverse gut microbial communities. These communities are influenced by factors such as host diet, habitat, and evolutionary history (Zheng *et al.* 2021; Huerta-García and Álvarez-Cervantes 2024). Furthermore, the highlighted complexity could partially be related to the nutritional uptake ratio in relation to the original body volume or weight of the parasitic mite. During the parasitic phase, the mite's body volume and weight change significantly. The ratio of nutritional uptake to original body volume/weight may impact the composition of the mite bacteria. Moreover, the complexity might indeed be related to the life cycle of the Parasitengona mite. The calyptostatic protonymph plays a pivotal role in the start of digestion of nutritional resources acquired during the parasitic phase and re-organization of tissues and structures during the ontogenetic development of the heteromorphic active post-larval instars.

The bacteria detected in our study may belong to the parasitic mite or originated from its prey. While complete assurance is challenging, there are some strategies that can be implemented in future researches to address this issue: 1) Include control groups where mites are reared under specific conditions (e.g., sterile diets) to assess baseline microbial communities. However, this will be rather difficult in case of *E.* (*E.*) *pistacicus* hosts, firstly because only few antibiotics act against *Wolbachia*, secondly because *E.* (*E.*) *pistacicus* larvae will not parasitize dead hosts and additionally, the host specificity of *E.* (*E.*) *pistacicus* remains uncertain at present; 2) Compare the gut microbiota of mites fed on different diets including their natural prey and also hosts with and without specific symbiont infections. However, it should be noted that extracting gut contents from mite larvae without risking cross-contamination by mite tissues presents a significant challenge. A more feasible approach would involve rearing the mite to its next mobile stage (deutonymph). It would also be helpful to have molecular sequencing from the mite and its host in parallel – in case *Wolbachia* is not present in the host of a particular *E.* (*E.*) *pistacicus* larva which is positive for *Wolbachia*, this would strongly support the hypothesis of *Wolbachia* presence in *E.* (*E.*) *pistacicus* populations. The widespread presence of *Wolbachia* sp. in arthropods is known according to different studies (Breeuwer and Jacobs 1996; Jeyaprakash and Hoy 2000; Kittayapong *et al*. 2003; Zchori-Fein and Perlman 2004; Xie *et al*. 2006). Although *Wolbachia* have been detected in some species of spider and predatory mites (Breeuwer and Jacobs 1996; Gotoh *et al*. 2003), for many species of Acari, there has not been conducted any survey for *Wolbachia*.

Cardinium has been found only in 13% of hitherto tested arthropod species, and it seems to be less widespread compared to *Wolbachia* (Weinert *et al*. 2015). Nonetheless, the presence of *Cardinium* genus in mites has been reported with high prevalence (e.g., 41% and 31% of screened species of spider mites and Opiliones) (Nakamura *et al*. 2009; Chang *et al*. 2010). It has been

demonstrated that *Cardinium* infection can increase the fecundity rate in some predatory mite species (Weeks and Stouthamer 2004).

In this study, *Planomicrobium* sp. was isolated from *E.* (*E.*) *pistacicus* specimens. The genus *Planomicrobium* is composed of aerobic, motile, gram-positive to gram-variable rod- to coccus-shaped bacterial strains. *Planomicrobium* consists of eleven species and has been reported from different natural habitats such as fermented seafood, marine mud, salt lakes, cold desert soils, intertidal sediments, and macroalgae (Saini *et al*. 2023). Among insects, *Planomicrobium* sp. has so far only been detected from the gut of *Dastarcus helophoroides* adults (Col.: Bothrideridae) (Wang *et al*. 2014). It is noteworthy to mention that certain symbionts, notably *Wolbachia* and *Cardinium*, exert considerable influence on the biology of their host organisms. In contrast, bacteria like *Planomicrobium* have been identified as potential constituents of the host intestinal fauna. However, the functional significance of *Planomicrobium* and its potential benefits to the host organism remain largely unexplored and merit thorough examination. It should be noted that the preservation method using ethanol for the available specimens proved insufficient to provide definitive insights into the biology of bacterial symbionts associated with this mite, so this aspect remains open to future investigation and research endeavors.

In a study similar to ours, DiBlasi *et al*. (2011) investigated *Leptus* mites belonging to the Erythraeidae family. They observed the presence of DNA of *Spiroplasma* 16S rRNA in populations of two parasitic *Leptus* mites, *Leptus sayi* Southcott, and *Leptus lomani* (Oudemans). The study found that 15.4% of *L. sayi* populations and 14.3% of *L. lomani* populations tested positive for *Spiroplasma*. In contrast, our investigation of *E.* (*E.*) *pistacicus* mites did not reveal any contamination with *Spiroplasma*.

Infection by multiple symbionts is relatively common in arthropods (Duron *et al*. 2008). Co-infections of *Wolbachia* and *Cardinium* in the same host mite species has been demonstrated in several previous studies (Ros and Breeuwer 2009; Ros *et al*. 2012; Xie *et al*. 2016; Sakamoto *et al*. 2019). The presence of *Wolbachia* and *Cardinium* in the Feizabad population might be such a co-infection in this population of *E.* (*E.*) *pistacicus*. Nonetheless, the possibility of co-infection in only its hosts should not be neglected (Ren *et al*. 2020). Although *Wolbachia* and *Cardinium* can both manipulate their host reproduction, fairly little information is available on their impacts and interactions on their host (Xie *et al*. 2016). Although our study had a relatively small sample size, our finding indicated a higher detection rate of *Wolbachia* (55%) compared with *Cardinium* (10%) in the studied mite populations. This finding is consistent with the results of Zchori-Fein and Perlman (2004) and Weeks *et al*. (2003), who found that *Wolbachia* (24% and 22%, respectively) was more widespread than *Cardinium* (6% and 7.2%, respectively) in tested insect and mite species.

More detailed studies are required to improve our understanding of infection dynamics and the fundamental factors determining symbiont frequencies in mites and elucidate their role in genetic differentiation/reproductive isolation between various populations.

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شناسایی میزبان جدید کنهٔ انگل *Erythraeus pistacicus* (Trombidiformes: Erythraeidae) از ایران و بررسی آلودگی احتمالی آن با باکتری‌های همزیست

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چکیده

طی بررسی شته‌های گال‌زا روی درختان پسته استان خراسان رضوی، لارو یک کنهٔ انگل از داخل گال شتهٔ *Forda hirsuta* (Aphididae) جمع‌آوری شد. این کنه بر اساس داده‌های ریخت‌شناختی و مولکولی به‌عنوان کنهٔ *Erythraeus* (*Erythraeus*) *pistacicus* Haitlinger, Mehrnejad & Šundić شناسایی شد. این نخستین گزارش کنهٔ انگل از شتهٔ میزبان *F. hirsuta* و هم‌چنین سومین گزارش از وقوع این کنه در ایران است. بررسی آلودگی‌های احتمالی با همزیست‌های باکتریایی در جمعیت‌های طبیعی *E.* (*E.*) *pistacicus* از مناطق مشهد و فیض‌آباد در شمال شرق ایران، حضور سه همزیست باکتریایی *Wolbachia*، *Planomicrobium* و *Cardinium* را با میزان آلودگی متفاوت (به‌ترتیب ٪۵۵، ٪۳۰ و ٪۱۰) نشان داد، در حالی‌که حضور باکتری‌های همزیست *Arsenophonus*، *Rickettsia* و *Spiroplasma* در این کنه تأیید نشد. بررسی همزیست‌های باکتریایی در حشرات شکارگر و کنه‌ها می‌تواند چارچوب ارزشمندی برای درک بهتر فعل و انفعالات پیچیده بین همزیست‌ها و میزبان‌های‌شان فراهم کند و منجر به توسعهٔ راهبردهای مهار زیستی مؤثرتر به‌ویژه برای کسانی شود که به دنبال کاهش اتکا به آفت‌کش‌های شیمیایی هستند.

واژگان کلیدی: *Cardinium*، شته‌های گال‌زا، همزیست‌های میکروبی، شتهٔ پسته، *Planomicrobium*، *Wolbachia*.

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