Original Article

Morphological Aberrations of the Dirofilariasis, Sindbis Fever and West Nile Fever Vector *Culex Theileri* (Diptera: Culicidae) in Iran

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Abstract

Background: *Culex theileri* (Diptera: Culicidae) is a known vector of pathogens that cause dirofilariasis, Sindbis fever and West Nile fever in Iran. The species is one of the country's most abundant and widely distributed species.
Methods: In order to conduct a faunistic and bionomic study of mosquitoes, larval collection was carried out in Chahar Mahal and Bakhtiari Province of western Iran using dipping method.
Results: In total, some 2096 larvae of *Cx. theileri* were identified. Among the larvae, 1024 (48.9%) displayed aberrations and anomalies in the development of cranial seta 1-C (preclypeal seta). Morphological aberrations and anomalies of seta 1-C in the third- and fourth-instar larvae and abdominal terga of the adults of *Cx. theileri* collected in Iran are discussed and illustrated.
Conclusion: More studies are needed throughout the distribution of *Cx. theileri* to analyze the variation of the species, especially using both morphological and molecular data.

Keywords: Anomalous; Abnormal; Character; Morphology; Variation

Introduction

The genus *Culex* Linnaeus (Diptera: Culicidae) currently includes 779 species comprising 26 subgenera (1). The genus consists of at least 34 species of seven subgenera in southwestern Asia (2) and 19 species representing five subgenera in Iran (3). The genus comprises several important vectors of pathogens that cause infections in humans and domesticated animals (3). *Culex theileri* Theobald belongs to the Theileri Subgroup of the Pipiens Group of *Culex* (*Culex*) (2). The species is found in the Afrotropical, Palearctic and Oriental Regions; however, it is mainly a southern Palearctic species (2).

Culex theileri is a known vector of avian *Plasmodium* in Portugal (4), Turkey (5) and Spain (6); avian *Haemoproteus* in Spain (6); *Dirofilaria immitis* (canine heart worm) in Portugal (7), Iran (8) and Canary Islands of Spain (9); West Nile virus in South Africa (10) and Iran (11); Sindbis virus in South Africa (10, 12) and Iran (13); Japanese encephalitis virus in China (14); and Rift Valley fever virus in South Africa (15, 16). Additionally, some insect-specific flaviviruses (ISFs) were isolated from this species in Myanmar (17), Portugal (18, 19), Spain (18) and Turkey (20) that are designated *Culex theileri* flavivirus (CxthFV or CTFV). Also, the *Wolbachia pipientis* endosymbiont has been found in *Cx. theileri* in Portugal (21) and Iran (22).

Lewis (23) noted some variation in the num-

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ber of the branches of setae 5,6-C (inner and median frontal setae) in *Cx. theileri* larvae in Sudan. Harbach (2) recorded the morphological variation of the larval and pupal chaetotaxy and gynandromorphism has been reported by Bedford (24) and Sanchez-Murillo et al. (25). Azari-Hamidian et al. (8) showed molecular variation of the barcode region of the cytochrome c oxidase I (COI) gene in the species for the first time in Iran. Demirci et al. (26, 27) studied genetic and morphometric variation in *Cx. theileri* in Turkey. Azari-Hamidian and Harbach (28) observed one abnormal seta 1-C (preclypeal seta) in a larva of *Cx. theileri* collected in Kurdistan Province of Iran.

Culex theileri is widely distributed in Iran, being found in 28 of the 31 provinces of the country (8, 29–32). The species is one of the most abundant species in the country (33, 34). The larvae occur in a wide range of aquatic habitats (29, 33).

During a faunal study of mosquitoes in Chahar Mahal and Bakhtiari Province, *Cx. theileri* was the most widely distributed and abundant species collected (33, 35). Among the larvae collected, 1024 specimens displayed various aberrations and anomalies in the cranial chaetotaxy. Also, variation in the abdominal terga of adults was observed in different areas of Iran. The present paper illustrates these anomalies, aberrations and variations.

Materials and Methods

The larval collection was carried out in Chahar Mahal and Bakhtiari Province using dipping method. Larvae were temporarily preserved in lactophenol solution and later mounted on microscope slides using Berlese's medium. Larvae and adults were identified using the keys of Azari-Hamidian and Harbach (36). The morphological terminology of Harbach and Knight (37, 38), revised and updated in the Mosquito Taxonomic Inventory (1), is used. Photographs were taken by the authors. Voucher specimens were deposited in the Museum of Medical and Veterinary Entomology, School of Health, Guilan University of Medical Sciences and the Museum of Medical Entomology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord. Azari-Hamidian and Harbach (28) could be consulted for the definitions of aberration, abnormally, anomaly and variation. A brief explanation of the terms, which were used for anomalies and aberrations, is listed in Table 1.

Results

A total of 2096 larvae of *Cx. theileri* (25.1 % of all mosquito larvae collected) were identified in collections made in Chahar Mahal and Bakhtiari Province. Among the larvae, 1024 (48.9%) displayed aberrations and anomalies in the development of cranial seta 1-C whereas 1072 (51.1%) had normally spiniform seta 1-C (Fig. 1). Six larvae (0.28%) had one anomalous additional seta 1-C (Fig. 2) and 1018 (48.57 %) exhibited aberrations in the development of seta 1-C that are listed in Table 2. The most prevalent aberrant feature was bilateral (26.71 %) and unilateral (17.56%) spiculation, receptively (Fig. 3). Interesting and rare features observed only once in a single larva (0.05%) included a forked seta 1-C on one side and its mate on the other side with 3 branches (Fig. 6) and a normal spiniform seta 1-C on one side and a filiform seta 1-C on the other side (Fig. 7). Additionally, aberrant scaling of the abdominal terga was observed in adults. The abdominal terga of *Cx. theileri* normally have basal pale bands that are produced medially into somewhat triangular patches and apical dark scaling is not interrupted by pale scaling (Fig. 9) but in a few specimens, the posterior dark scaling included two patches of pale scales (Fig. 10).

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Table 1. Brief description of the anomalies and aberrations of seta 1-C in *Culex theileri* larvae encountered in the present investigation, Chahar Mahal and Bakhtiari Province, Iran

Normal	
Normal	Spiniform, normally developed 1-C without any abnormalities including aberrations (spiculation and branching) and anomalies (extra 1-C) (Fig. 1)
Abnormal	
Anomalous (unilaterally double)	Extra 1-C on one side (Fig. 2)
Aberrant	
Unilateral or bilateral spiculation	One or both sides of 1-C with spiculation at mid-length (Fig. 3)
Unilateral or bilateral branching	One or both sides of 1-C with a branch at mid-length (Fig. 4)
Unilaterally or bilaterally forked	One or both sides of 1-C forked apically (Fig. 5)
Unilaterally forked on one side and other side with 3 branches	1-C on one side forked; its mate on the other side with 3 branches (Fig. 6)
One side spiniform and the other side filiform	1-C on one side spiniform; its mate on the other side filiform (Fig. 7)
Abnormal shape	Developed differently than the above conditions (Fig. 8)

Table 2. Characteristics of seta 1-C observed in *Culex theileri* larvae collected in Chahar Mahal and Bakhtiari Province, Iran

Characteristics	**n**
Normal	1072
Abnormal	
Anomalous (Unilaterally double)	6
Aberrant	
Unilateral branching	7
Unilaterally forked	23
Unilateral spiculation	368
Unilateral spiculation and unilateral branching	6
Unilateral spiculation, unilateral branching and unilaterally forked	1
Unilateral spiculation and unilaterally forked	8
Bilateral branching	1
Bilateral spiculation	560
Bilateral spiculation and bilateral abnormal shape	1
Bilateral spiculation and bilateral branching	2
Bilateral spiculation and bilaterally forked	2
Bilateral spiculation and unilateral branching	22
Bilateral spiculation, unilateral branching and unilaterally forked	1
Bilateral spiculation, unilateral branching and bilaterally forked	1
Bilateral spiculation and unilaterally forked	13
One of pair spiniform the other filiform	1
One of pair unilaterally forked the other with 3 branches	1
Subtotal	1024
Total	2096

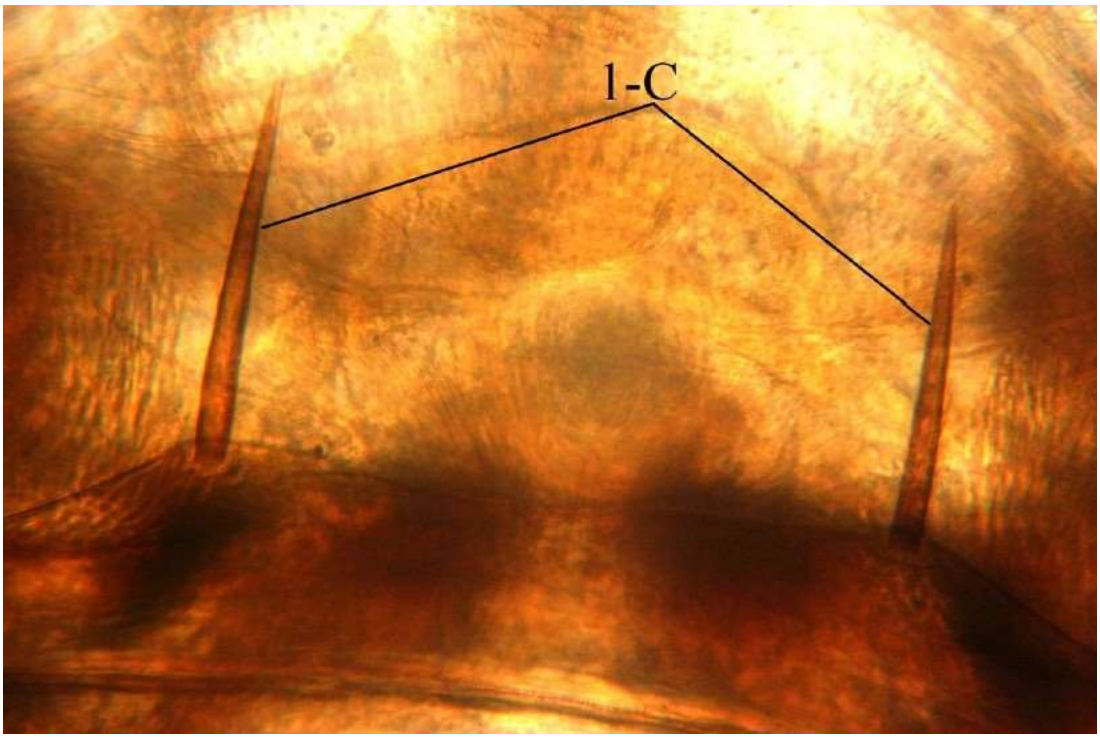

Fig. 1. *Culex theileri*, normal spiniform seta 1-C, Ardal, Chahar Mahal and Bakhtiari Province, Iran (original photo)

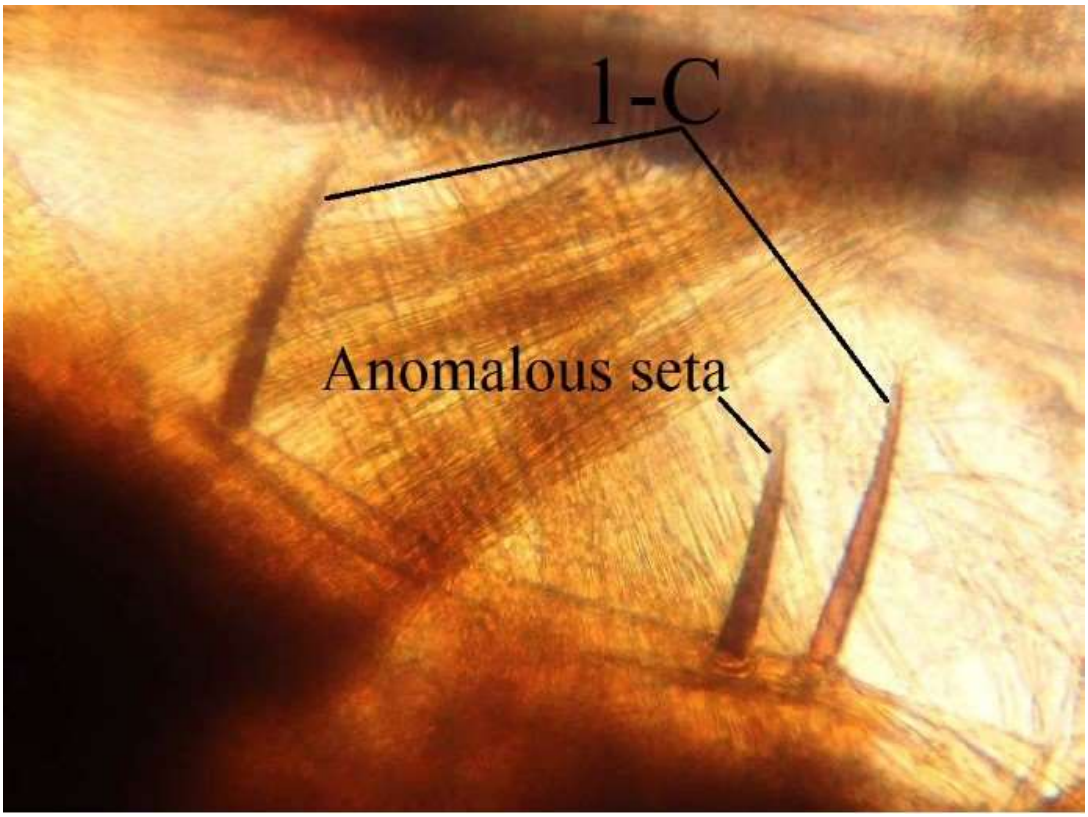

Fig. 2. *Culex theileri*, anomalous (extra) seta 1-C on one side (unilaterally double), Esaabad, Chahar Mahal and Bakhtiari Province, Iran (original photo)

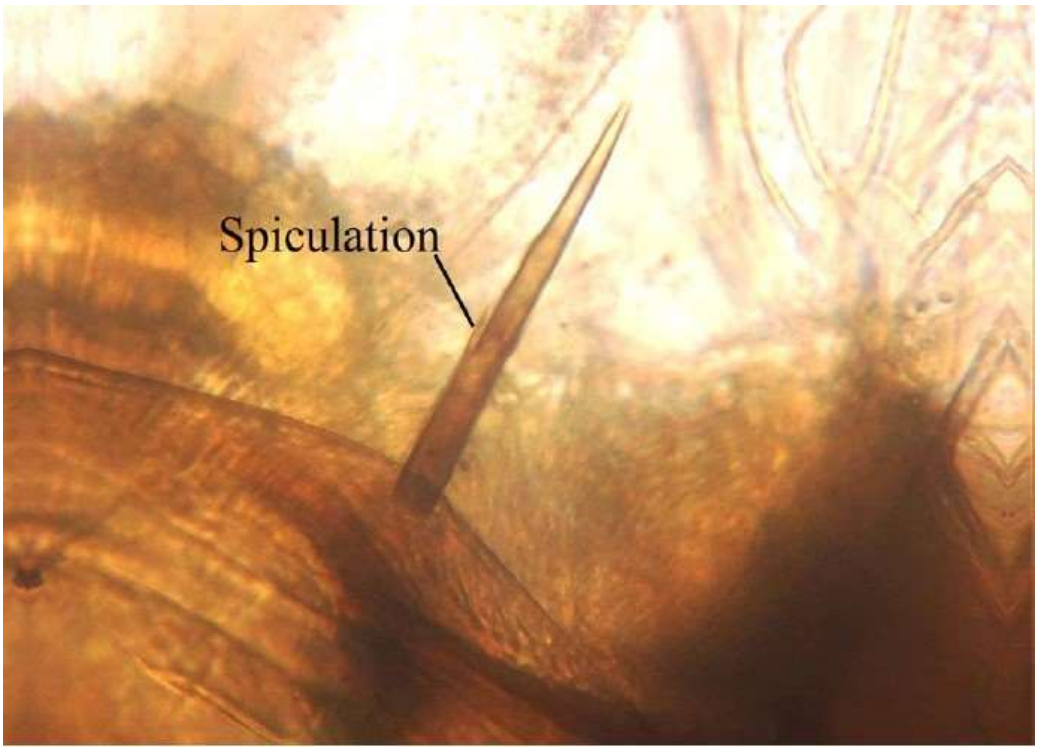

Fig. 3. *Culex theileri*, aberrant seta 1-C with spiculation at mid-length, Chamnar, Chahar Mahal and Bakhtiari Province, Iran (original photo)

Fig. 4. *Culex theileri*, aberrant seta 1-C with bilateral branching, Sarkhon, Chahar Mahal and Bakhtiari Province, Iran (original photo)

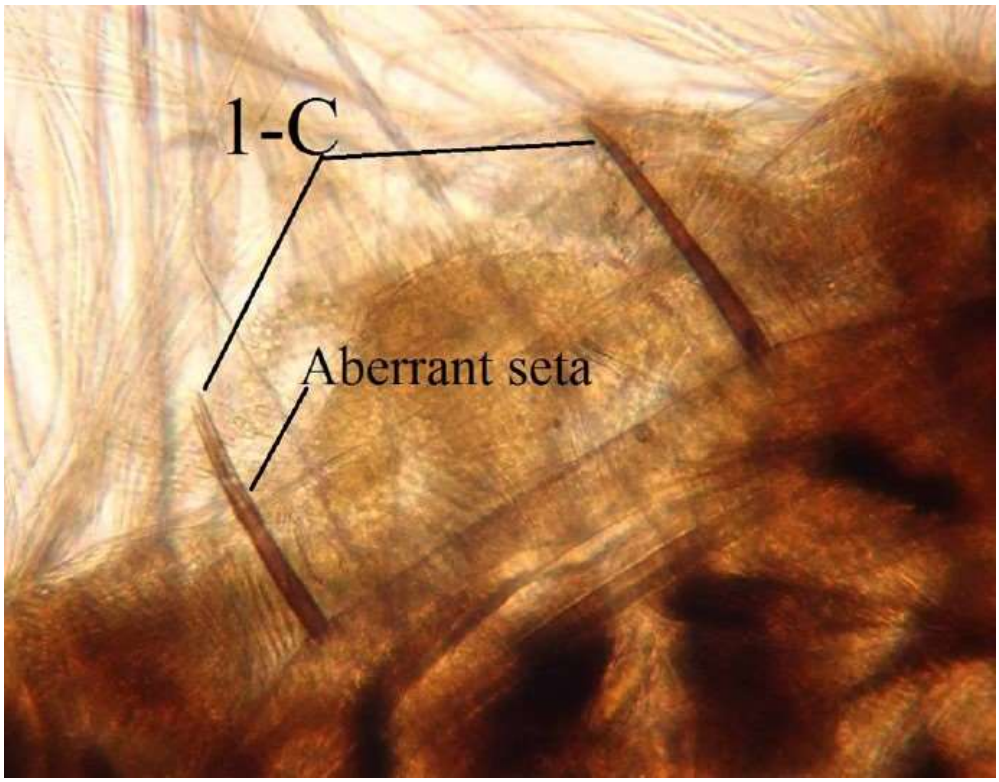

Fig. 5. *Culex theileri*, one the pair of seta 1-C forked, Saman, Chahar Mahal and Bakhtiari Province, Iran (original photo)

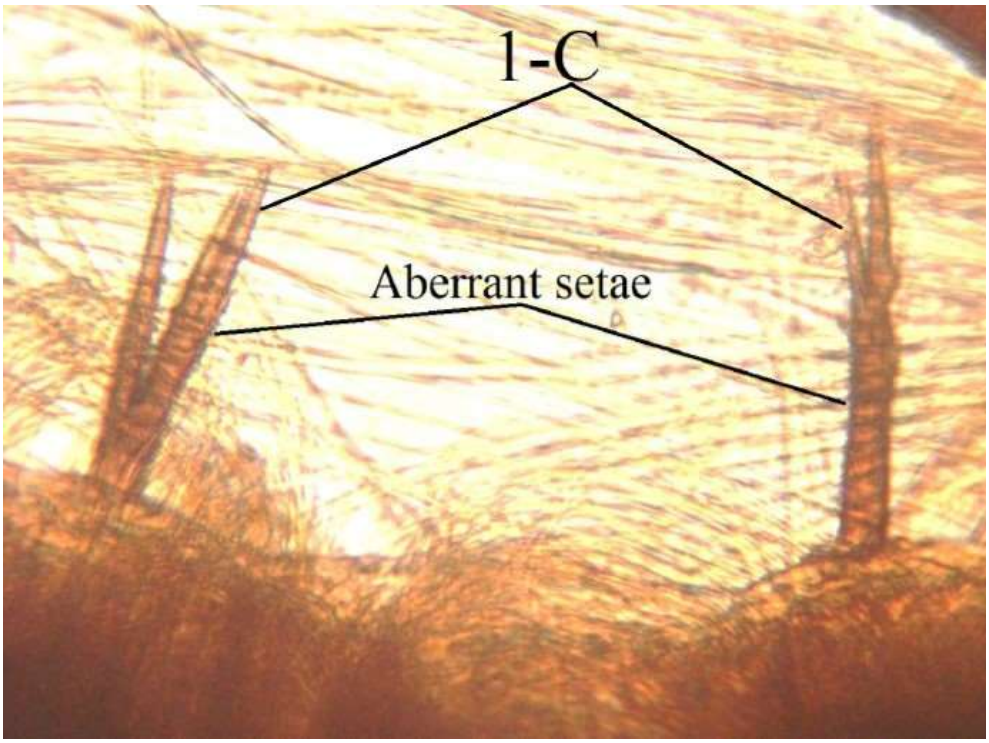

Fig. 6. *Culex theileri*, one seta 1-C forked and the other with 3 branches, Sarkhoon, Chahar Mahal and Bakhtiari Province, Iran (original photo)

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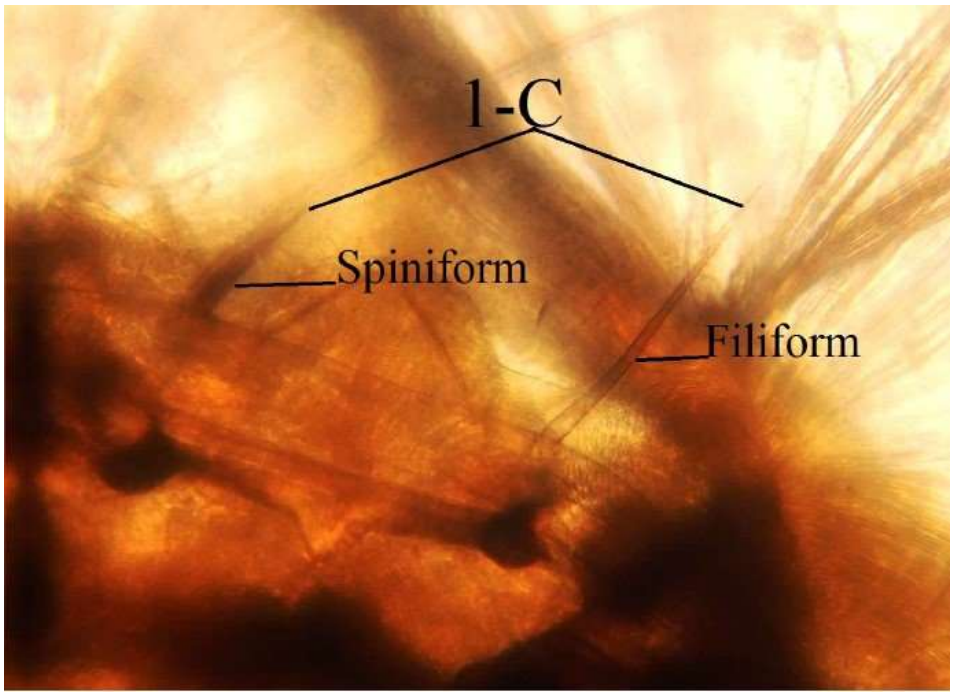

Fig. 7. *Culex theileri*, seta 1-C spiniform (normal) on one side and filiform on the opposite side, Razgah, Chahar Mahal and Bakhtiari Province, Iran (original photo)

Fig. 8. *Culex theileri*, seta 1-C with abnormal shape, Alooni, Chahar Mahal and Bakhtiari Province, Iran (original photo)

Fig. 9. *Culex theileri*, abdominal terga with normal basal pale bands, Kojanagh, Ardebil Province, Iran (original photo)

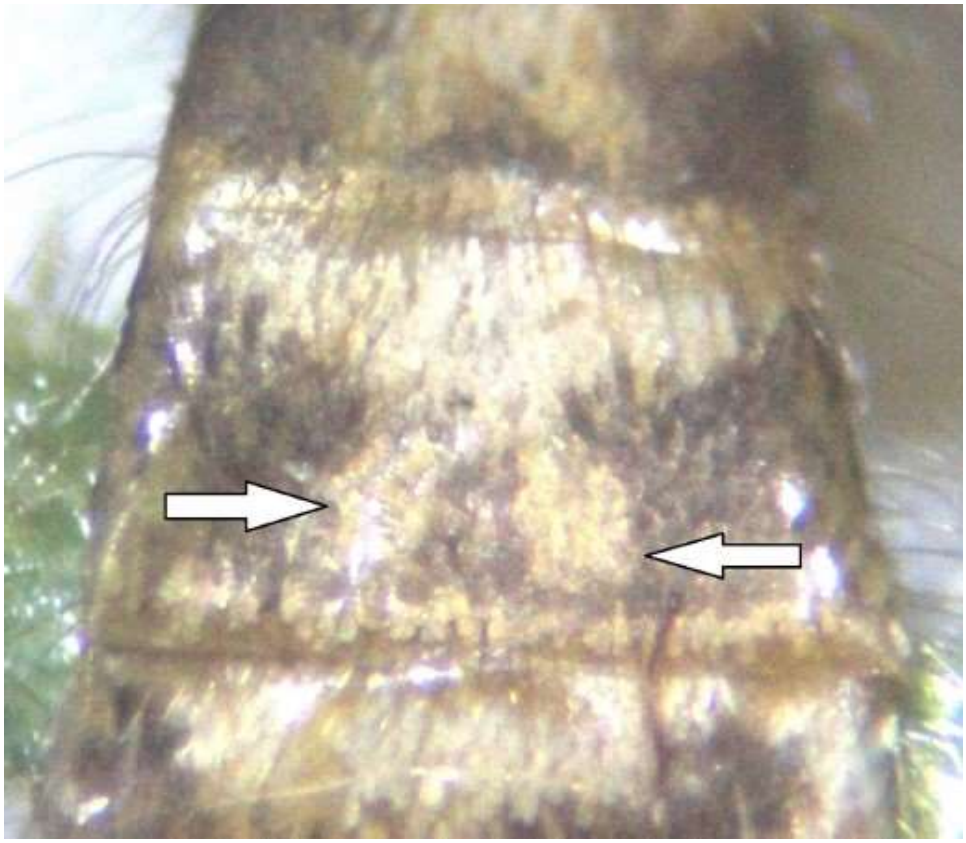

Fig. 10. *Culex theileri*, abdominal terga with a pair of postmedian patches of pale scales, Astara, Guilan Province, Iran (original photo)

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Discussion

Regarding the variability of *Cx. theileri*, Harbach (2) stated: "the larvae and adults, excluding the male genitalia, are extremely variable and exhibit the same degree of variation within local populations that is observed throughout the entire range. Therefore, it appears that only a single widespread and variable species is involved, yet it is possible that the taxon actually consists of an aggregate of biologically distinct, isomorphic species". Therefore, it seems that after more than 30 years the situation is the same.

For the first time, Azari-Hamidian et al. (8) found a substitution at position 518 (G/A) in the barcode region of the COI gene of *Cx. theileri* collected in a northwestern province of Iran (Ardebil) which caused one amino acid change (V to M). Later, Demirci et al. (26) studied genetic and morphometric variation in *Cx. theileri* in northeastern Turkey and found that although populations did not display genetic differentiation, there was a positive correlation between wing (body) size/shape and altitude. Also, Demirci et al. (27) observed a single genetic polymorphism in *Cx. theileri*.

Conclusion

Despite the wide distribution of *Cx. theileri* and its role in the transmission of pathogens and parasites of diseases in humans and domesticated animals, there is little genetic information about populations in different countries. More investigations are needed throughout the distribution of *Cx. theileri* to shed light on possible relationships between morphological and molecular variation that may be correlated with biologically distinct populations.

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Ethical considerations

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Conflict of interest statement

Authors declare that there is no conflict of interest.

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