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Variation in different populations of common pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer, 1989 (Hemiptera: Sternorrhyncha: Aphalaridae) in Iran

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Abstract. The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer 1989, is one of Iran's most important pests of pistachio cultivars. To recognize variation in different populations of this pest, 227 individuals of adult pistachio psyllid from 21 geographic populations were collected from the distribution region in Iran during 2016– 2017. The right fore wing was used for a geomorphic morphometric analysis. The results showed a range of variation at each landmark from individual specimens and the shape analysis revealed a significant difference among the shape components of the populations. The Alborz populations were clustered distinctly from the others based on the cluster analysis. The Esfahan and Kerman populations were similar. Also, Kermanshan and Kordestan were clustered together and could be considered the sister-group of the remaining populations.

Keywords: *Pistacia*, psyllid, morphometric, geomorphic, Iran.

Introduction

Pistachio is a subtropical plant from the Anacardiaceae family and belongs to the genus *Pistacia*. Among the eleven species of this genus, *Pistacia vera* L. is the only one whose fruits are big enough and economical (Seyedoleslami et al. 2002). *P. vera* is an essential economic horticultural crop that is currently grown in Asia, the Americas, Europe, Africa, and Oceania (Nezam Kheirabadi et al. 2021). The native range of pistachio is in the vast region of Khorasan between the borders of Iran, Afghanistan, and present-day Turkmenistan, which was later introduced from this region to other parts of the world. In addition to Iran, this plant is found in Syria, Jordan, Cyprus, and is cultivated in Lebanon, Palestine, Italy, Greece, France, Mexico, Spain, and especially the USA and Turkey (Abrishami 1995).

Pistacia vera is one of Iran's most important agricultural products, and it has a very high economic value. The pistachio tree has been cultivated in different parts of Iran in the past, and its cultivated area has increased significantly. Kerman province, especially Rafsanjan county, is considered the most important pistachio area in Iran and the world (Razavi 2005, Sheibani 1995, Torabipour et al. 2022).

Among the pistachio pests, the common pistachio psyllid is known as the main pest (Mehrnejad 2010, Mohammadi et al. 2015). Common pistachio psyllid *Agonoscena pistaciae* Burckhardt and Lauterer 1989 belongs to the order of Hemiptera, superfamily Psylloidea, family Aphalaridae, and subfamily Rhinocolinae (Burckhardt et al. 2021). Common pistachio psyllid is widely distributed in most pistachio-growing areas of the world, such as Iran, Turkey, Iraq, Armenia, and Turkmenistan, as well as in Mediterranean areas such as Syria and Greece (Anagnou-Veroniki et al. 2008, Bolu 2004, Burckhardt & Lauterer 1989, Mart et al. 1995). Önuçar (1983) states that *A. pistaciae* primarily feeds on various species of pistachios, including *P. vera*, *P. terebinthus*, *P. mutica*, and *P. khinjuk*, which serve as its host plants. Both immature stages and adults of this pest become detrimental by penetrating and feeding on the plant's shoots, leaves, and fruits. Depending on their population density, they can lead to the early dropping of leaves, flowers, and fruit and stunted growth or decreased quality of the fruits due to the sweet substance they produce (Bolu 2023, Hassani et al. 2009). A high density of psyllid immatures and adults can greatly decrease kernel development, resulting in bud drop and defoliation (Mehrnejad 2001).

Furthermore, the synergy of a multivoltine life cycle and high reproductive rate frequently results in outbreaks, causing substantial damage and remarkable crop losses in various provinces of Iran, including Sistan-Baluchestan, Hormozgan, Kerman, and Fars, which are known for their pistachio cultivation (Mehrnejad 2001). Cultivated pistachio (*Pistacia vera*) is more susceptible to psylla infestation than wild pistachio species (Mehrnejad 2003).

Evidence exists regarding the impact of host plants on the development and morphology of insects spanning various genera and taxonomic families, exemplified by Psylloidea such as *Spondyliaspis* cf. *plicatuloides* and *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) (Hodkinson 2009, Makunde et al. 2023, Perring et al. 1993).

The structural composition and secondary metabolite profiles of cultivated plants are distinguishing features that influence herbivorous insects' feeding and oviposition behaviors. This phenomenon has been observed with the psyllid species, *Boreioglycaspis melaleuca* Moore, feeding on *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtales: Myrtaceae), where changes in leaf nitrogen and carotene levels act as factors that alter the behavior of the mentioned psyllid (Casteel et al. 2006, Hodkinson et al. 2001). The influence of host plants on insect morphology is crucial for comprehending the morphological diversity within insect species and is regarded as evidence supporting the determination of "biotypes" (Vargas-Madríz et al. 2013).

Morphometrics describe and statistically analyze changes in shape within and among populations, including the analysis of shape changes due to growth and development (Pavlinov 2001, Rohlf 2009). Variations in the samples may reflect variances in geography, development, genetics, environmental influences, and other similar factors. This approach has a significant advantage in that the specimens being examined do not need to be alive, and it also avoids harming organisms' bodily tissues (Gillham & Claridge 1994).

A series of studies in Iran have utilized geometric morphometric methods to analyze the shape and size variations in different insect populations. Researchers found this method was more precise and easier than standard morphometrics for discriminating insect populations. Abdolahi Mesbah et al (2015) used this approach to identify significant differences in the wing shape and size of the Spotted Amber Ladybird in different regions. Mozaffarian et al. (2007) used this method to evaluate the morphological variation of the Carob Moth among different geographic populations. Lashkari et al. (2015) found significant differences in wing shape and size between Asian citrus psyllid populations in Iran and the USA and suggested a genetic basis for this variation. Research has shown that the population of the common pistachio psyllid, *Agonoscena pistaciae*, is affected by chemical control levels, for example, in high-stress environments, wing deformation has been observed (Mostafavi et al. 2017). Morphometric identification methods have been successfully applied to the study of *Agonoscena pistaciae* (Lashkari et al. 2019). Roohollahi (2013) tried to prove the existence of genetic differences among different populations of common pistachio psyllid in Kerman province (Iran) with molecular methods. Nadi (2014) constructed a dendrogram employing the Neighbor-Joining method and incorporating COI gene sequences from three species of Psylloidea (*Euphyllura olivine* (Costa, 1839) (Liviidae), *Cacopsylla* (*Hepatopsylla*) *pyricola* (Foerster, 1848) and *Diaphorina citri* Kuwayama, 1907 (Psyllidae)); the results indicated a clear separation of *A. pistacia* from the species above.

This study aims to investigate the variation in different populations of the common pistachio psyllid, *Agonoscena pistaciae*, in Iran. This may impact the management and control of this pest species in pistachio orchards. By understanding the factors influencing population variation, researchers may be able to develop more effective strategies for pest management and conservation of pistachio crops in Iran. Ultimately, the study aims to contribute to the sustainable management of the common pistachio psyllid and preserving pistachio orchards in Iran.

Material and methods

Extensive surveys were carried out to collect adult psyllids, covering various regions in Iran from February to May to collect the *A. pistachio* specimens. Adults were captured on pistachio trees in the primary pistachio cultivation regions across different provinces of Iran using sweep nets and aspirators, then preserved in 1.5 ml tubes filled with 96% ethanol.

Adult psyllids were gathered from pistachio trees in Ahmad Aghaei's orchards throughout the provinces of Iran between 2016 and 2017. In total, twenty-one geographically diverse populations of *A. pistaciae* were sampled from pistachio trees in the primary pistachio-producing areas of Iran (Table 1). In each population, 60 psyllids were collected and preserved in 90% ethanol at -20°C for subsequent analysis.

Table 1. The collecting sites of *pistachio psyllid, Agonoscena pistaciae* populations

Province	Region	N	E	Altitude	No.	F.No	Code
Alborz	Karaj	35°58'31.9"	50°29'37.2"	1164	60	30	ALB
Ardabil	Ardabil	38°18'24.59"	48°16'20.38"	1332	60	30	ARD
East Azerbaijan	East Azerbaijan	36°53'37.76"	76°09'07.17"	1307	60	30	AZE
Chaharmahal - Bakhtiari	Shahre kord	32°09'25.75"	50°50'22.08"	2026	60	30	CHB
Esfahan	Gaz	32°42'27"	51°03'22"	1889	60	30	ESF
Fars	Marvdasht	29°30'32"	53°14'06"	1396	60	30	FARS
Ilam	Ilam	33°36'02.14"	46°22'17.37"	1239	60	30	ILA
Kordestan	Kordestan	35°40'23"	47°02'56"	2113	60	30	KOR
Kerman	Kerman	30°20'46"	56°59'59"	1748	60	30	KER
Kermanshan	Kermanshah	34°20'14.16"	47°12'56.72"	1336	60	30	KERSH
Lorestan	Khoramabad	33°28'14.74"	48°27'10.13"	1326	60	30	LOR
Markazi	Saveh	35°22'51.06"	50°20'17.20"	1359	60	30	MAR
Qom	Qom	34°51'52"	50°42'46"	895	60	30	QOM
Qazvin	Boein Zahra	35°50'28.97"	50°06'07.46"	1179	60	30	QAZ
Sistan - Baluchestan	Khash	28°11'34.76"	61°13'37.89"	1403	60	30	SB
Semnan	Damghan	36°09'21.77"	54°22'30.57"	1127	60	30	SEM
Tehran	Tehran	35°08'33.67"	51°36'40.30"	827	60	30	TEH
Yazd	Yazd	31°59'32.06"	54°07'53.09"	1165	60	30	YAZ
Khorasank-e Shomali	Shirvan	37°23'59.42"	57°51'06.44"	1057	60	30	KHN
Khorasan Razavi	Mashhad	35°06'11.15"	59°13'50.57"	1154	60	30	KHR
Khorasan-e Jonoubi	Boshrouye	33°52'57.81"	57°27'23.32"	872	60	30	KHS

More than 2P-4 specimens were chosen for each province, where P represents the number of landmarks in the partial warp matrix, including the uniform component (W matrix) (Zelditch et al. 2004). Therefore, a sample size of 60 male specimens was randomly selected, exceeding the number of variables in the W matrix. The right fore wing of each specimen was utilized to create microscopic slides (using Canada balsam), and photographs were taken with a stereomicroscope. This study focused on the primary pistachio cultivation regions.

Preparing specimens and analysis

Geometric morphometrics utilizes a set of landmarks to provide detailed insights into the shape characteristics of specimens (Bookstein 1996). The right fore wings were used for geomorphic morphometric analysis of the pistachio psyllid populations, as outlined in Lashkari et al (2014). The wings of each specimen were carefully mounted on slides using Canada balsam as the mounting medium. A selection of eleven standard morphological characters on the fore wings was made for analysis (Figure 1). Images of the wings were captured using a digital camera (Dino digital microscope) connected to a stereo microscope with 40X magnification. Eleven homologous landmarks of type I were digitized and utilized in geometric morphometric analysis.

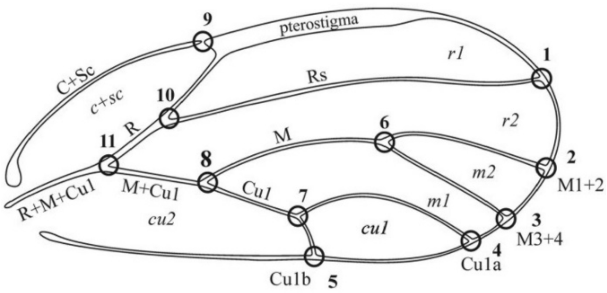

Figure 1. Position of landmarks (circles) in the right fore wing of Pistachio psylla (*A. pistaciae*) that follows that of Lashkari et al. 2013.

The raw x and y coordinates of the homologous landmarks in biological specimens were aligned and superimposed through the least-squares method, utilizing the generalized Procrustes analysis (GPA) within a non-Euclidean Kendall's shape space. This process was then transferred to a linear Euclidean tangent space, facilitating the elimination of non-shape-related variations like rotation and movement (Rohlf 1999, Slice 2001).

The captured images were converted to the TPS format (Rohlf 2010). Then, 11 homologous landmarks (type I) were digitized utilizing the TPSDIG program. (Rohlf 2004). Landmark positions adhered to guidelines set by Lashkari et al. (2013; 2014) (Table 2). Procrustes superimposition was carried out using generalized Procrustes analysis (GPA) with the tpsRelw program (Rohlf 1999, Slice 2001) and individuals that were aligned were compared utilizing the TpsRelw program (Rohlf 2010), which employed a thin-plate spline interpolation function to project the data onto a Euclidean plane.

The consensus shape and maximal shape variation are shown along the first two relative-warp axes (RW1 and RW2). The shape variable data set (weight matrix) was subjected to MANOVA and canonical variate analysis (CVA) (Rohlf et al. 1996). The MANOVA and CVA analyses were performed using the SAS statistical program, version 9.1 (SAS Institute 2003) and the NTSYS-pc program, version 2.02 (Rohlf 1998), respectively. The UPGMA clustering method was performed using the NTSYS-pc program version 2.02 (Rohlf 1998). ANOVA and Tukey pair-wise comparisons were conducted using the SAS statistical program, version 9.1 (SAS Institute 2003).

Table 2. Description of the landmark positions in the fore wing of *Agonoscena pistaciae* (Lashkari et al. 2013).

Landmarks	Description
1	Rs
2	M1+2
3	M3+4
4	Cu1a
5	Cu1b
6	M
7	Cu1
8	M+ Cu1
9	Pterostigma
10	R
11	R+M+Cu1

Results

The superimposition of 227 *A. pistaciae* specimens revealed differences in each landmark among individual specimens (Figure 2). The vectors on marginal landmarks, especially landmarks 1– 5, 7, and 11, show more inclination to the margin parts of the wing (Figure 2). The veins R, Rs and M in the negative extreme were longer than those in the positive one, resulting in a larger size of the wings (Figures 2 and 3). RW1 and RW2 explained 20.9628% and 18.5497% of the shape variation among specimens. The positive and negative extremes of wing shape variation along the RW1 axis are shown in Figure 3.

The shape analysis revealed a significant difference among the shape components of the populations (Wilksí lambda = 0.0016, F= 1.81, P < 0.0001). Three-dimensional ordinations using the mean canonical variate scores (CV1 and CV2) of the 21 populations showed the same result (Figure 4).

The Alborz populations were clustered distinctly from the others based on the cluster analysis. The Es and Ker populations were similar. Also, Kersh and Kor were clustered together and could be considered the sister groups of the remaining populations (Figure 5).

Varying wing size, as assessed by a one-way ANOVA, indicated a significant difference in centroid size (F = 5.71, df = 20, P < 0.0001) among the populations. A pair-wise comparison using the HSD post-hoc test (Alpha= 0.01) is shown in Table 3 and Figure 6.

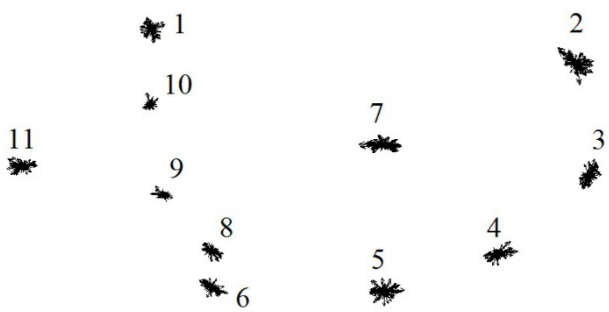

Figure 2. Superimposition of the fore wing's images of pistachio psyllid, *Agonoscena pistaciae*, populations

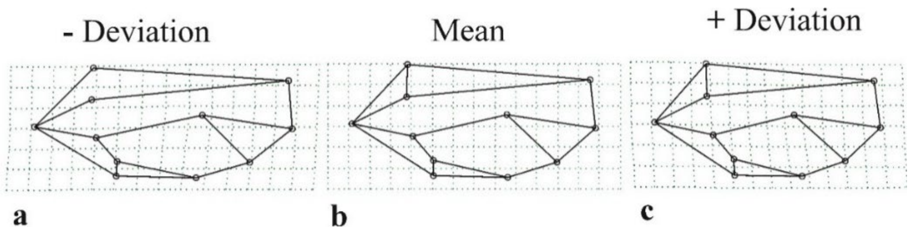

Figure 3. Deformations in shape variables along the RW1 in the fore wings of pistachio psyllid, *Agonoscena pistaciae*, populations. (a) consensus configuration, (b) negative deformation, and (c) positive deformation

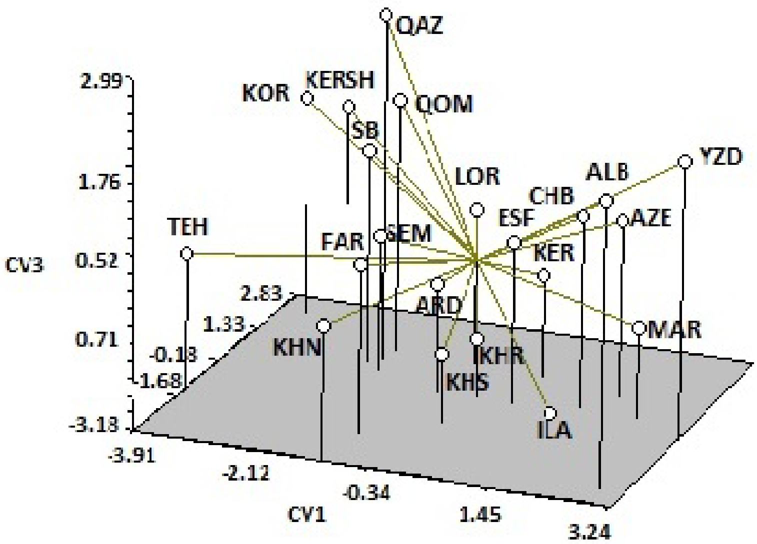

Figure 4. Ordination of the group means along the first three canonical variate (CV) axes (ALB.: Alborz; ARD.: Ardabil; AZE.: East Azerbaijan; CHB.: Chaharmahal – Bakhtiari; ESF.: Esfahan; FARS: Fars; ILA.: Ilam; KOR.: Kordestan; KER.: Kerman; KERSH.: Kermanshan; LOR.: Lorestan; MAR.: Markazi; QOM: Qom; QAZ.: Qazvin; SB.: Sistan – Baluchestan; SEM.: Semnan; THE.: Tehran; YAZ.: Yazd; KHN.: Khorasank-e Shomali; KHR.: Khorasan Razavi; KHS.: Khorasan-e Jonoubi)

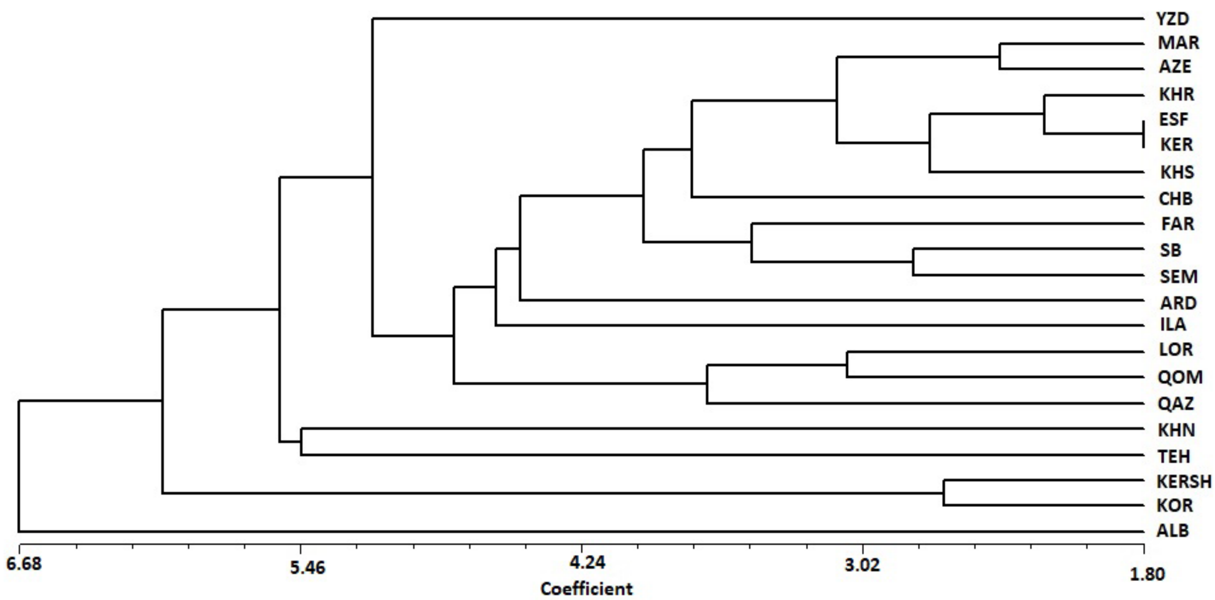

Figure 5. Dendrogram plotted by UPGMA method based on the generalized distance matrix of pistachio psyllid, *Agonoscena pistaciae*, populations. (See table 1 for abbreviations.)

Table 3. Results of HSD (post-hoc) test on the wing centroid size of pistachio psyllid, *Agonoscena pistaciae*, populations. See Table 1 for abbreviations (* Comparisons significant at the 0.01 level).

	AZ	CHB	IL	LOR	KER	KHS	Y	MR	ESF	KHN	SEM	FAR	SB	ARD	KHR	KERSH	ALB	TEH	QM	KOR	QZ
AZ																					
CHB	1.67																				
IL	30.00	28.33																			
LOR	73.33	71.67	43.33																		
KER	92.62	90.95	62.62	19.29																	
KHS	92.67	91.00	62.67	19.33	0.05																
Y	94.17	92.50	64.17	20.83	1.55	1.50															
MR	95.00	93.33	65.00	21.67	2.38	2.33	0.83														
ESF	103.67	102.00	73.67	30.33	11.05	11.00	9.50	8.67													
KHN	145.00	143.33	115.00	71.67	52.38	52.33	50.83	50.00	41.33												
SEM	**149.17***	147.50	119.17	75.83	56.55	56.50	55.00	54.17	45.50	4.17											
FAR	**152.22***	150.56	122.22	78.89	59.60	59.56	58.06	57.22	48.56	7.22	3.06										
SB	**154.44***	**152.78**	124.44	81.11	61.83	61.78	60.28	59.44	50.78	9.44	5.28	2.22									
ARD	**156.67***	155.00	126.67	83.33	64.05	64.00	62.50	61.67	53.00	11.67	7.50	4.44	2.22								
KHR	**164.44***	162.78	134.44	91.11	71.83	71.78	70.28	69.44	60.78	19.44	15.28	12.22	10.00	7.78							
KERSH	**166.67***	165.00	136.67	93.33	74.05	74.00	72.50	71.67	63.00	21.67	17.50	14.44	12.22	10.00	2.22						
ALB	**173.33***	171.67	143.33	100.00	80.71	80.67	79.17	78.33	69.67	28.33	24.17	21.11	18.89	16.67	8.89	6.67					
TEH	**175.00***	173.33	145.00	101.67	82.38	82.33	80.83	80.00	71.33	30.00	25.83	22.78	20.56	18.33	10.56	8.33	1.67				
QM	**201.67***	200.00	171.67	128.33	109.05	109.00	107.50	106.67	98.00	56.67	52.50	49.44	47.22	45.00	37.22	35.00	28.33	26.67			
KOR	**206.67***	205.00	176.67	133.33	114.05	114.00	112.50	111.67	103.00	61.67	57.50	54.44	52.22	50.00	42.22	40.00	33.33	31.67	5.00		
QZ	**216.67***	215.00	186.67	143.33	124.05	124.00	122.50	121.67	113.00	71.67	67.50	**64.44**	62.22	60.00	52.22	50.00	43.33	41.67	15.00	10.00	

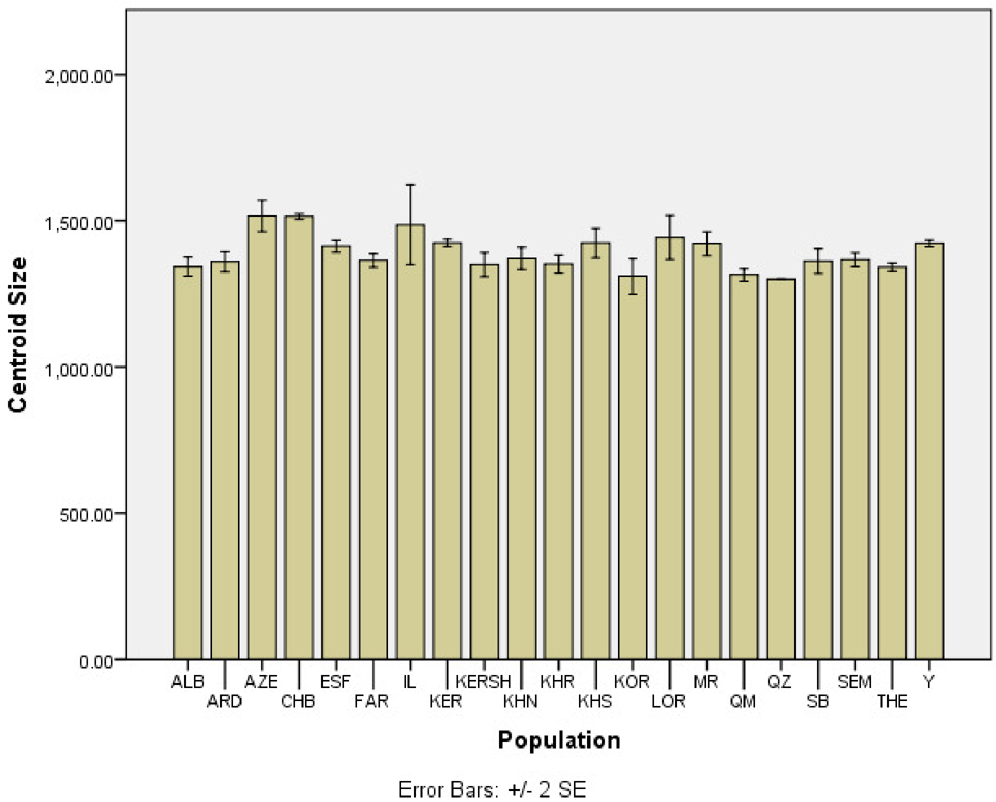

Figure 6. Centroid size of populations of pistachio psyllid, *Agonoscena pistaciae*. Whiskers show standard error (see Table 1 for abbreviations).

Discussion

The common pistachio psyllid, *Agonoscena pistaciae*, is a significant pest in Iran, causing economic damage to pistachio trees (Mostafavi et al. 2017). Molecular and morphometric identification methods have been used to distinguish between different pistachio psyllid species, with the niche modelling for *A. pistaciae* being identified in Eastern, Southern, and some parts of Central Iran (Lashkari et al. 2019). In Iran, research on the common pistachio psyllid, *A. pistaciae*, has revealed significant population variation, as Mostafavi et al. (2017) showed differences in wing shape, with a narrower shape in stressed environments, of populations exposed to different levels of pesticide application. This suggests the development of evolutionary resistance.

The demographic characteristics may be affected by the differing climatic conditions at the collection sites in various provinces of the country. For instance, areas like Razavi Khorasan, Qom, Tehran, and certain parts of Isfahan have warm and semi-arid climates, characterized by high evaporation and transpiration rates. On the other hand, Kerman and Yazd provinces experience dry climates with cold winters and extremely hot summers. At the same time, regions such as Azerbaijan, Kurdistan, and Kermanshah have mountainous climates with increased rainfall and lower temperatures (Mostafavi et al. 2018).

Topographical maps of different country regions show that temperature, humidity, and altitude can indicate population dispersion. This observation suggests that relative humidity may significantly impact distinguishing the various populations of common pistachio psyllids in the country (Sadr & Eslami 2021). Alternatively, the variation could be the outcome of the interaction of all the mentioned factors. However, further investigations are necessary to confirm this hypothesis.

By assessing the topography and temperature maps of the sampled locations in this study, it is evident that areas with lower altitudes and warmer climates may contribute to the smaller wing size observed in populations from these regions, compared to other areas in the country.

This concept is rooted in Bergmann's rule, which posits a correlation between body size and latitudinal variations. According to Bergmann's rule, individuals in lower latitudes, characterized by warmer climates, tend to have smaller body sizes, while those in higher latitudes exhibit larger ones. Studies showed that Individuals with smaller size and shorter, blunter fore wings were primarily found in regions with lower latitudes and mountainous terrain, characterized by higher temperatures and greater precipitation. On the other hand, larger grasshoppers with longer fore wings, specifically a more extended radial sector, tend to inhabit areas with opposite environmental conditions (Bai et al. 2016).

The distance can also affect gene flow among populations, even in species capable of travelling long distances. This phenomenon is referred to as "isolation by distance" (Britten et al. 1995). Isolation by distance (IBD) is a typical pattern in population genetics where gene flow decreases with increasing geographic distance (Jensen et al. 2005). This phenomenon occurs even in species capable of long-distance travel, such as the sedentary butterfly *Euphilotes enoptes* (Peterson 1996). Gene flow among populations can boost local adaptation when it brings in new genetic diversity for selection, but strong gene flow can hinder adaptation by overwhelming beneficial local genes. These consequences are influenced by factors such as population size, genetic diversity, and the surrounding environment. The direction of gene flow may correspond with geographic proximity (IBD-Isolation by Distance), where the rate of gene exchange decreases as populations are farther apart. Alternatively, gene flow may adhere to patterns of isolation by environment (IBE), with higher gene flow rates occurring between populations in similar environments (Sexton et al. 2014).

The current study's results revealed that the examined populations of common pistachio psyllid can be classified into two distinct groups and Alborz populations were clustered distinctly from the others. Additionally, the morphometric data obtained in this study are consistent with those from Nadi's (2014) research, which demonstrated that geographically distinct populations of common pistachio psyllids from Khorasan, Razavi, and Kerman provinces differ from each other and can be categorized into two separate groups.

Kim and McPheron suggest that evolutionary changes in essential abilities and responses occur as a result of genetic changes to better adapt to environmental conditions (Kim & McPheron 1993). Morphological differences between populations are more influenced by genetic factors than environmental conditions (Dujardin et al. 1999)

This phenomenon may arise from the widespread application of various agricultural pesticides in orchards to combat the common pistachio psyllid, leading to as many as eight sprayings annually, potentially causing genetic alterations among populations.

In this case, additional biological traits of the mentioned populations may undergo alterations. Therefore, it is recommended that further research be conducted to identify and assess the molecular characteristics of these populations.

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