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Article

Classical and geometric morphometric methods reveal differences between specimens of *Varroa destructor* (Mesostigmata: Varroidae) from seven provinces of Iran

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ABSTRACT
Varroa destructor Anderson & Truman is the most serious pest of honey bee colonies all over the world. Its specimens were assessed by morphometric and geometric morphometric methods using six morphometric traits including body length and width, length and width of the right metapodal shield, length of epigynal and the anal shield for classical morphometrics and six landmarks in ventral surface of mite's body, in seven provinces including Ardabil, Markazi, Qom, Qazvin, Alborz, North Khorasan, and Semnan. Twenty and ten individuals were used for morphometric and geometric morphometric studies, respectively. The ventral surface of each mite slide was prepared and used Digimizer and Tps package software for morphometric and geometric morphometric measurements, respectively. Data were analyzed with Past software. Furthermore, principal component analysis (PCA) was used for both two methods. In addition, Cluster analysis was used to categorize specimens of provinces. The results demonstrated significantly morphometric and geometric morphometric (or shape and size) differences among the specimens of provinces. Qazvin and Ardabil were located in one group with the PCA and Cluster analysis in the classical morphometric method and Alborz mite's dimensions showed a significant difference in morphometric measurements with other provinces. However, in the geometric morphometric method, Ardabil, Markazi, North Khorasan, and Semnan specimens were located in another group. The difference in distribution can be related to geographical factors and methods of measurement.

KEY WORDS: Cluster analysis; geometric morphometrics; morphometrics; PCA analysis; varroa mite.

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INTRODUCTION

Varroa mite is the most serious pest of honey bees all over the world, which annually cause damages qualitatively and quantitatively to honey bee colonies (De Jong 1997). This mite is the ectoparasite of honey bees first in larval and pupal stages and then adults. Originally, varroa mite was thought to be a single species until Anderson and Trueman (2000) identified that *Apis mellifera* is infected by a separate species, *Varroa destructor* Anderson and Trueman, 2000. So far a lot of researches including biology, control, resistance, and morphology were performed on the varroa mite (Rosenkranz *et al*. 2010).

One of the major problems in the mite populations is the resistance to acaricides (Le Conte *et al.* 2010). The problem may be related to the honey bee races (some race of honey bees are able to be more tolerate varroa), environmental conditions, genetic factors and maybe morphological characters of varroa mite (Delfinado-Baker 1984). On the other hand, differences between hosts

such as honey bee races and environmental conditions of the ectoparasite can have an effect on morphological characters of varroa mite (Kelomey Emniside *et al*. 2016). Different morphotype of *V. destructor* was seen in some geographic area of Argentina (Maggi *et al*. 2009) which confirms that the geographical condition affects the varroa morphology. Some honey bees have geographical variations that originate in their genetic (Oleksa and Tofilski 2014). When the host of mite has variation, it is believable that parasite also changes with this condition; in a similar study in Uruguay, the relationship between honey bee and varroa mite was detected by geometric morphometric method (Giménez Martínez *et al*. 2017). Also, size of comb cells affects on mite's body size so that significant reduction in varroa size was observed in small comb cells (Borsuk *et al*. 2012).

A lot of methods were used for evaluating variation of varroa population and other arthropods. Furthermore, many molecular and morphological studies are applied for this purpose (Franck *et al*. 1998; Jensen *et al*. 2005; Bodur *et al*. 2007; Miguel *et al*. 2011; Gruber *et al*. 2013). In some studies, these methods are correlated with each other and results are accepted but others have discrepancies between different methods (Franck *et al*. 2000; De la Rúa *et al*. 2001; Radloff *et al*. 2001). For choosing the best controlling methods it is necessary to identify the population of mites with morphometric characters then we should action based on races of their hosts, economic and environmental conditions.

In this study, we aim to analysis morphological differences between varroa mite specimens in seven provinces of Iran and to compare the performance of two morphological methods, i.e. classical and geometric morphometrics.

MATERIAL AND METHODS

Sampling

A total of 140 varroa mites (20 specimens per province) were collected from seven provinces including Markazi, Ardabil, Alborz, North Khorasan, Qazvin, Qom, and Semnan. Sampling from phoretic mites was conducted in the summer and autumn (September) in 2016 from stayed apiaries without migration. Then the samples were immediately transferred into 96% alcohol. Slides were prepared from the ventral surface of all of the samples. Following, photos of each sample were taken with the binocular and CCD video camera (Sony Dino-lite 2). These photos were used for morphometric and geometric morphometric measurements.

Classical Morphometric measurement

Distances and angles are used in the classical morphometric measurements. In this study, six distances including body length and width, length and width of the right metapodal shield, the length of epigynal and the anal shield were measured with the Digimizer software (Fig. 1). The software was calibrated before measurement. Data are transferred to Excel for further analysis and were analyzed by the Past software. Principal component analysis (PCA) was used and statistically significant at the 5% level was observed in the data (Table 1).

Geometric morphometric measurement

For this purpose, a total of six landmarks were selected (Fig. 2). Landmarks with high accuracy present the coordination of the sample body parts in photos. All photos have similar zoom so angle and the landmarks were easily definable in all samples. For the geometric coordination of each landmark was used software tpsDig2 v2.16 (Rohlf 2010a). The raw data were obtained from the landmarks coordination was used for subsequent analysis. Weight matrices of partial warps achieved from the tpsRelw package were used for the cluster analysis and principal components analysis (PCA) (Rohlf 2010b).

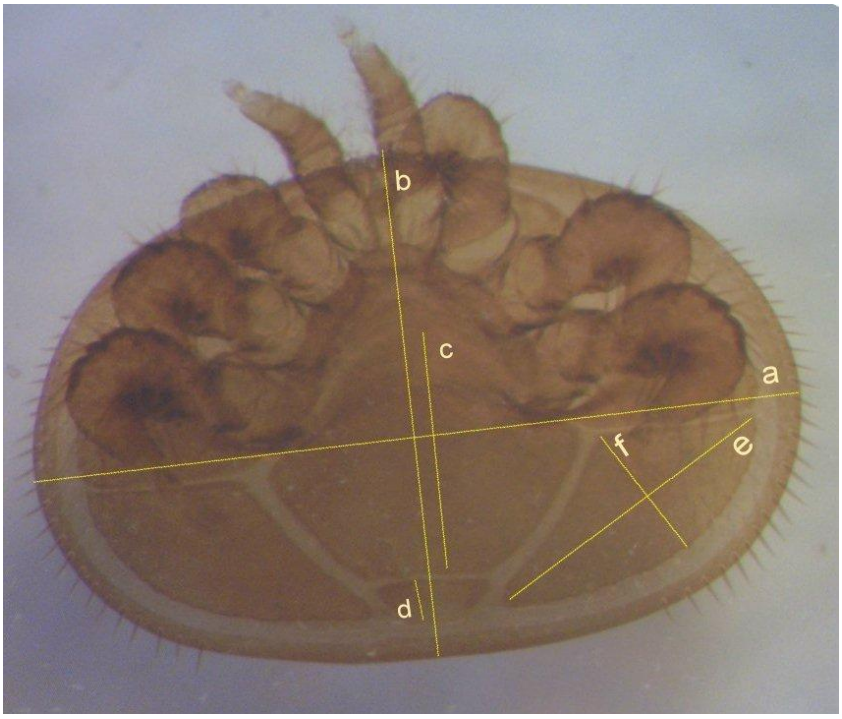

Figure 1. Morphometric parameters measured on the ventral surface varroa mite – a: body width, b: body length, c: length of the epigynal shield, d: length of the anal shield, e: metapodal shield's width, f: metapodal shield's length.

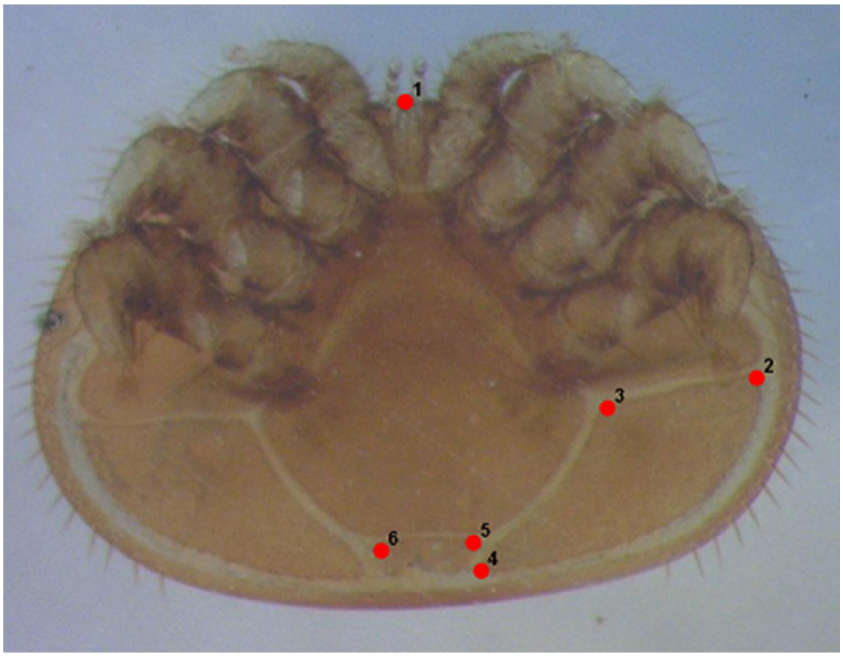

Figure 2. Distribution of six landmarks on the ventral surface of varroa mite for geometric measurement.

RESULTS

Classical morphometric results represent that there was a difference in mite length in Markazi, Qazvin, and Semnan specimens. In addition, based on the Duncan test there was a difference in mite's width and the average of it in the Ardabil, Qom, Markazi, and North Khorasan was higher than Semnan and Alborz specimens. In the anal shield attribute, significant differences in Alborz

and other provinces were observed. Furthermore, length of the anal shield was different between Semnan and Ardabil, Tehran and Qazvin. The attribute metapodal width between the Ardabil, Markazi, Qazvin, Semnan, and North Khorasan was not showed the significant difference but they have a difference with Alborz provinces; also the metapodal length between Alborz and Semnan with other provinces was significantly diverse. The epigynal shield in the Semnan province was different with other provinces. The results of the PCA analysis showed that there was a little distance between Qom and Markazi although Qazvin and Ardabil are in a cluster (Fig. 3). However, Qom and Semnan provinces had a large distance with the other specimens of provines. As it was shown in Table 2, the first component (PC1) had the most contribution (69.447%) in describing variation. In addition, cluster analysis in this method shows that Qazvin, Ardabil, and Alborz are located in one group but Qazvin and Ardabil are close together and Markazi and North Khorasan are located in another group (Fig. 4).

Table 1. ANOVA analysis of morphometric characters.

Morphometric Characters		df	F	Sig.
length	Between Groups	6	1.877	0.104
	Within Groups	49		
	Total	55		
width	Between Groups	6	1.928	0.095
	Within Groups	49		
	Total	55		
metapodal shield Length	Between Groups	6	1.641	0.156
	Within Groups	49		
	Total	55		
metapodal shield Width	Between Groups	6	1.540	0.185
	Within Groups	49		
	Total	55		
epigynal shield	Between Groups	6	2.600	0.029
	Within Groups	49		
	Total	55		
anal shield	Between Groups	6	1.831	0.112
	Within Groups	49		
	Total	55		

Table 2. Principal Component Analysis (PCA) of the ventral surface of varroa mites in seven provinces based on morphometric measurement.

PC	Eigenvalue	% Variance
1	219.365	69.447
2	70.7893	22.411
3	17.5098	5.5433
4	5.63186	1.7829
5	2.53648	0.803
6	0.043225	0.013684

Results in the morphometric geometric method are different from the classical morphometric method. In the geometric morphometric method which the analysis was performed based on weight matrices, the populations of Ardabil and Markazi are close together. Furthermore, Semnan and North Khorasan are situated in one group (Fig. 5). The first principal component (PC1) is (30.964%) describing variation (Table 3). Cluster analysis based on UPGMA method presents that Qom, Ardabil, and Markazi mite's populations can be located in one group, although Ardabil and Markazi are close together, while North Khorasan, Qazvin, and Semnan are situated in one group (Fig. 6).

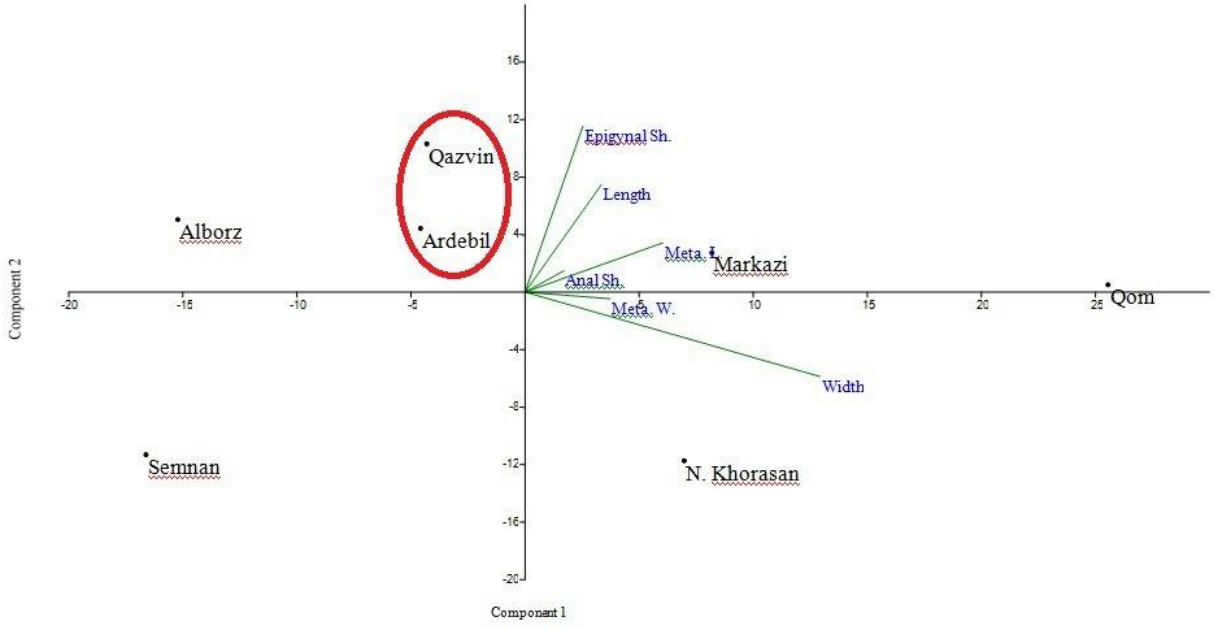

Figure 3. Distribution of morphometric characters in PCA analysis. This graph is based on the average size of the characters, is drawn.

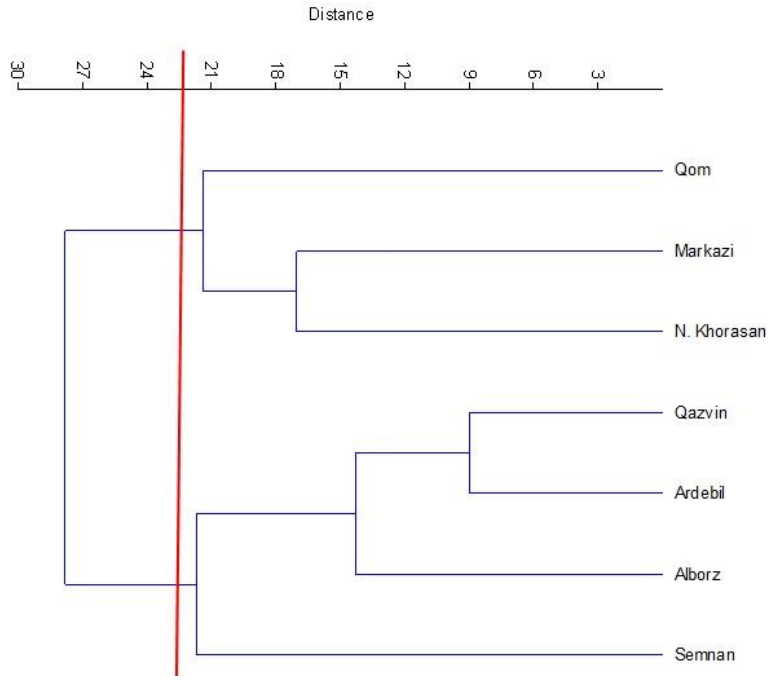

Figure 4. Dendrogram plotted by on UPGMA method based on morphometric measurement. The vertical line is the benchmark for population categories.

Table 3. Principal Component Analysis (PCA) of the ventral surface of varroa mites based on geometric morphometric measurement.

PC	Eigenvalue	% Variance
1	0.000213327	30.964
2	0.000157697	22.89
3	0.000104297	15.139
4	8.20937E-05	11.916
5	6.54377E-05	9.4982
6	4.38975E-05	6.3717
7	1.31074E-05	1.9025
8	9.09203E-06	1.3197

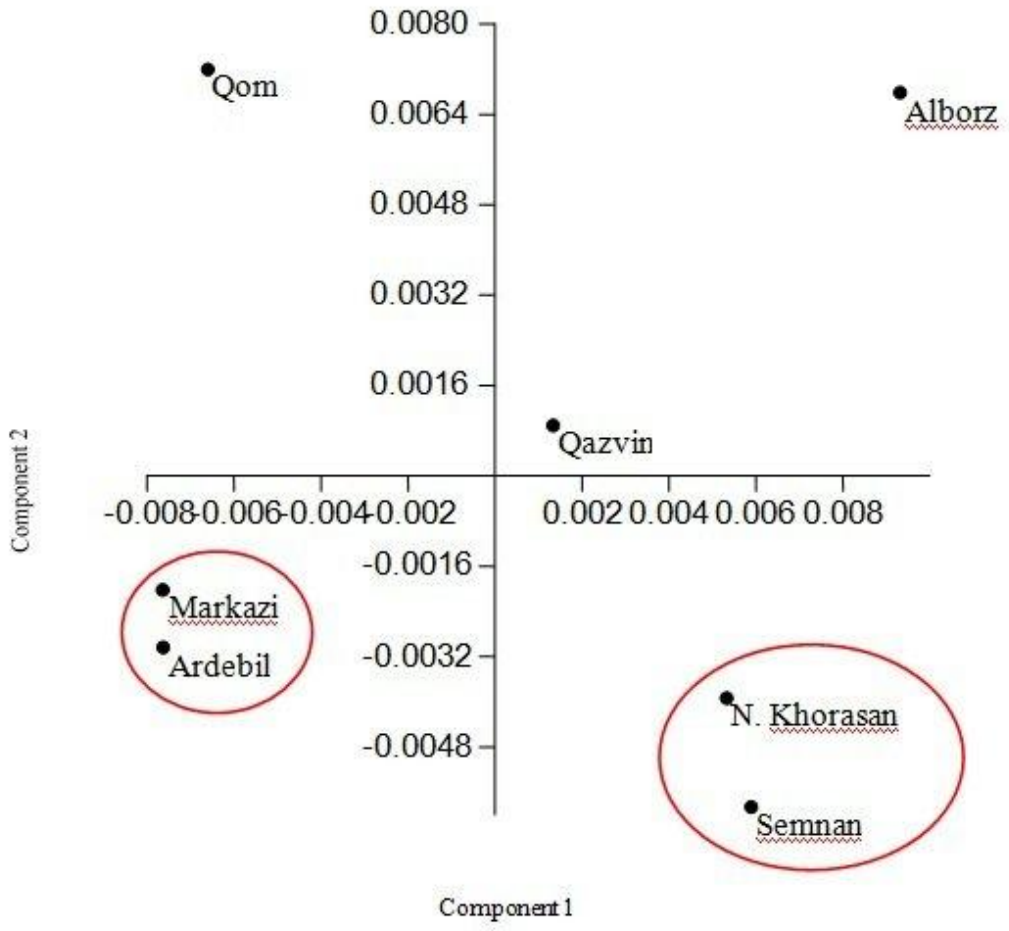

Figure 5. Distribution of varroa mite based on a landmark in PCA analysis. Weight matrices data are used for this analysis. Circles show the closer groups.

As a result, it was observed that the populations of mites in some provinces were morphologically different in some characters such as length and width of the body but, there was no significant difference in the shape, size, and distance of samples. Analysis showed the geometric morphometric and morphometric methods for morphological measurements were acceptable. In this study, geometric morphometric method isolated varroa mite population and morphometric method

discriminate population with a distance of ventral characters of mites that can be used for comparing mite populations of Iran with other countries.

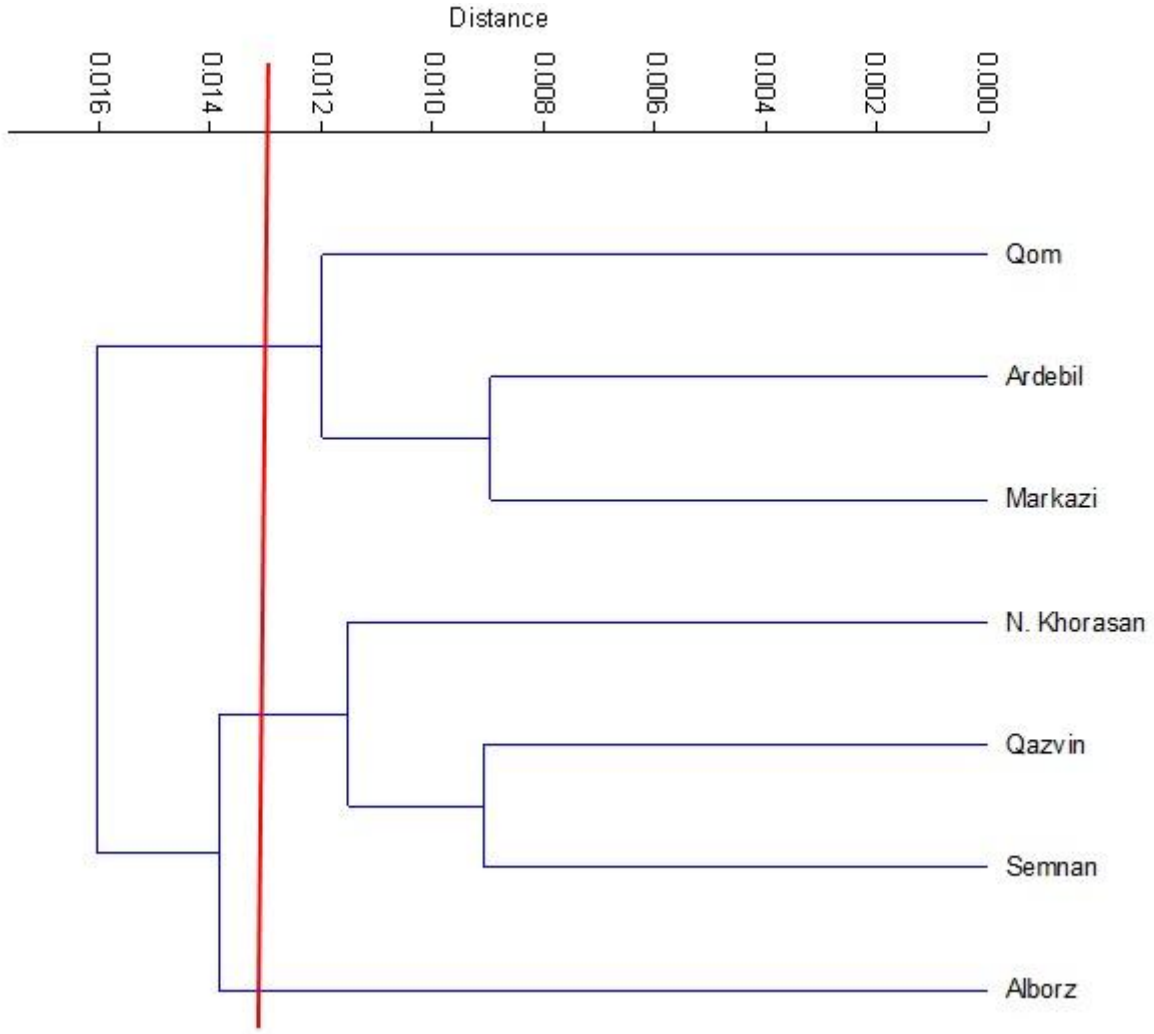

Figure 6. Cluster analysis by UPGMA method based on geometric morphometric measurement.

DISCUSSION

Behavioral and morphological changes were made in different populations due to genetic differences. Differences were observed in mite population of some provinces based on the morphometric and geometric morphometric analysis. These morphological differences in other arthropods and insects have been reported previously (Sarafrazi *et al.* 2004; Schachter-broide *et al.* 2004; Mozaffarian *et al.* 2007; Lashkari *et al.* 2013).

According to the classical morphometric analysis of the varroa's width body attribute showed the greatest variation. Shield attribute also has the lowest variation (Fig. 3). In the morphometric analysis, Qazvin and Ardabil were close in terms of variations and in the cluster analysis was performed in one group but in the geometric morphometric analysis, Markazi and Ardabil populations were close and also North Khorasan and Semnan specimens were in one group (Fig. 5). These differences in the results of the research scholars were observed in many studies such as Frank *et al*. (2000) that differences in the type of genetic measurements showed different

haplotypes in the honey bee and (Radloff *et al*. 2001) which examined the morphological dependence of the bee at several points in Spain. In a research conducted in Argentina, the geographical variation caused the variation in varroa morphology which in the present study also confirmed morphological changes based on geography (Maggi *et al*. 2009). These geographic changes depend on altitude, temperature, relative humidity, latitude, and even climatic conditions which ultimately lead to a difference in the morphology of the mite. It should be noted that these differences occur in populations at different geographic distances. Rahmani *et al*. (2006) compared the morphological characters of *Varroa* mite in Tehran Province and unlike the present study, there was no significant difference between these characters.

Semnan and North Khorasan are near in geographical coordination in comparison with other provinces and can be placed in one group because of their climate features; so it could be said the geometric morphometric method showed creditable results when compared to the classical morphometric method. Besides, in some studies, morphological and molecular methods were used and suggested that there is a correlation between results obtained by using these two morphological and molecular methods (De la Rúa *et al*. 2007; Miguel *et al*. 2011). The similarity between geometric and genetic results in these studies represent that geometric methods are more decisive and attributable.

REFERENCES

Anderson, D.L. & Trueman, W.H. (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, 24: 165–189.

Bodur, C., Kence, M. & Kence, A. (2007) Genetic structure of honeybee, *Apis mellifera* L. (Hymenoptera: Apidae) populations of Turkey inferred from microsatellite analysis. *Journal of Apicultural Research*, 46: 50–56

Borsuk, G., Olszewski, K., Strachecka, A., Paleolog, J. & Kasperek, K. (2012) Genetic and morphometric variation of the *Varroa destructor* developing in standard and small comb cells. *Veterinary medicine Science and Practice*, 68: 599–602.

De la Rúa, P., Galian, J., Serrano, J. & Moritz, R.F.A. (2001) Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. *Molcular Ecology*, 10: 1733–1742

De la Rúa, P., Radloff, S., Hepburn, R. & Serrano, J. (2007) Do molecular markers support morphometric and pheromone analyses? A preliminary case study in *Apis mellifera* populations of Morocco. *Archive Zootechnia*, 56: 33–42.

Delfinado–Baker, M.D. (1984) The nymphal stages and male of *Varroa jacobsoni* Oudemans a parasite of honey bees. *International Journal of Acarology*, 10 (2): 75–80.

De Jong, D. (1997) Mites: *Varroa* and other parasites of brood. *In*: Morse, R.A. & Flottum, K. (Eds.), *Honey bee pests, predators, and diseases*. 3rd edition, Cornell University Press, Ithaca, pp. 279–328.

Franck, P., Garnery, L., Solignac, M. & Cornuet, J.M. (1998) The origin of West European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. *Evolution*, 52: 1119–1134.

Franck, P., Garnery, L., Solignac, M. & Cornuet, J.M. (2000) Molecular confirmation of a fourth lineage in honeybees from the Near East. *Apidologie*, 31: 167–180.

Gruber, K., Schöning, C., Otte, M., Kinuthia, W. & Hasselmann, M. (2013) Distinct subspecies or phenotypic plasticity? Genetic and morphological differentiation of mountain honey bees in East Africa. *Ecology and Evolution*, 3(10): 3204–3218.
DOI:10.1002/ece3.711

Giménez Martínez, P., Mendoza, Y., Invenizzi, C., Fuselli, S., Alonso Salces, R., Fernández Iriarte, P. & Maggi, M. (2017) Morphometric correlation between *Apis mellifera* morphotypes

(Hymenoptera) and *Varroa destructor* (Acari) from Uruguay. *Journal of Apicultural Research*, 56(2): 122–129.

Jensen, A.B., Palmer, K.A., Boomsma, J.J. & Pedersen, B.V. (2005) Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. *Molcular Ecology*, 14: 93–106.

Aude, K.E., Armand, P., Francoi A., Charlemagne, G., Georg, G., Manuelle, T. & Lamine B.-M. (2016) Morphometric characterization of parasite *Varroa* sp. of bee *Apis Mellifera* L. in Benin. *European Scientific Journal*, 12(33): 221–234.
DOI: 10.19044/esj.2016.v12n33p221.

Lashkari, M.R., Sahragard, A., Manzari, S., Mozaffarian, F. & Hosseini, R.A. (2013) Geometric morphometric study of the geographic populations of Asian citrus psyllid, *Diaphorina citri* (Hem.: Liviidae), in Iran and Pakistan. *Journal of Entomological Society of Iran*, 33 (2): 59–71.

Le Conte, Y., Ellis, M. & Ritter, W. (2010) *Varroa* mites and Honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41: 353–363.

Maggi, M.D., Sardella, N.H., Ruffinengo, S.R. & Eguaras, M.J. (2009) Morphotypes of *Varroa destructor* collected in *Apis mellifera* colonies from different geographic locations of Argentina. *Parasitology Research*, 105: 1629–1636.

Miguel, I., Baylac, M., Iriondo, M., Manzano, C., Garnery, L. & Estonba, A. (2011) Both geometric morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera* M evolutionary branch. *Apidologie*, 42: 150–161

Mozaffarian, F., Sarafrazi, A. & Ghanbalani, G.N. (2007) Host plant-associated population variation in the carob moth *Ectomyelois ceratoniae* in Iran: A geometric morphometric analysis suggests a nutritional basis. *Journal of Insect Science*, 7: 1–11.

Oleksa, A. & Tofiliski A. (2014) Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. *Apidologie,* 46: 49–60.
DOI: 10.1007/s13592-014-0300-7

Radloff, S.E., Hepburn, H.R., Hepburn, C. & De la Rúa, P. (2001) Morphometric affinities and population structure of honey bees of the Balearic Islands (Spain). *Journal of Apicultural Research*, 40: 97–104.

Rahmani, H., Kamali, K., Saboori, A. & Nowzari, J. (2006) Report and survey of morphometric characteristics of *Varroa destructor* (Acari: Varroidae) collected from Honeybees in Tehran Province, Iran. *Journal of Agricultural Science and Technology*, 8: 351–355.

Rohlf, F. J. (2010a) *TpsDig v2.16. Free software available*. Available on: http://morphometrics.org/morphmet.html (accessed on 22 June 2011).

Rohlf, F. J. (2010b) *TpsRelw v1.49. Free software available*. Available on: http://morphometrics.org/morphmet.html (accessed on 22 June 2011).

Rosenkranz, P., Aumeier, P. & Ziegelmann, B. (2010) Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103: 96–119.

Sarafrazi, A., Loxdale, H.D., Hemingway, J., Abdollahi, G. & Murray, D.A. (2004) Host plant associated variation and sexual dimorphism in size and shape in Iranian geographic populations of sunn pest, *Eurygaster integriceps* Puton. *Second International Conference on Sunn Pest*, *Aleppo*, *Syria*: *ICARDA*, p. 18.

Schachter-broide, J., Dujardin, J.P., Kitron, U. & Gürtler, R.E. (2004) Spatial structuring of *Triatoma infestans* (Hemiptera, Reduviidae) populations from northwestern Argentina using wing geometric morphometry. *Journal of Medical Entomology*, 41(4): 643–649.

روش‌های مورفومتریک سنتی و ژئومتریک مورفومتریک اختلاف بین نمونه‌های کنه *Varroa destructor* (Mesostigmata: Varroidae) در هفت استان ایران آشکار می‌کنند

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چکیده

کنه *Varroa destructor* یکی از مهم‌ترین آفات زنبورعسل در سراسر جهان است. در این پژوهش کنه واروا به دو روش مورفومتریک سنتی و ژئومتریک مورفومتریک مورد ارزیابی قرار گرفتند که در روش مورفومتریک سنتی شش صفت شامل طول و عرض بدن، طول و عرض صفحهٔ پس‌پایی سمت راست، طول صفحهٔ جنسی و طول صفحهٔ مخرجی استفاده شد. هم‌چنین شش لندمارک در سطح شکمی برای اندازه‌گیری‌های ژئومتریک مورفومتریک مورد استفاده قرار گرفت. تعداد ۲۰ و ۱۰ نمونه به ترتیب برای اندازه گیری مورفومتریک سنتی و ژئومتریک مورفومتریک از هفت استان اردبیل، مرکزی، قم، قزوین، البرز، خراسان شمالی و سمنان جمع آوری شد. از سطح شکمی کنه‌ها اسلاید میکروسکپی تهیه و از نرم افزار Digimizer برای اندازه‌گیری مورفومتریک سنتی و نرم افزارهای Tps package برای اندازه‌گیری ژئومتریک مورفومتریک استفاده و داده‌ها با نرم افزار Past تجزیه و تحلیل شدند. برای هر دو روش اندازه‌گیری تجزیه مولفه اصلی (PCA) صورت گرفت. هم‌چنین آنالیز خوشه‌ای برای دسته‌بندی نمونه‌های کنه در استان های مختلف مورد استفاده قرار گرفت. براساس نتایج، اختلاف معنی‌دار بین اندازه‌گیری‌های مورفومتریک و ژئومتریک مورفومتریک در استان‌های مختلف مشاهده شد. استان‌های قزوین و اردبیل بنا بر آنالیز PCA در اندازه گیری مورفومتریک سنتی در یک گروه و بر اساس آنالیز خوشه‌ای استان البرز در گروهی متفاوت نسبت به دیگر استان‌ها قرار گرفت. درحالی‌که نتایج حاصل از اندازه‌گیری ژئومتریک مورفومتریک استان‌های اردبیل و مرکزی در یک گروه و استان‌های خراسان شمالی و سمنان در گروهی دیگر قرار گرفتند. می‌توان این طور استنباط کرد که شرایط جغرافیایی و اقلیمی نقش مهمی در نوع پراکندگی و سازگاری مورفولوژیک کنه واروا در نقاط مختلف ایران ایفا می کند.

واژگان کلیدی: آنالیز خوشه‌ای؛ ژئومتریک مورفومتریک؛ مورفومتریک سنتی؛ تجزیه مؤلفه اصلی؛ کنه واروآ.

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