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TRADITIONAL MORPHOMETRIC STUDY OF FOREWING OF *APIS FLOREA* L. (HYMENOPTERA: APIDAE) IN TROPICAL PROVINCE OF SOUTHWESTERN OF IRAN

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ABSTRACT

The dwarf honey bee regarded as one of the most important insects in southern, southeastern and western provinces of Iran due to crop pollination and honey production. The first step for these main goals is understanding the morphology of this economic Hymenoptera. Diversity of forewing of *Apis florea* was studied by traditional morphometric methods. In total 90 samples were randomly collected from three regions of the Khouzestan province, front wing size and shape of these populations were compared using morphometric methods. Ten important traits including forewing length, width, length/width of forewing ratio, length of anal, A4, D7, G18, G7, K19 angles and cubital index were measured. Morphometric analysis softwares including tps, NTSYS and SPSS were used for every stage. Three *Apis florea* studied populations divided into two main groups based on studied characters which Dezful and Sushtar were in a group and Ramhormoz was in the separate cluster. Among the studied traits of forewing of *Apis florea*, forewing length, length/width of forewing ratio, A4, G18, K19 angles and length of anal of the forewing traits play important role in differentiation.

Keywords: *Apis florea* L., Forewing, Iran, Morphometric

INTRODUCTION

The honeybee is one of the most important social insects known in the world. Honeybees have been largely studied, especially during the last three decades. The importance of this insect is clear and it plays a critical role in the field of agriculture and medicine (production of important substances like: honey, royal jelly, bee wax, propolis, pollen and bee venom). It has a much wider distribution than its sister species, *Apis* and *reniformis* (Ruttner, 1988). First identified in the late 18th century, *Apis florea* is unique for its morphology, foraging behavior and defensive mechanisms like making a piping noise. *A. florea* have open nests and small colonies, which makes them more susceptible to predation than cavity nesters with large numbers of defensive workers. These honey bees are important pollinators. The distribution area of *A. florea* is generally confined to warm climates. In the west, the species is present in the warmer parts of Oman, Iran and Pakistan, through the Indian sub-continent and Sri Lanka (Ruttner, 1988). It is found as far east as Indonesia, but its primary distribution centre is Southeast Asia. Rarely found at altitudes above 1500 m, the bee is absent north of the Himalayas. It is frequently found in tropical forests, in woods and even in farming areas. In southeast Asia it is not rare to find a nest of *A. florea* in a village (Ruttner, 1988).

The common method for the characterization and classification of honey bee subspecies is based mainly on measuring honey bee wing characters, which were considered as strong tool. Various honey bee colonies, races and species were discriminated by employing morphometric analysis (Raina and Kimbu, 2005; Shaibi *et al*., 2009; Rattanawannee *et al*., 2010).

Morphomteric of insects and in particular honey bees is a developing technique. This technique has been developed from the use of body characteristics or standard morphometrics (Rinderer *et al*., 1995) to the use of coordinates of the wing venation characters (Cartesian coordinate) or geometric morphometrics (Tofilski, 2008; Çakmak *et al*., 2011). Due to the importance of the standard and geometric analyses many studies have been performed worldwide on honey bees, and the morphometric analysis methods were reviewed intensively (Bouga *et al*., 2011) while wing venation characters were reviewed by Abou-Shaara

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(2013).

Standard morphometric was used in honey bee studies by measuring different wing angles, indices and distances (Ruttner, 1988) while geometric morphometric was used in honey bee studies by measuring the coordinates of fore wing points to calculate the centroid size (Tofilski, 2008).

The morphometric analysis methods either geometric or standard have been used separately or as integrated methods for subspecies discrimination (Thiripurasundari *et al*., 2017; Tofilski, 2008; Francoy *et al*., 2008; Abou-Shaara, 2013), and regional classification or cluster analysis (Shaibi *et al*., 2009) as well as for other purposes including; testing of races purity (Radloff *et al*., 2003; Miladenovic *et al*., 2011), the prediction of colonies productive characteristics (Mostajeran *et al*., 2006) and to monitor the changes within honey bee population over time (Abou-Shaara, 2013) beside other reasons.

The dwarf honey bee morphometrics were study by several researches (Bhandari, 1983; Ruttner *et al*., 1995; Tahmasebi *et al*., 2002; Chaiyawong *et al*., 2004; Ozkan *et al*., 2009; Haddad *et al*., 2009; Hepburn *et al*., 2011; Al-Kahtani and El-Kazafy, 2014; Zewdu *et al*., 2016;; Thiripurasundari *et al*., 2017).

The right fore and hind wings were used by some authors during their morphometric analysis (Miladenovic *et al*., 2011 and Abou-Shaara, 2013) while left wings were used by others (Tofilski, 2008). Fluctuating asymmetry and directional asymmetry were studied by some authors (Smith *et al*., 1997 and Schneider *et al*., 2003).

This research was carried out on three population of *A. florea* in Khouzestan provinces in southwestern of Iran.

MATERIALS AND METHODS

Sampling: Samples of honey bee workers were collected from different cities of Khouzestan Province in south western of Iran (Table 1) and subsequently killed at -20°C and dissected by using forceps to separate forewings. Separated right wings were slide to obtain wing images (Table 2) then the following investigations were performed.

Table 1

Geographical positions of sampled population.

Code	Sample places	Geographic coordinates	Above mean sea level (m)
RMZ	Ramhormoz	N 31.26308854 E 49.627368450	184
DZE	Dezful	N 32.416486682 E 48537597656	153
SHTR	Shushtar	N 32.14340533 E 48. 34087234	70

ForeWing Characteristic: Forewing length and width, length/width ratio, anal length, cubital index and wing angles (A4, D7, G7, G18 and K19) were measured by Image Tool 3.0 program. The mean values of measurements of 30 worker bees in each colony were calculated.

Table 2

Some studied traits of *A. florea* L. forewing.

Code	Studied traits
X1	Fore Wing Length (FWL)
X2	Fore Wing Width (FWW)
X3	Length/Width ratio (L/W ratio)
X4	D7 angle
X5	A4 angle
X6	G18 angle
X7	G7 angle
X8	K19 angle
X9	Length of anal (AL)
X10	Cubital Index (CI)

Data analysis: Analysis of Variance (ANOVA) was performed and means of fore wing were compared by using L.S.D.0.05 to identify significant differences between the three populations. To discriminate the honey bee populations based on morphometric characters, multivariate statistical analyses (MANOVA) were performed on mean values of measurements. Percentages of correct identification were calculated by Discriminant Function Analysis (DFA). ANOVA, MANOVA and DFA calculated by SPSS ver 19 software. Cubital index calculated by Beemorph software (http://www.hockerley.plus.com)

A UPGMA cluster analysis (Rohlf, 2004) was performed on Mahalanobis distances of morphometric data to show the clustering among honey bee populations by NTSYS program package (STAND, SMINT, SAHN, TREE program).

Table 3

Analysis variance of means of *A. florea* traits in three studied populations.

Places	Sample number	FWL (mm)	WWL (mm)	L/W ratio	A4 angle	D7 angle	G18 angle	G7 angle	K19 angle	AL (mm)	CI
SHTR	30	2584.24a	886.42a	2.916a	32.56b	91.23a	96.12a	23.31a	63.28a	1411.66ab	2.65ab
RMZ	30	2633.06a	895.49a	2.936a	34.02a	88.53b	93.81b	23.57a	61.04a	1379.01b	2.83a
DZE	30	2663.67a	901.46a	2.955a	32.93ab	90.57a	96.87a	23.25a	62.45a	1449.51a	2.57b

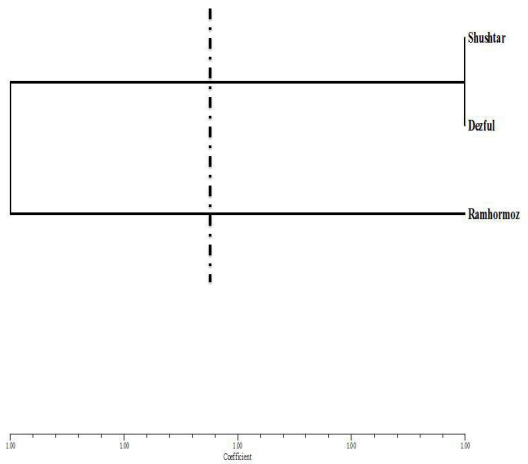

Fig. 1.

UPGMA phenogram of *A. florea* populations from different geographic regions based on standard morphometrics

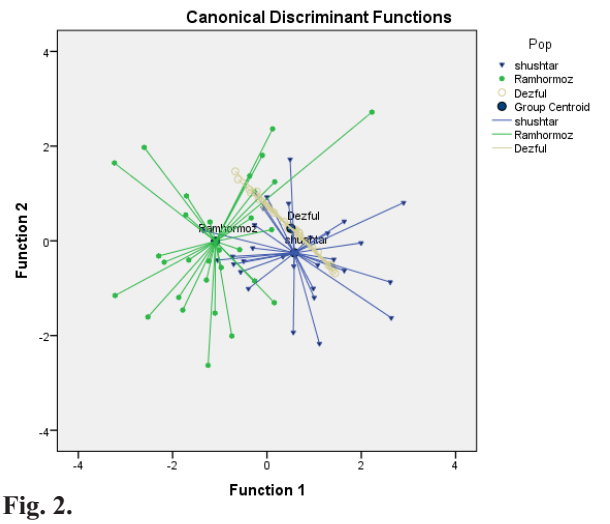

Fig. 2.

Scatter diagram with respect to first and second canonical discriminant functions

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) of morphological characters showed that 5 out of 10 characters displayed statistically significant differences among honey bee populations ($P < 0.05$). The most common preferable characters to study honeybee biodiversity have been cubital index, fore wing lenght and some wing venation angles (Kandemir *et al*., 2000; Güler *et al*., 2010). Analysis variance of ten traits of fore wing showed that length, width and its ratio, G7 and K19 angeles did not significant difference among three *A. florea* populations (Table 3). In general, Ramhormoz specimens traits showed more variance compared with two other populations. Wing characters were found to be affected by different factors e.g. temperature and season (Mattu and Verma, 1984) and bee age (Herbert *et al*., 1988).

Three *A. florea* studied populations divided into two main group based on studied characters which Dezful and Sushtar were in a same group and Ramhormoz were in the separate cluster, weather condition and geographic distance of Ramhormoz from other two cities regarded as one of main factor in this separation. Ruttner (1988) obtained three morphoclusters for *A.florea* including 1. India and Sri lanka, 2. Thailand, 3.Oman, Pakistan and Iran. Similar climate condition and same geographic flore led to same cluster groups in studied researches. Tirgari (1971) reported that *A. florea* migrated to areas with maximum sun light exposure in autumn and return to dense foliage with less sunlight exposure for comb production. Same floral components of Dezful and Shushtar cities inserted these two groups in one cluster (Figs. 1 and 2).

Canonical variate analysis of wing measurements plotting function 2 against function 1 could clearly separate the 3 honey bee populations. The results of this study clearly showed that measurements of size and angle characters can be sufficient to identify or discriminate dwarf honey bee populations according climate condition.

The new approach geometric morphometric is much more effective than traditional morphometric in discrimination/ identification of dwarf honey bee populations in the world

(Tofilski, 2008; Francoy *et al*., 2008). We suggest more studies on all traits of this species in all provinces during all season.

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AUTHORS' CONTRIBUTION

Rouhollah Radjabi*, Azadeh Kalantar-Hormozi and Maryam Seifouri conceived the idea. Rouhollah Radjabi, Azadeh Kalantar-Hormozi and Maryam Seifouri conducted the experiment. Rouhollah Radjabi1, Azadeh Kalantar-Hormozi and Maryam Seifouri conducted the data analysis.

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