

Morphological discrimination of geographical populations of *Macrosiphoniella sanborni* (Gillette, 1908) (Hem.: Aphididae) in Iran

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Abstract. Multivariate morphometric studies are often carried out on different aphid taxa in order to analyze population variation and species discrimination in relation with different factors such as host plant, temperature and geography, which have significant effect on aphid morphology. The chrysanthemum aphid, *Macrosiphoniella sanborni* (Hemiptera: Aphididae), is a widespread pest on cultivated chrysanthemum throughout the world. In the present study 21 morphological characters in 11 populations of *M. sanborni* collected from several geographic locations in Iran were examined. Multivariate morphometric techniques, canonical variates analysis (CVA) and cluster analysis were used to determine whether these populations can be reliably morphologically discriminated. The results showed such discrimination between populations. The Kermanshah population (located in western Iran) clearly separated from other populations, indicating the presence of a morphologically distinct group. It could be concluded that presence of a geographic barrier and climatic conditions are among the most important factors which contribute to the differentiation between populations. Other complimentary studies such as genetic investigation will provide a better understanding of the evolutionary implications of the population structure.

Key words: *Macrosiphoniella sanborni*, Chrysanthemum aphid, Morphometric analysis, Geographical variation, Iran.

Introduction

Aphids of the subfamily Aphidinae are among the most important agricultural crop pests (Blackman & Eastop 1984, 2000). Some described species are comprised of complexes of morphologically closely related species, biotypes, host-races or subspecies (Blackman & Paterson 1986, Blackman & Spence 1992, Jörg & Lampel 1995, Mopper & Strauss 1997, Jensen 1998, Clements et al. 2000, Jensen & Holman 2000, Poullos et al. 2007). Lack of distinguishable morpholo-

gical characters, the presence of sibling species and intraspecific variation (Mopper & Strauss 1997) among members of this group make their identification and separation based on morphological characters alone very difficult (Jensen 1997, Margaritopoulos et al. 2006). These difficulties have made it necessary for taxonomists to look for other techniques such as morphometrics in order to delimit species and populations (Powell & Walton 1989, Smith et al. 1998).

Multivariate morphometric analyses can be used either to group organisms together

(cluster) or separate them (discriminate) and provide statistical methods which allow the study of interrelationships between several variables (Sneath & Sokal 1973, Sokal & Rohlf 1981, Reyment et al. 1984, James & McCulloch 1990). Multivariate morphometric studies have been carried out on different aphid taxa to analyze population variation and species discrimination (Pungerl 1986, Blackman & Paterson 1986, Tizado & Nieto Nafria 1994, Rubin-de-Celis et al. 1997, Rakauskas 1998, Margaritopoulos et al. 2000, Margaritopoulos et al. 2006, Poullos et al. 2007). Shape of body and the relative length of appendages are among the most important taxonomic characters in aphids (Illharco & van Harten 1987). Different factors such as host plant (Margaritopoulos et al. 2000, 2007, Wool & Hales 1997), temperature (Blackman & Spence 1994) and geography (Sokal et al. 1980, Riska 1985) have significant effect on aphid morphology.

Morphometric approach can provide useful information at the subspecific level for investigating the geographical structure and host plant adaptation of insect populations (Blackman 1987, Blackman & Brown 1991, Via & Shaw 1996). Numerous authors have stressed the relationships between morphological characters and environmental factors in insects (Bodenheimer & Swirski 1957, Anderson 1973, Hespeneide 1973, Powell 1974, Wool 1977, Haas & Tolley 1998). Most of these studies suggest a correlation between morphological variation and environmental factors. The country of Iran covers an area of 1,623,779 km² and constitutes a large part of the Iranian plateau. The diverse topography and climate of Iran, from cool and humid mountains to hot and dry deserts, makes the country very

well suited ecologically for taxonomic studies (Zehzad et al. 2002).

The chrysanthemum aphid, *Macrosiphoniella sanborni* (Gillette, 1908) (Hemiptera: Aphididae), is a widespread pest on cultivated chrysanthemum throughout the world. It is an anholocyclic species with East Asian origin (Heie 1995, Blackman & Eastop 2000, Blackman & Eastop 2006). *Macrosiphoniella sanborni* is a vector of chrysanthemum viruses, vein mottle and virus B (Blackman & Eastop 1984). It is a serious pest of chrysanthemum in Iran which feeds mainly on young leaves and developing flower buds and can become very abundant on them. In the case of a high infestation, the aphid causes significant damage which results in deformation and disturbance of flower development. All of these factors together cause significant economic damage to chrysanthemum crops by decreasing their beauty and the value of cut flowers (Zahedi 1999).

So far no morphometric study had been carried out on populations of *M. sanborni*, so little is known about the morphological variation in this taxon. In the present study morphological characters in populations of *M. sanborni* collected from several geographic locations were examined. The aim of this study was to investigate morphological variation between geographical populations of *M. Sanborni* in Iran using multivariate morphometric analysis of distance measurements among morphological landmarks.

Materials and methods

The study was based on chrysanthemum aphid samples collected from different geographic locations in Iran from 12 to 23 October 2007. The studied material consisted of 269 apterous vivi-

parous females from 11 locations (Fig. 1). Aphids were collected from different bushes from at least three different places in each location (cities). Since the chrysanthemum is considered a decorative plant, there are several productive centres in different parts of the country that reproduce this plant and send it to other regions. There is a possibility that cultivated chrysanthemums available in different cities have been transferred from mentioned centres, so the aphids may be transferred along with the host plant to those places. Thus, the aphid samples were collected from locations in which they had no imported chrysanthemum plants for several years or at least aphid samples were collected from perennial chrysanthemum plants in order to be sure that the collected aphids belong to the same place. Aphids

were collected gently from different colonies in each location using paint brush. They were preserved in tubes filled with ethyl alcohol (75%) until they could be mounted on slides according to the method described by Ilharco (*Pers. comm.*). In total, 21 morphological characters were measured for each specimen (Table 1). The measurements were done according to Ilharco & van Harten (1987), using phase contrast microscope (Olympus CH2) fitted with a calibrated micrometer eyepiece.

Canonical variates analysis (CVA) was performed using the SPSS ver. 12.0 (SPSS Inc., 2003) statistical package. The interrelationship among populations from different geographic locations was examined using hierarchical cluster analysis (Sneath & Sokal, 1973) based on squared Euclidean distances.



Figure 1. Collection sites of *Macrosiphoniella sanborni* populations in Iran. The geographic positions and elevations are represented.

Table 1. Morphometric characters used for analysis in populations of *Macrosiphoniella sanborni*.

Character No.	Acronym	Character
1	BL	Body length
2	ANTIII	Antennal segment III length
3	ANTIV	Antennal segment IV length
4	ANTV	Antennal segment V length
5	ANTVIb	Basal part of antennal segment VI length
6	PT	Processus terminalis
7	ANT	Antenna length
8	BDANTIII	Basal diameter of antennal segment III
9	LHANTIII	Longest hair on antennal segment III length
10	URS	Ultimate rostral segment length
11	BDURS	Basal diameter of ultimate rostral segment
12	2HT	2 nd segment of hind tarsus length
13	SIPH	Siphunculus length
14	PRSIPH	Polygonal reticulation of siphunculus length
15	BDSIPH	Basal diameter of siphunculus
16	Cauda	Cauda length
17	BDCauda	Basal diameter of cauda
18	HFEM	Hind femora length
19	HTIB	Hind tibia length
20	CaudaH	Number of caudal hairs
21	RHIN	Number of rhinaria on antennal segment III

Results

Individual specimens were projected on the first and second canonical variates (CV1 and CV2) using the stepwise method. A summary of canonical discriminant functions considering the 21 morphological variables for apterous females of *M. sanborni* across the 11 geographical locations is given in Table 2, including the level of variance and the significant for three functions. Low values of Wilks' Lambda test (the smaller the Lambda, the greater difference among populations) and statistically significant

Chi-square values (Table 2) show that the first three functions in particular are significant predictors. The first function accounted for 53.1% of total variation and the second function for 28.5% which together accounted for 81.6% of the total variation. Table 3 shows the standardized canonical discriminant function coefficients that determine the relative importance of the characters in discriminating the populations. The characters SIPH, BDURS and BDSIPH show the highest magnitudes at CV1 whereas the character 2HT shows the highest magnitudes at CV2 (Table 3). The

Table 2. Canonical discriminant functions of *Macrosiphoniella sanborni* populations across Iran.

Function	Eigenvalue	Variance explained (%)	Cumulative variance (%)	Wilks' Lambda	Chi-square	df	Sig.
1	2.961	53.1	53.1	.043	412.000	50	.000
2	1.590	28.5	81.6	.171	231.666	36	.000
3	.757	13.6	95.2	.442	106.998	24	.000

result of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership indicates that in Zahedan and Mahalat populations 48%, in Shiraz and Khoramabad populations 47.8%, in Kerman population 56.5%, in Yazd population 76%, in Isfahan population 68%, in Kermanshah population 60%, in Hamedan population 28%, in Khoy population 24% and in Karaj population 56% of individuals are reclassified correctly into their original groups.

Table 3. Standardized canonical discriminant function coefficients for the first three functions on the basis of 11 *Macrosiphoniella sanborni* populations.

Characters	Function		
	1	2	3
URS	.038	-.101	.885
BDURS	-.535	.543	.349
2HT	-.338	.753	-.558
SIPH	.907	-.281	.453
BDSIPH	.469	.321	-.773

The dendrogram of hierarchical cluster analysis based on data of squared Euclidean distances using average linkage between populations of *M. sanborni* across 11 geographic locations is shown in Figure 2.

Two main branches are shown; the first one combines Kermanshah, Khoy and Hamedan populations, the second one combines the rest of populations. In the first cluster Kermanshah population showed a large phenotypic distance from two other populations in the cluster. In the second cluster Isfahan and Mahalat grouped with each other while Yazd population is separated from other populations in the cluster. Other populations that are grouped with each other are Zahedan and Kerman as well as Shiraz and Khoramabad. Considering the presence of important morphological variation between some populations located in various geographical locations, four populations which located along South-east to North-west of Iran (Zahedan, Yazd, Isfahan and Kermanshah) were chosen for analysis. With these, additional analysis was performed to see if a better between-group separation could be achieved. Therefore a second CVA was performed using samples from Zahedan, Yazd, Isfahan and Kermanshah populations, which were found the most distinct ones in the first CVA. A graphical presentation of the first two canonical variates is shown in Figure 3, indicating the degree of inter-population differences. CV1 explains 52.9% of the total variation of the data. The first two CVs accounted for 93.2% of total variation in the data and clearly separated the aphids into

four groups. The morphological characters with the highest CV1 coefficients were SIPH, BDSIPH and BL. The character with the highest CV2 coefficient was HTIB (Table 4). Table 5 shows the result of reclassification of individuals according to the original discriminant functions. The correct self-classification rate is 91.7% for Zahedan population, 92.0% for Yazd and Kermanshah populations and 100% for Isfahan population. As a whole 93.9% of the original grouped cases were correctly classified (Table 5).

The dendrogram of cluster analysis based on data of squared Euclidean distances using average linkage between four populations of *M. sanborni*, indicating morphological distances between populations, reveals two main branches; the first represents Kermanshah population and the other combines Zahedan, Yazd and Isfahan populations. Zahedan and Yazd populations are grouped with each other while Isfahan population is separated. Kermanshah population shows a very large pheno-

typic distance from the other three populations.

Table 4. Standardized canonical discriminant function coefficients for the first three functions on the basis of four *Macrosiphoniella sanborni* populations.

Characters	Function		
	1	2	3
BL	-.633	-.214	1.313
BDURS	-.410	.424	.392
SIPH	.862	-.655	1.116
BDSIPH	.711	.001	-.377
HTIB	.115	1.383	-1.814

Discussion

There are no studies concerning multivariate analysis of morphological characters in *M. sanborni* associated with chrysanthemum crops. The aim of this study was to investigate morphological variation in 11 *M. sanborni* populations collected from various

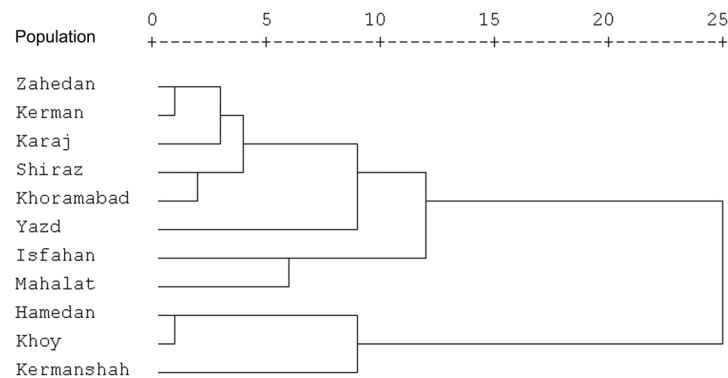


Figure 2. Dendrogram of hierarchical cluster analysis based on squared Euclidean distances using average linkage between populations of *Macrosiphoniella sanborni* from Iran.

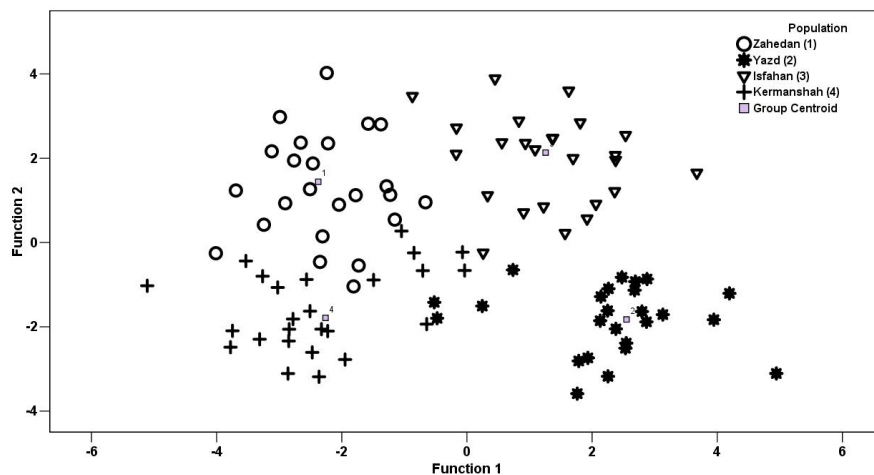


Figure 3. Scatter-plot of the scores of the first two canonical variates for four populations of *Macrosiphoniella sanborni* from Iran.

Table 5. Self-classification rates from the discriminant analysis of *Macrosiphoniella sanborni* from four locations. Percent of individuals correctly classified are in the diagonal. Percentage of misclassifications into each group is also reported.

Population	Zahedan	Yazd	Isfahan	Kermanshah
Zahedan	91.7	.0	.0	8.3
Yazd	.0	92.0	.0	8.0
Isfahan	.0	.0	100.0	.0
Kermanshah	8.0	.0	.0	92.0

geographic locations in Iran. The results of this morphometric analysis reveal size variation in some morphological characters such as body length, SIPH length, 2HT length and antennal length among geographic populations of *M. sanborni*.

Considering the canonical variates analysis and cluster analysis on all 11 populations, despite the occurrence of some overlap between individuals of some populations, it is clear that these are separated from each other and that populations lo-

cated in the same geographical regions are grouped together. The reason for these groupings are probably the geographical proximities and also similar climatic conditions of locations. For example, populations from Isfahan and Mahalat cities are close to each other and are grouped together; similarly, populations from Kerman and Zahedan, located in South-east Iran, are grouped together. These cities have relatively similar climatic conditions. Considering the second CVA analysis based on four populations,

(Zahedan, Yazd, Isfahan and Kermanshah), a complete separation can be seen on both CVs (except a few overlapping) (Fig. 3). The result of cluster analysis again confirms this separation between geographic populations which are located on a cline from south-east to north-west Iran. In a morphometric study, the geographic variation of 33 morphological characters of the gall-forming aphid, *Pemphigus populicaulis*, was investigated in Eastern North America (Sokal et al. 1980). Canonical variates analysis has been extensively used as a powerful tool to discriminate and resolve the taxonomic status among related insect species and closely-related aphid taxa (Rakauskas 1998, Sanmartin & Martin Piera 1999, Blackman & de Boise 2002, Williams & Langor 2002, Barbagallo & Cocuzza 2003, Margaritopoulos et al. 2006, Poullos et al. 2007).

The result of the present study demonstrates that according to CVA analysis on four populations (Zahedan, Yazd, Isfahan and Kermanshah), the SIPH, BDSIPH, BL and HTIB were the most important characters that showed differentiation between the four mentioned populations in Iran.

The first two canonical variates for four *M. sanborni* populations accounted for 93.2% of total variation, so it can be concluded following Reyment et al. (1984) that the variation more than 80% is biologically meaningful in this case. The present study demonstrates that morphometric analysis of morphological characters can discriminate among geographic groups based on size variation and classify individuals of *M. sanborni* populations in Iran. The above four mentioned populations can be separated into two morphological groups. The first group contains Zahedan, Yazd and Isfahan populations and the second group includes

Kermanshah population, located on a line extending from South-east to North-west of Iran. Intra-specific geographic variation is widespread in animal populations that have a large geographic distribution (Mayr 1963). Every local population is under influence of physical and biotic factors of its environment giving rise to the adaptation to local climatic conditions (Ricklefs & Miles 1994).

Several factors can affect the morphology of aphids such as temperature (Blackman & Spence 1994), host plant conditions (Wool & Hales 1997, Dixon 1998, Margaritopoulos et al. 2000) and geography (Wool 1977, Sokal et al. 1980, Riska 1985). Considering the dendrogram of cluster analysis in four populations (Zahedan, Yazd, Isfahan and Kermanshah), Kermanshah is completely separated which might indicate the presence of a morphologically different group. There are several reasons to explain this differentiation. Firstly, the presence of Zagros Mountains, extending from North-west to South-east Iran. These mountains can be regarded as an important geographic barrier separating Kermanshah population from the other three populations. Kermanshah city is located on the west side of Zagros Mountains so the aphid samples of this location have been completely separated from the other three populations. Secondly, there are differences in climatic condition between Kermanshah and the other locations. Kermanshah city has a temperate mountainous climate and longer winter (with 91 frosty days in a year [Darvich 2001] whereas the other cities have semi-desert climatic condition. This can lead to restriction in the seasonal activity of aphids. The annual rainfall in Kermanshah is also more than in the other three locations (486mm) (Darvich 2001), being under the influence of the Mediterranean climate and

warm whether of Iraq.

The second cluster comprises Zahedan, Yazd and Isfahan populations. Isfahan population is separated. There are several factors which could explain morphological differences between this population in comparison to Yazd and Zahedan populations. Firstly, Isfahan city is located approximately in the west of the Iranian central plateau. This place has semi-desert climate which has more annual rainfall (116 mm) in comparison to Yazd (62 mm) and Zahedan (83 mm) cities (Darvich 2001). Secondly, one of the most important permanent rivers, Zayandeh-rud, is passing Isfahan city, contributing to a higher rate of relative humidity in this area than in Zahedan and Yazd (Darvich 2001). So the effect of climatic condition on morphology of aphids in this area is considerable.

Several entomological studies suggest that latitude is among the most important factor affecting morphological characters of insects (Stalker & Carson 1947, Bryant 1977, Haas & Tolley 1998). These studies suggest that increasing latitude is in correlation with increasing body size and fecundity. Increased fecundity is advantageous in high latitudes because of a relatively short reproductive season. Zahedan, Yazd, Isfahan and Kermanshah cities are located on a straight line (approximately 1400 Km distance), with increasing latitude, extending from South-east to North-west of Iran. However our results are not in accordance with these studies since in our study body size and the length of appendages are not in association with latitude. One explanation is that our study was developed in a relatively fine-scale geographical location while we may get different result on a larger scale. In *Baizongia*, the gall forming aphid, body size is larger in the cooler northern regions than

in the south (quoted from Wool 1977). This study is not in accordance with our results since the individual aphids from Kermanshah population have smaller body size. Kermanshah city has temperate mountainous climate and the weather is cold during winter. Although the interaction of evolutionary forces which led to differentiation among the four Iranian populations is still not established at this time, one explanation for the observed geographical divergence in morphological characters among the four populations of *M. sanborni* is that they are not homogeneous morphological populations and represent two spatially separated groups. Therefore these two geographical forms of *M. sanborni* may be considered as cryptic species and the probable explanation is that morphological differentiation among mentioned geographic forms may be due to climatic and environmental factors. Other studies beside morphology, e.g. life cycle, host adaptation and performance in regards to environmental conditions are necessary in order to clarify these populations as cryptic species. These studies, especially if supplemented by genetic investigations using molecular genetic markers in association with other complementary studies can lead to a better understanding of the evolutionary implications of the population structure and of its geographical, environmental and historical determinants.

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