

Wing size and shape variation of *Phlebotomus papatasi* (Diptera: Psychodidae) populations from the south and north slopes of the Atlas Mountains in Morocco

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ABSTRACT: The wing shape and size morphology of populations of the medically important phlebotomine sand fly, *Phlebotomus papatasi*, were examined in two endemic (south of the Atlas Mountains) and nonendemic (north of the Atlas Mountains) foci of cutaneous leishmaniasis by using geometric morphometrics in Morocco. Although it is present in all of Morocco, *P. papatasi* is the main vector of *Leishmania major* in only southern part of the Atlas Mountains. There are four major mountain ranges that serve as geographical barriers for species distribution in the study area and at least four gaps were recognized among these barriers. We found statistically significant differences in wing shape morphology between southern and northern populations. Analysis clearly recognized two main groups of populations on both sides of the mountains. The graphical depiction of Principal Component Analysis (PCA) and Canonical Variates Analysis (CVA) confirmed our morphometric study suggesting that the difference in wing morphology between the populations indicates that the population of *P. papatasi* shows phenotypic plasticity in the study area. According to centroid size analyses, which were used as measures of wing size differences among different sites, the north population of *P. papatasi* had relatively larger wings than the south population. *Journal of Vector Ecology* 37 (1): 137-147. 2012.

Keyword Index: *Phlebotomus papatasi*, geographic variation, geometric morphometrics, Morocco.

INTRODUCTION

Leishmaniasis is a major vector-borne disease endemic in Morocco, with two clinical types that exist in the country. Visceral leishmaniasis (VL), caused by *Leishmania infantum* and transmitted by species of the subgenus *Larrousius*, is widespread in the country and is more frequent in the northern part. Cutaneous leishmaniasis (CL) is widely distributed in Morocco and caused by either *Leishmania tropica* or *L. major*. CL due to *L. tropica* is widespread in the semi-arid regions with *Phlebotomus* (*Paraphlebotomus*) *sergenti* Parrot as the vector. CL due to *Leishmania major* is localized in the south, particularly in the per-arid area and southern part of the Atlas Mountain ranges. This CL has been known since 1914 (Foley and Vialatte 1914) and is caused by *L. major* zymodeme MON-25 (Rioux et al. 1986) transmitted by *Phlebotomus* (*Phlebotomus*) *papatasi* Scopoli (Guernaoui et al. 2006). Leishmaniasis is still a growing public health problem in Morocco. According to the Moroccan Ministry of Public Health, 359 cases of CL caused by *L. major*, 1,669 cases of CL by *L. tropica*, and 116 cases of VL by *L. infantum* were reported in 2001. In 2006, 2,240 new cases were reported.

Previous studies in Morocco (Rioux et al. 1984, 1997, Rioux 2001, Rispail et al. 2002) identified 17 species belonging to two genera, *Phlebotomus* and *Sergentomyia*,

and showed that the distribution of sand flies was related largely to the bioclimate (Guernaoui et al. 2006). There are five different bio-climatic zones in the country as Saharan, arid, semi-arid, sub-humid, and humid (Rioux et al. 1984). *P. papatasi*, the proven vector of *L. major*, is one of the dominant and widespread species in Morocco. In fact, *P. papatasi* is the dominant species in the arid plain in the southern part of the Atlas Mountains (Rioux et al. 1984). The high occurrence of *P. papatasi* in the lowland and its low frequency in the mountains could be explained by its preference for arid and peri-arid areas (Guernaoui et al. 2006). Moreover its density increases with increased aridity (Croset et al. 1974). Along with bioclimatic zones, altitude also acts on *P. papatasi* distribution by the habitats, relief, and by the climatic gradient on present in Morocco. According to Guernaoui et al. (2006) *P. papatasi* shows a negative correlation with the altitude. The density of this species is very important at lower altitudes between 400 and 600 m, and lower at the other altitudes. It is absent from 1,200 m altitude. Though *P. papatasi* populations exist throughout Moroccan territory, Gaud (1947) clearly showed that the biggest part of the population is distributed in the southern part of the Atlas ranges with numerous subpopulations. Rioux and de La Rocque (2003) confirmed this result. Rioux and de La Rocque (2003) also demonstrated that the main reservoir of *L. major*, *Meriones shawi*, is also present

in the whole country; however CL caused by *L. major* is only transmitted by the southern populations of *P. papatasi*. The questions are now whether there are any differences in terms of phenotypic or genetic features between southern and northern populations of *P. papatasi* and if so, whether phenotypic differences by themselves could be effective in transmitting *L. major* by only the southern populations of *P. papatasi*.

Size and shape variation are important in understanding ecological relationships because they generally correlate with the biology of many species. There has been considerable interest in the determinants of body size and how size varies through time and space. Geographic variation in body size is particularly controversial because both the patterns and their underlying mechanisms are matters of contention (Chown and Klok 2003). Similar to size, biological shape is one of the most conspicuous aspects of an organism's phenotype and provides a link between the genotype and the environment (Ricklefs and Miles 1994). In sand flies, significant morphometric wing shape variations were found in populations from different geographic regions, forming clusters or latitudinal clines in previous studies (Kasap and Alten 2006, Aytekin et al. 2007, Aytekin et al. 2009).

The main goals of this study were to utilize geometric morphometric approaches, which have the highest statistical power among alternatives (Rohlf 2000), to understand the possible phenotypic differences of *P. papatasi* populations between two geographically and climatologically distinct areas and to determine the importance of the Atlas Mountains as a barrier between southern and northern populations. It was hypothesized here that the body size of *P. papatasi* might change with bioclimatic differences between both sides of Atlas Mountains.

MATERIALS AND METHODS

The study area

Field studies were conducted from July to September 2010, in seven locations on southern, and in three locations on northern sides of the Atlas Mountains, Morocco. Table 1 shows information about altitudes, geographical references, bioclimatic and vegetation zones, eco regions and climatic conditions of the sampling stations located in the north (N) and south (S) portions of the Atlas Mountains, shown in Figure 1.

Morocco is subject to the different influences of the Mediterranean, the Atlantic Ocean and the Sahara. However, the Atlas, an imposing mountain range separating the northwest and southeast portions of the country, plays an important role in the climate of the area. The climate of Morocco lies between the Mediterranean climatic belt and the Saharan subtropical desert, with high mountain ranges introducing an altitudinal climatic gradient that overlies the latitudinal gradient. The southern slopes of the Atlas Mountains, which divert the moisture-laden Atlantic winds, have a rigorous pre-Saharan climate (desertic), while the northern slopes are relatively cool and well-watered (temperate). There are five different bioclimatic

zones: humid, subhumid, arid, semiarid, and saharian that create vegetation zones and eco-regions (Table 1) across the wetlands, rivers, desert, and mountains (UNESCO-FAO 1963, Rioux et al. 1984).

Sand fly collection and identification

Phlebotomus papatasi adults were collected using CDC miniature light traps (John W. Hock Co. FL, U.S.A.) during summer (July-September) in 2010. On each trapping night, one to three light traps were placed in each of the sampling localities. Light traps were placed 1 to 1.5 m above ground, and they were operated between 18:00 and 06:00 around/inside animal barns and houses used as main sampling stations. In addition, the light traps were also set near rodent burrows, poultry houses, and/or livestock. At the end of the sampling period, specimens from the traps were transferred into 1.5 ml Eppendorf tubes one by one with 96% ethanol.

Prior to mounting, heads, genitalia, and wings of the sand flies were removed. The heads and genitalia were cleared in KOH 19% for 2 h in Marc-Andre solution (chloral hydrate/acetic acid), then dehydrated and placed in creosote (beech wood oil) for a night. They were mounted in Euparal (Abonnenc 1972). The identification was made by examining the morphology of male genitalia, female spermathecae, and pharynges using the standard keys of Perfiliew (1968), Abonnenc (1972), Artemiev (1991), and Killick-Kendrick et al. (1991).

Morphometric analysis

The number of specimens used in the geometric morphometrics analysis is shown in Table 1. All the specimens were screened under a light microscope for the presence of endoparasites to prevent possible variations which might affect the morphometric data (Mayr and Ashlock 1991). The wings were cleaned of scales and using the method of Belen et al. (2004), colored for viewing the veins with the modification of keeping the wings in methylene blue for 6 min at room temperature. They were then placed in 75% alcohol for a few seconds, after which they were transferred to cups containing distilled water for washing. The wings were again put in 75% alcohol for a few seconds and finally soaked in xylene for 2 min for fixation. As last step, all wings were mounted in Euparal on labelled slides. Slides were photographed using a Leica Z16 APOA stereoscopic zoom dissection microscope with DFC 425 digital camera system, digitized, and archived. All specimens were scored by a single experimenter. Photographs were first entered into tps-UTIL1.28 (Rohlf 2007).

A total of 32 specimens was used for the geometric morphometrics analysis (10 for the north and 22 for the south) (Table 1). Rohlf and Slice (1990) advised that for the paired organs, the one on the right side or left side should be used consistently to avoid possible negative allometric effects. Left and right wings for both southern and northern specimens were compared by using centroid sizes of the wings. No statistically significant difference was found between the right and left wings from southern (Wilcoxon-Mann-Whitney, $p=1$) and northern specimens

Table 1. Site description and sampling size of the stations in the study area.

Coordinates	Bioclimatic zones	Vegetation zone	Ecoregions	Annual Mean Precip. (mm)	Annual Mean Temp. (°C)	No. collected	No. used
34°55'N, 2°41' W	semi-arid	thermomediterranean	sclerophyll forests	400-500	21	11	2
28°45'N, 8°54'W	arid	inframediterranean	macaronessian tree	100-200	23	18	3
33° 45'N, 4° 47'W	sub-humid	thermomediterranean	sclerophyll forests	600-700	15.5	12	1
31°33'N, 4°15'W	saharian	maroc saharien	arboreous and steppe	100-200	23.5	7	1
31°13'N, 6°08' W	arid	thermo-mesomediterranean	sclerophyll forests, arboreous, presteppe and steppe	200-300	15.5	24	1
31°22'N, 5°59'W	semi-arid	supramediterranean	high altitude Atlasique steppe	300-400	15.5	15	1
31° 55'N, 4° 24'W	arid and saharian	maroc saharien and thermomediterranean	arboreous and steppe	100-200	18.5	24	13
29° 58'N, 9° 24'W	semi-arid	inframediterranean	macaronessian tree	400-500	15.5	10	2
34° 16'N, 4°22'W	sub-humid	supra-mesomediterranean	humid and sclerophyll forests	700-900	18.5	15	1
31°21' N, 8°4'W	sub-humid	supra-mountain mediterranean	sclerophyll forests	400-600	15.5	31	7

(Wilcoxon-Mann-Whitney, $p=-0.8833$). In this study, the right wings from females were only used in the size and shape analyses. Sixteen landmarks were chosen to be used according to Belen et al. (2004). The data were collected in the form of 2-D coordinates of the 16 landmarks. Landmarks were used for the analysis following the method of Rolf and Slice (1990) (Figure 2). The landmarks are the intersections of wing veins with the wing margin and intersections of cross veins with major veins. All wings were digitized twice in order to reduce the measurement error (z) (Arnqvist and Mårtensson 1998). Scaled, translated, and rotated landmark configurations were made using the GPA Procrustes superimposition method (Bookstein 1991, Rohlf 1993, 1999, Alibert et al. 2001). The coordinates were analyzed using tps-RELW 1.34 (Rohlf 2007) to calculate the eigen values for each principal warp. The variability in wing shape was assessed using the scores obtained for each individual on the first two relative warps by the realization of a principal component analysis (PCA). The relative warps correspond to the principal components and define a shape space in which individuals are replaced (Alibert et al. 2001). To better visualize the shape variation, we added only the deformations on the positive and negative extremes of the first two PCAs on the graph. A Canonical Variates Analysis (CVA) was also performed on landmark data by IMP CVAGEN60 (Zelditch et al. 2004) for comparing the populations collected from the south and the north, using group membership information and Barlett's test to examine differences among groups. "CVAGEN60" is the preferred version among CVAGEN programs when the sample size is smaller than the number of landmarks used. We used a number of PC axes equal to one less than the specimen count instead of all PC axes, while no variance is lost and difficulties related to matrix inversions in the CVA

are eliminated (Zelditch et al. 2004). The size morphometry of the sand flies were investigated using the centroid sizes of the wings as an estimator with the nonparametric Wilcoxon-Mann-Whitney test. Centroid size is the square root of the sum of squared distances of a set of landmarks from their centroid, i.e., the square root of the sum of the variances of the landmarks about that centroid in x- and y- directions (Bookstein 1991).

The coordinates of the landmarks obtained from tps-DIG were manipulated in Morpheus (Slice 1998) to make comparisons among different populations by means of spline plots. The results were exaggerated twice for a better visualization. Twenty *P. papatasi* specimens from east of Anatolia, Turkey were included in the analysis as an out-group.

RESULTS

Insect wings are the most appropriate structures for geometric morphometric studies (Dvorak et al. 2006). When a PCA was conducted on 16 wing landmarks, the first two PCs summarized 60.67% of the total variances, respectively (PC1; 47.18% and PC2; 13.49%). The first PC suggests some differences in the relative positions of the landmarks regarding the apical part of the wing (Figures 3, 4). Main deformations centered on the medial of the wing on landmarks 1, 9 and 10-12 (Figure 4). The distribution of individuals along the two PCs is shown in Figure 5. PCA shows some overlap among individuals from all *P. papatasi* specimens examined in both sides of the Atlas Mountains. However, specimens of *P. papatasi* from the north tended to cluster together on the positive axis of PC1 suggesting a higher phenotypic distance relative to specimens from the south. When the shape differences of populations

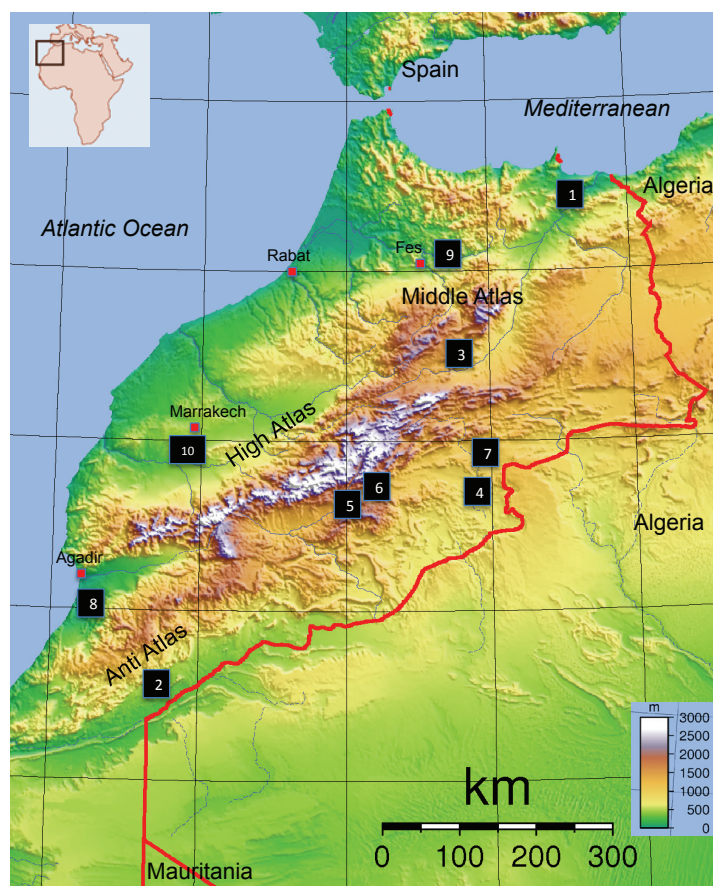


Figure 1. Map of the study area showing the main sampling stations, the main geographical barriers and altitude. 1. Sidi Athmane; 2. Tigmi Jdid; 3. Ait Brahim; 4. Es Sfalate; 5. Mirna; 6. Boumaine; 7. Errachidia; 8. Tafat; 9. Dar Bachir; 10. Akhlij (see Table 1 for geographical references and properties).

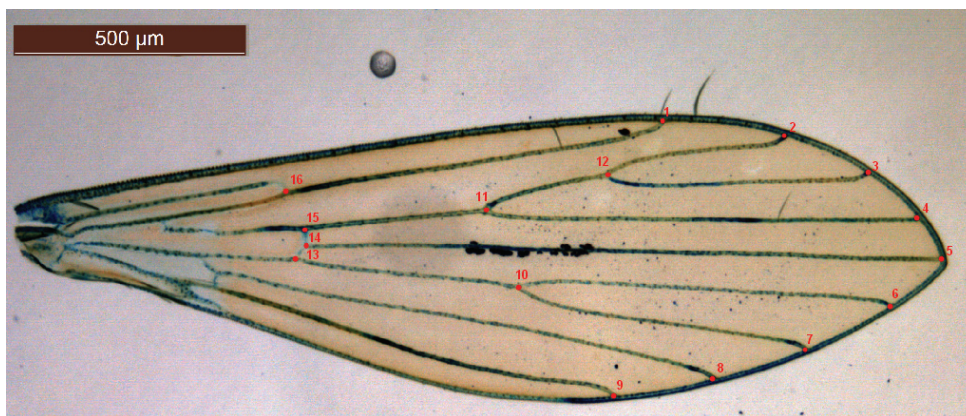


Figure 2. Location of the 16 wing landmarks used in the morphometric analysis of *Phlebotomus papatasi*.

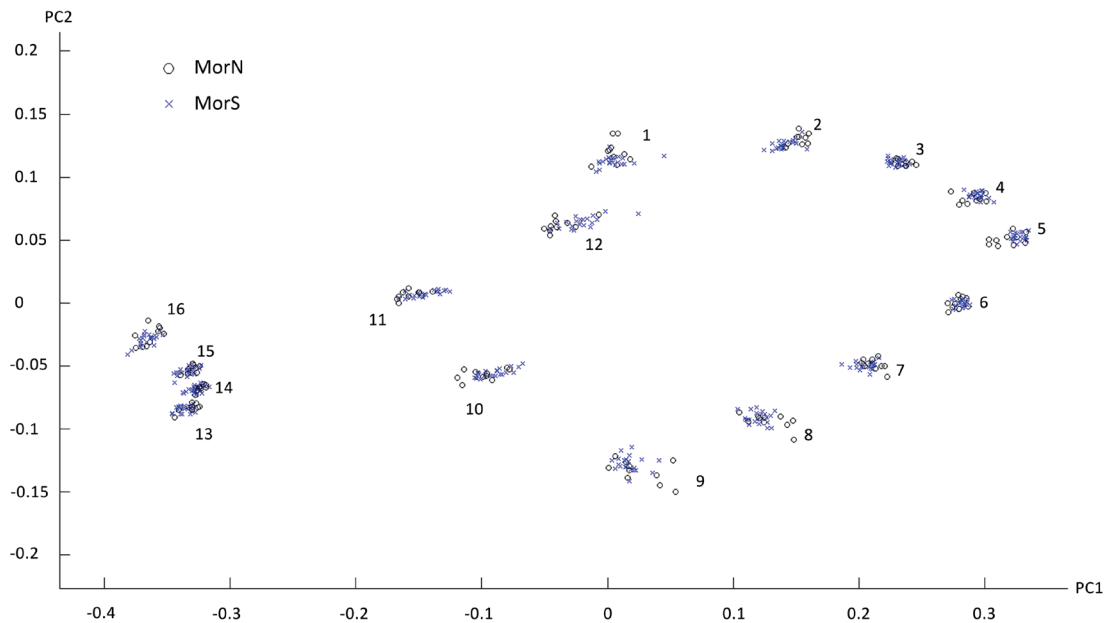


Figure 3. Output of consensus configuration by the GPA Procrustes superimposition method locating the 16 landmarks for each individual wing of the two populations. x: south of Atlas Mountains, o: north of Atlas Mountains.

were analyzed, CVA also showed some overlap in local populations of Morocco. There were distinct CVs in which Axis 1 Lambda= 0.6748, $\chi^2= 11.4075$, $df=2$, $P=0.00333342$. On the other hand, the cluster analyses conducted clearly recognized two main groups of populations that Morocco (Mor) formed a distinct group from Turkey (Tur) for the wings measured along the CV2 (Axis 1 Lambda= 0.2508, $\chi^2=66.3828$ $df=6$ $P=2.25076e-012$; Axis 2 Lambda= 0.7132 $\chi^2=16.2222$ $df=2$ $P=0.000300181$). In the first group (Morocco), it appeared that North Morocco (MorN) and South Morocco (MorS) populations of *P. papatasi* were different from each other and from the Turkish population along the first two axes in terms of phenotypic features (Figure 6).

Centroid sizes were used as measures of overall wing size differences among populations. The size differences among the populations were significant (Wilcoxon-Mann-Whitney Test: $p=0.0004583$) with the specimens from the north of the Atlas and Turkey as an out-group displaying considerably larger wings (Figure 7).

DISCUSSION

This is the first comparative morphometric study of different populations of *P. papatasi* occurring in endemic and non-endemic foci of CL in Morocco. The results for size and shape presented here indicate that there are some significant differences among populations. This may be associated with a set of biological and/or environmental factors such as altitude, host population, ecological, and climatic effects.

Body size is correlated with a large number of ecological, physiological, and genetic variables. A complete understanding of the effect of bio-geographical factors

requires information on the genetic and environmental components of body size (Kuclu et al. 2011). For instance, it is generally accepted that the body size of mosquitoes is ultimately a result of larval habitat quality (Schneider et al. 2004, 2007, Jirakanjanakit et al. 2007) and larval competition (Gimnig et al. 2002). Because the properties of larval habitats and crowding conditions were not recorded, we cannot exclude the influence of their variation on the observed size changes. Therefore, the studies conducted here are based only on size and shape differences, and we cannot predict how much of the geographic variation in body size is a result of genetic differences and how much is due to phenotypic plasticity of individuals growing in different environments. Geometric morphometrics provides us with a powerful tool to describe and quantify subtle shape and size variations. However, it is obvious that environmental effects are not the only contributions to the formation of phenotypes. Accordingly, genetic effects on body size and shape should be considered in future studies.

Phlebotomus papatasi is widely distributed around the Mediterranean basin, North Africa, throughout the Middle East, and eastward across the entire Indian subcontinent. Depaquit et al. (2008) demonstrated that genetic variability in *P. papatasi* contrasts with that observed within other species having a wide distribution like *P. (Paraphlebotomus) sergenti* in the Old World or *Lutzomyia (Lutzomyia) longipalpis* in the New World. The authors hypothesized that all populations (26) of *P. papatasi* over its distribution area (18 countries) have similar vectorial capacities. However, its distribution is highly disjunctive within that range, depending on locally occurring environmental factors, precipitation, temperature, physical barriers, altitude, latitude, habitat availability, and the distribution and abundance of vertebrate hosts (Ghosh et al. 1999). If we

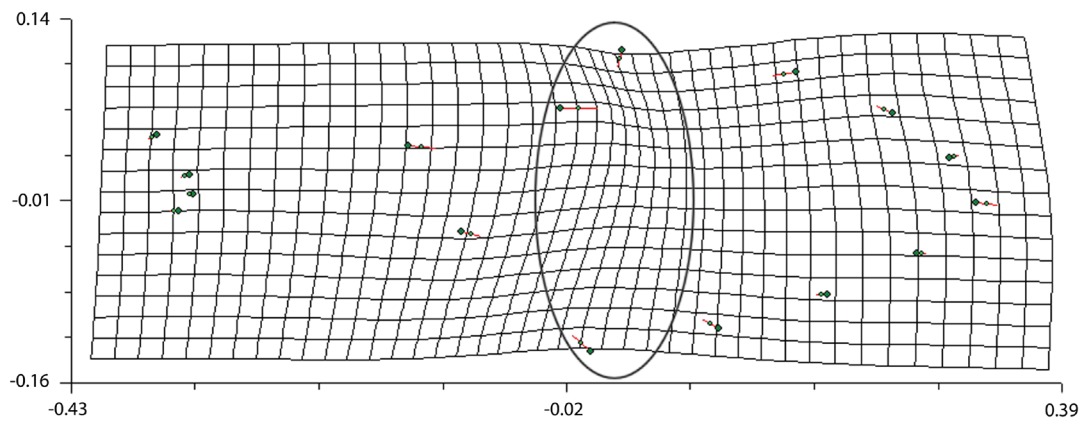


Figure 4. Thin-plate spline deformation grid showing the differences between the mean of the *P. papatasi* wings from the south and north parts of the Atlas Mountains (individuals magnified 2x; vectors are also shown).

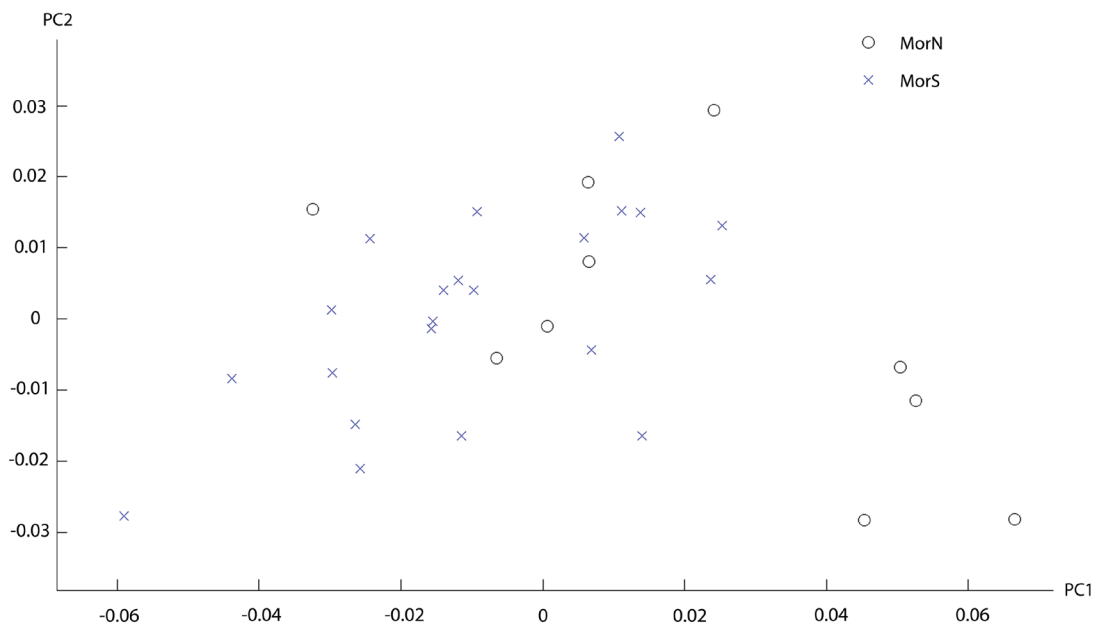


Figure 5. Distribution of the individuals of *P. papatasi* along the first two PCs. Principal component analysis of tangent space coordinates derived from GPA of the original coordinates that was conducted for the 16 landmarks digitized from the wings. Horizontal axis, PC1; vertical axis PC2. Signs indicate each individual. x: south of Atlas Mountains, o: north of Atlas Mountains.

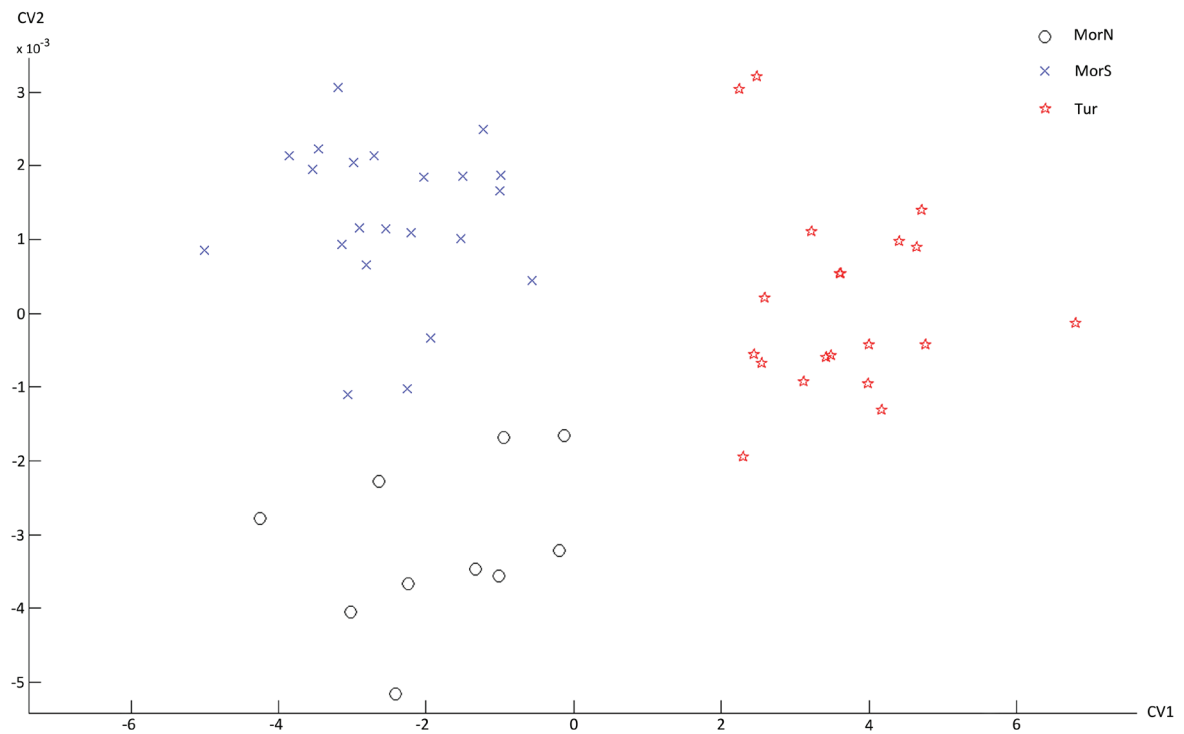


Figure 6. Distribution of *P. papatasi* individuals along the first two CVs. Canonical Variates Analysis of tangent space coordinates derived from GPA of the coordinates that was conducted for the 16 landmarks digitized from the wings. Horizontal axis, CV1, Vertical axis, CV2. Signs indicate each individual. x : south of Atlas Mountains, o : north of Atlas Mountains, * : individuals from Turkey (out-group).

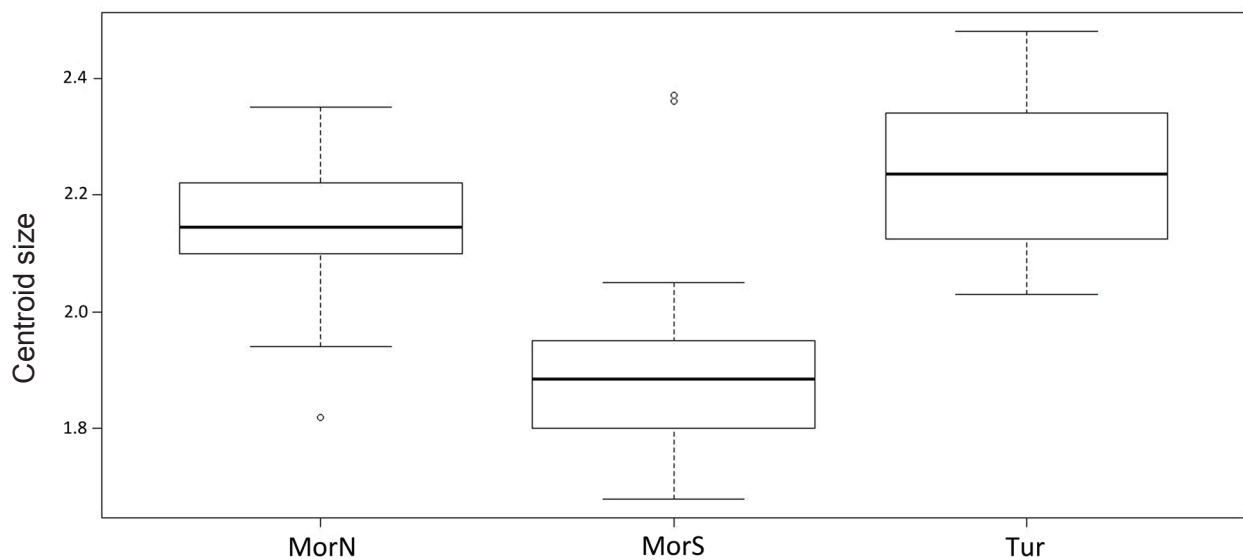


Figure 7. Mean, standard deviation, and error of centroid wing sizes of each population in *P. papatasi*. MorS: South of Atlas Mountains, MorN: North of Atlas Mountains, Tur: Turkey.

take into account the wide-range distribution of this species and also any kind of flying insects, full isolation should not be expected at first. On the other hand, Boussaa et al. (2008) compared four Moroccan populations with other Mediterranean basin populations from Spain, Cyprus, and Syria using isoenzymatic analysis, and the authors clearly revealed the genetic distance between populations of western Mediterranean countries (Morocco and Spain) and eastern countries according to nine polymorphic and three monomorphic enzymes. However, sand flies are poor fliers and the individuals rarely disperse more than several hundred meters, so it may be assumed that they may exist as local populations. Thus, genetic differences may be expected not only between geographical ecophenotypes from widely separated origins but also between local populations of *P. papatasi*. In Iran, genetic differentiation was detected between populations of this species sampled from different biotypes (Parvizi et al. 2003). Previous studies in Turkey clearly showed significant genetic and morphological differences between low altitudes and high altitudes and also between urban and rural populations of *P. papatasi*. (Volf et al. 2002, Alten et al. 2003). Dvorak et al. (2006) carried out a study to understand possible intraspecific variability of *P. sergenti* populations between Israel and southeast Anatolia by geometric morphometrics and RAPD analysis. The authors found statistically significant shape differences between the two populations and this result was fully supported by RAPD analysis to explain the genetic difference.

Guernaoui et al. (2010) demonstrated the phenotypic variation in the genitalia of male *P. papatasi* between the populations from central (north side of Atlas) and south (south side of Atlas Mountains) Morocco. They suggested that these malformations in male genitalia are associated with some specific environmental factors. The results for size and shape presented here indicate that there are differences among populations located in southern and northern parts of the Atlas Mountains in spite of a low number of examined specimens. The differences among the populations primarily occurred in the size and in the shape, respectively. This may also be associated with factors such as ecological and climatic effects. Similarly, four *P. papatasi* populations from southeast Anatolia were found to be significantly different according to altitude, and the differences among the populations primarily occurred in the wing size and shape (Belen et al. 2004). In our study, when whole size differences of the landmark locations were analyzed by centroid size analysis, two groups of populations on both sides of Atlas Mountains were recognized.

We obtained similar results when the wing shape differences of populations were analyzed. Similar to size, wing shape might be influenced by many factors and micro-environmental variation of some climatic parameters such as temperature and/or humidity. Kasap and Alten (2005, 2006) for *P. papatasi* and Aytekin et al. (2009) for *An. superpictus* demonstrated remarkable effect of temperature and humidity under laboratory conditions. Variations in wing shape can be related to flight performance and

nonlinear patterns in shape might also arise from selection by environmental variables such as relative humidity or saturation deficit of atmosphere showing nonlinear changes with altitude (Hoffman and Shirriffs 2002). We also identified the considerable deformation of wings between the populations (Figure 4). Thin-plate spline deformation analyses showed that this deformation on the medial part of the wings was mostly due to the relative effect of the southern populations. The medial of the wing of flying insects plays the most important role in flying capability (Johansson et al. 2009). If we consider this information, determining the deformation on the wings of especially the south population may explain why the biggest part of the population is located south of Atlas Mountain.

Temperature is one of the main factors preventing the spread of both visceral and cutaneous leishmaniasis. This ecological factor varies with altitude according to the thermal altitudinal gradient and latitude. The possible relationship between the leishmaniasis transmission and altitude may be closely related to many factors such as the temperature suitable for the evolution of *Leishmania* in sand flies (Rioux et al. 1985). Aytekin et al. (2007) found that even though there are three major mountain ranges that may serve as geographical barriers for sand fly species distribution and four main gaps were recognized in southern Anatolia, Turkey, there are no significant differences in wing morphology in *P. papatasi* specimens. It means that this species may disperse in large areas, vertically and horizontally. But in same study, the researchers also confirmed that there was a negative correlation among *Leishmania* transmission based on species, geographical barriers and altitude. In Turkey, *P. papatasi* can transmit HCL (human cutaneous leishmaniasis) vectored by *L. tropica* in the arid lowland of the hyper endemic area in southeast Anatolia. Guernaoui et al. (2006) proposed that altitude has an influence upon the spatial distribution and density of the sand fly species. The authors showed the distribution of *P. papatasi* populations between the northern and the southern parts of the Atlas Mountains has been limited by altitude (1000 m). The occurrence of *P. papatasi* in the lowlands and its low frequency in the mountains could be explained by its preferences for the arid and peri-arid areas. Moreover, its density increases with the aridity (Croset et al. 1974). Cross and Hyams (1996) predicted that raising the ambient temperature by 1-5° C through global warming would result in the expansion of the geographical distribution of *P. papatasi* in southwest Asia and extend the transmission season of *L. major*. In addition, this species is active throughout the year in the urban area of Marrakech, Morocco (Boussaa et al. 2005). This suggests the necessity of preventing the risk of extension of *L. major* foci from southern Morocco as predicted by Rioux et al. (1997).

In temperate climates, phlebotomine populations display marked seasonal variations in abundance, reaching high densities only during the summer months. The high levels of phenotypic diversity suggest that *P. papatasi* populations are able to maintain large effective population sizes in spite of the marked seasonality imposed by the

cold winter temperatures. A similar scenario is also met by Afrotropical vector populations in dry savanna/sahelian regions. In *An. arabiensis*, the strong seasonal fluctuations in abundance do not seem to affect the overall genetic diversity and current effective population size in dry areas of Sudan and Senegal where rains last for less than five months (Taylor et al. 1993, Simard et al. 2000).

Phlebotomus papatasi is active throughout the year in Morocco, absent occasionally in some samples because of meteorological disturbances. These results suggest that this vector does not diapause in Morocco (Baussaa et al. 2005). Two density peaks were identified by the authors in June and November. Between these two peaks, the density presented significant micro-variations because of the effect of the wind. On the other hand, the significant role of temperature on *P. papatasi* distribution was demonstrated by Singh (1999) and Kasap and Alten (2005). According to these studies, this species is less active at lower temperatures. Moreover, Srinivasan et al. (1993) showed a significant negative correlation between *P. papatasi* density and rainfall. Together with phenotypic correlations with arid and humid bioclimate, these results may explain why *L. major* transmission by *P. papatasi* can be easier in the south of the Atlas Mountain compared to the north.

This study is one component of a large-scale multi-level work that has been planned on population genetics and bio-ecologic studies in the same areas. The levels of phenotypic variation in our samples are more likely to reflect the local environmental pressures to which these populations are subjected. However, possible correlation between phenotypic differentiation and genetic and/or geographic distances should be considered with larger sample sizes in future studies.

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