Keywords:
Generalized Procustes Analysis (GPA)
*C. barbarus*
Landmarks
Wing polymorphism

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**Introduction**

Amid *Calliptamus* genus, *C. barbarus* is the most polymorphic species. It is characterized by a chromatic polymorphism of the hind femora (femore red with three spots or orange with only one spot).

In Algeria, *C. barbarus* was found for two areas: Littoral region for the form with three femoral spots (3S), and extremely semiarid and dry inhabitants for the form with only one femoral spot (1S).

Different authors have studied this species in order to compare the two forms on the basis of morphometric characters (Clemente et al. 1987; Benzara 2004; Larrosa et al. 2004), females ovarioles and biochemistry (Benzara 2004), acoustic emissions (Larrosa et al. 2008) and sexual relationships (Larrosa et al. 2007). Our last research of this species is a phylogenetic study based on molecular data (Rouibah et al, 2016). In order to continue our investigations of this species,
we conducted a geometric morphometric analysis on wing-vein landmarks.

According to Clarke (1993), there are two types of asymmetry: fundamental asymmetry due to a specialization of one of the sides and fluctuating or occasional asymmetry due to developmental stress. In this study we investigate whether a geometric morphometric of wings approach has the potential to inform us on the influence of geographic and chromatic polymorphism on evolutionary transformations within population of *C. barbarus*.

This is a method that allows to eliminate differences in size between samples. It is also the field of analysis of the biological form (Slice 2007), allowing to characterize quantitatively, to analyze and to compare the biological form (Bookstein 1991). In term of the type of imput data three general types of morphometry can be distinguished: traditional morphometry, contour-based geometric morphometry and a landmark-based geometric morphometric analysis. In our study we have chosen the latter. The explanation of the principle of the Procustes method will be provided, and then this approach will be applied in the current work.

**Material and methods**

**Principle of the Procustes method**

An insect wing is most often a flattened structure formed by a network of veins supporting a membrane composed of two segregated tegumentary layers. The homology of the different veins of the wings has long been discussed. However, venation as a whole has long been regarded as a homologous structure across different groups of insects (Hamilton 1972, Comstock and Needham 1998). In this study, the landmark-based geometric morphometric method is applied to the wing shape of *C. barbarus*. The purpose was to quantify the asymmetry of the elytra of each individual. This can be determined by a fundamental asymmetry that may emerged as a result of specialization of one of the sides. It is the case generally of males of Orthoptera Ensifera where the mirror of the right elytra rubs against the teeth carried by the left elytra (Benfekkh, 2006). It also possible that there is a fluctuating asymmetry due to genetic and/or environmental stress during development (Palmer and Strobeck 1986; Clarke 1993; Frampton & Hardersen 1999).

Procustes Analysis is a technique for comparing forms. The goal is to eliminate non-conformational variations. After this step, the differences between the landmark configurations will therefore be solely due to the shape variation. This is done by means of a Generalized Procustes Analysis (GPA), based on the least squares method (Gower 1975). It is performed by three mathematical functions: rotation, translation and scaling (Figure 1). This step makes it possible to obtain a 'consensus' representing the average shape of samples. The Procustes residuals represent deviations of each samples from the consensus of landmarks and then Procrustes residuals can be analyzed with the methods of multivariate statistics.

**Application of the method**

To carry out this study, slides were used as a support for the flattening of the wings. Glycerin was used to fix these wings. A lens equipped with a digital camera was used for taking photographs. The samples have been collected in August 2014 in 3 regions: samples of 3S form of *C. barbarus* was collected in Texenna (36° 69’ N; 5° 77’ E); samples of 1 S form were collected in Kasr El Boukhari (2° 67’ E; 35° 86’ N) and Djelfa (34°40’ N; 3°15’ E). This work has been partially carried out within the Department of Zoology of the University of Murcia in Spain and at the Laboratory of Zoology at the Faculty of Science of the University of Jijel. A total of 38 specimens are used, of which almost half (8 males and 8 females) were the 3 S form. The others (10 males and 12 females) were the 3 S. The elytra were flattened and mounted between two blades and fixed with glycerine, then photographed and finally scanned and saved as a JPEG image file. The
Figure 1. The three steps of the Generalized Procustean Analysis. (Gower, 1975)

Figure 2. *C. barbarus* female wing at 3S. (original photo)

Figure 3. *C. barbarus* female wing at 1S. (original photo)

Figure 4. Elytra veins nomenclature and 11 landmarks position. ASC: Anterior Sub Costal; M: Median; PSC: Posterior Sub Costal; AR: Anterior Radial; PR: Posterior Radial; ACU: Anterior Cubital; PCU: Posterior Cubital; PM: Posterior Median; AM: Anterior Median. Note that some veins divide themselves into sub branches named 1 (anterior) and 2 (posterior).
The coordinates of landmarks (L) are digitized on each image by using the TPS dig2 version 2.17 software (Rohlf, 2013). These coordinates are then stored in a text file in the format required for TPS-RelW version 1.11 software (Rohlf, 1997). Landmarks were chosen by taking into account the difference observed in the median field between the wings of the two forms (Figure 2, 3). The wing veins nomenclature used in this study was proposed by Grauvogel-Stamm et al. (2000); Bethoux & Nel (2001); Petit et al. (2006). Landmarks were as follow (Figure 4):

- L1: insertion point of M with (A Cu + P Cu1)
- L2: insertion point of of P1Cu with P Cu2
- L3: the point of distal divergence of A Cu and P M
- L4: the point of distal divergence of A Cu and P Cu1
- L5: proximal starting point of A Cu and P M
- L6: point of distal divergence of M
- L7: proximal insertion point of A R with P R
- L8: point of proximal bifurcation of P R
- L9: insertion point of A SC with leading edge
- L10: insertion point of A R with distal edge
- L11: insertion point of M A with posterior border

The coordinates of 11 landmarks of the 38 elytra pairs allow us, after superimposition, to compute of the angles formed by the vectors of the landmarks of the right elytra and the left one. This calculation was conduct for each individuals, to test whether some (L) have a preferred angle of deformation (Figure 5). The displacement of the (L) of the right elytra by contribution to the left one is represented.
by a vector whose angle with respect to the horizontal axe and the Euclidean distance can be calculated as the follow. If the coordinates of the points (Li) left and (Li) right are respectively \((x1, y1)\) and \((x2, y2)\), then the Euclidean distance is: 
\[
\sqrt{(x2 - x1)^2 + (y2 - y1)^2}
\]
So the angle is \((y2 - y1) / (x2-x1)\).

The TPS-RelW version 1.11 program (Rholf 1997) allows to perform all the measured objects to the same centroid size and to superimpose them by the GPA method, in order to calculate the consensus. The components of relative warps on the first 2 axes are considered to compare the different individuals. The contribution of the different L to the overall variability was evaluated by calculating for each L the sum of the Euclidean distances of each individual with respect to the consensus. Then it is evaluated by comparing the sum obtained for each L with the sum of the set for the 11 L.

The centroid sizes of each elytra were measured using GMTP version 2.1 software (Taravati & Darvish, 2010). For this purpose, the center of gravity of each elytra is calculated from the 11 (L) by the average of all the coordinates of the points of this form. Then the average Euclidean distance between each L and the barycenter of the 11 L is evaluated (Figure 6). Finally, ANOVAs were performed using Statistica software version 10.0, considering sex and chromatic polymorphism as factors. The aim is to know is the difference between Euclidean distances and centroid sizes of different forms and sexes statistically significant.

- \(P > 0.05\): no significant difference; \(P < 0.05\): significant difference; \(P < 0.01\): very significant difference; \(P < 0.001\): highly significant difference

Figure 7 shows that L8 had the one of the largest scatter. While L 4, 5 and 6 have a low amounts of variability. From the point of view of the percentage of deformation, it must be noted that the L8 is the most unstable, with a contribution of almost 13% of the total variability.

Moreover, the angles between the vectors of the set of 76 elytra pairs were calculated for each L. The distribution histogram of the angles is shown for the L1 in Figure 9. The histogram shows that the majority of the values hovering around zero. This means that there is no privileged deformation within the angles of the 11 L of the right elytra by contribution to the left.

**Influence of sex and polymorphism on the wing asymmetry of C. barbarus**

The projections of the 38 pairs of elytra on the first 2 axes of relative flexion are placed in Figure 10. These projections make it possible to calculate, in a two-dimensional space, the Euclidean distances between each (L) of the right elytra and the left one. This makes it possible to estimate the measurement of the deviation leading to asymmetry.

From these last projections, there is a heterogeneity in the dispersion of the points on the morphospace. For example, points 7, 8 on one side and 21, 22 on the other side correspond successively to the right and left wings of a male and a female at 3S form. On the other hand, points 19, 20 on the one hand and 53, 54 on the other hand, correspond successively to the right and left wings of a female and a male at 1S form.

The patterns of the relative flexions of the elytra show the different variations of instability of the landmarks (Figure 11). It should be noted also a remarkable displacement especially for L8: Figures A, B and D, L10: Figures A, B and C: L11: Figures B, C and D: L9: Figures B and D, L7: Figure C, L2: Figure D and L4: Figure B and to a lesser extent for the others Ls.
To explain the variation of distances for each individual, a two-factor ANOVA (sex and polymorphism) without interaction was performed. The result indicates that there is no significant difference between the Euclidean distances of elytra of sexes (p = 0.7113) and forms of chromatic polymorphism (p = 0.0814) (Table 2).

The centroid sizes of the elytra of the 38 individuals are compared between sexes and forms of polymorphism. The result indicates a high significant centroid size differences (p = 0.004 <0.01) between sexes (Table 3), but there was no significant difference (p = 0.195> 0.05) between the polymorphic forms.

In a general way, the displacement of the 11 landmarks of the C. barbarous wings in 1S and 3S

### Table 1. Percentage of contribution of each L's in total variability.

<table>
<thead>
<tr>
<th>Landmarks</th>
<th>Contribution (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>7,54</td>
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<tr>
<td>2</td>
<td>9,7</td>
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<tr>
<td>3</td>
<td>7,84</td>
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<td>4</td>
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<tr>
<td>7</td>
<td>10,43</td>
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<tr>
<td>8</td>
<td>12,80</td>
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<tr>
<td>9</td>
<td>11,45</td>
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<tr>
<td>10</td>
<td>7,92</td>
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<tr>
<td>11</td>
<td>10,07</td>
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**Figure 7.** Alignment of 11 landmarks of 38 pairs of elytra after Procustes superposition.

**Figure 8.** Percentages of variability of the 11 landmarks. Red circles correspond to the most stable landmarks, blacks – to the most variable landmarks

**Statistical analysis**

The centroid sizes of the elytra of the 38 individuals are compared between sexes and forms of polymorphism. The result indicates a high significant centroid size differences (p = 0.004 <0.01) between sexes (Table 3), but there was no significant difference (p = 0.195> 0.05) between the polymorphic forms.

In a general way, the displacement of the 11 landmarks of the C. barbarous wings in 1S and 3S
Figure 9. Histogram of distribution of angles of landmark i vectors

Figure 10. Projection of 38 pairs of elytra on the first 2 axes of relative flexion. The letters A, B represent successively a male and a female with 3T, while the letters C and D represent a female and a male with 1S
forms is not significant. This means that the alar shape changes between the semi-arid population (Kasr El Boukhari and Djelfa) and that of the wetland (Texenna) are small. These differences are apparently due to the existing deformation in the alar morphology of the median sector of the elytra in the two forms. On the other hand, it should be noted that sex has no influence on the displacement of the 11 landmarks and that the influence of the polymorphism is small. It is also clear that the size of the elytra present in the two forms depends only on sex, the females were larger than the males, while the polymorphism has no influence on this size. Benfekih (2006) in his study of *Locusta migratoria*, found that the landmarks which are located in the median field, namely L4, 5, 6 and 7 are the most stable. On the other hand, L10 (insertion point of subcostal vein ASC with the anterior border) is the most unstable. In addition, Petit et al. (2006) reported in their study on the subject of wing geomorphometry of some Acridinae, Gomphocerinae and Oedipodinae that L7 (insertion point of the anterior margin with ASC), L1 (insertion point of median M with Cu cubital veins), (L)5 (insertion point of ACu and PM with CuP2) and the L8 (insertion point of AR with PR) are the subject to the strongest displacements. For its part, Petit (2007) in its study on *Chortipus corsicus* and *C. pascuorum* (Acrididae: Gomphocerinae) reported that for both species, L2 (the point of distal divergence of ACu and PCu1), L4 (the point of distal divergence of M), L7 (insertion point of ASC with the anterior border) and L8 (the proximal insertion point of AR with PR) were the most varied landmarks. The asymmetry observed between the right and left elytra of the 38 studied individuals can be determined as a fluctuating asymmetry. Consequently, there is no influence of chromatic and geographic polymorphism on the wing asymmetry of *C. barbarus*.

**Conclusion**

To prove that the population of *C. barbarus* of Jijel region belongs to the 3S form (3 spots), it is essential to carry out a comparative geometric morphometric study between the two forms. For this purpose, specimens are collected from wet area: Texenna and then compared with those collected from semi-arid regions: Kasr El Boukhari and Djelfa. The aim of this study was to investigate possible wing asymmetry between the two forms of this Orthopteran species. This asymmetry may have a fundamental nature, or it may be defined as a fluctuating asymmetry, that is a result of environmental stress. The benefit of geometric morphometric approach is to obtaining a two-

<table>
<thead>
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<th>Table 2. ANOVA of Euclidean distances of elytra according to sex and forms of chromatic polymorphism.</th>
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<tr>
<td><strong>Factors</strong></td>
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<tr>
<td>Sex</td>
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<tr>
<td>Polymorphism</td>
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<td>Error</td>
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<td>SC: sum of squares; DDL: degree of freedom; MC: mean of squares; F: Fisher statistics; P: Probability to meet the computed value of the statistic</td>
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<th>Table 3. Results of ANOVA: the effect of sex ad polymorphic forms on the centroid sizes of elytra</th>
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<td><strong>Factors</strong></td>
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dimensional (2D), graphical and statistical representation of the set of individuals studied separately. This method allowed to observe and to compare the variability of the wing conformation between the populations of the two forms of *C. barbarus*. The results obtained made it possible to note first that the landmarks located at the level of the median field (4,5 and 6) are the most stable and

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**Figure 11.** Patterns of relative flexion of right and left elytra of both forms.
superimposed in *C. barbarus* as well as in other locusts as it has been reported by various authors. Moreover, it was indicated that the displacement of landmarks is poorly represented between the individuals of the two populations. This means that the only type of asymmetry present between the two forms and this is a fluctuating asymmetry due to development stress.

References


