

# Morphological discrimination between two populations of shemaya, *Chalcalburnus chalcoides* (Actinopterygii, Cyprinidae) using a truss network

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Bagherian, A. & Rahmani, H., 2009. Morphological discrimination between two populations of shemaya, *Chalcalburnus chalcoides* (Actinopterygii, Cyprinidae), using a truss network. *Animal Biodiversity and Conservation*, 32.1: 1–8.

## Abstract

*Morphological discrimination between two populations of shemaya, Chalcalburnus chalcoides (Actinopterygii, Cyprinidae), using a truss network.*— Several body measurement methods used to identify stock have recently been criticized because of inherent biases and weaknesses. As an alternative, a new system of morphometric measurement called the truss network has been increasingly used for stock identification. We studied the morphometric differentiations between two populations and sexes of shemaya fishes (*Chalcalburnus chalcoides*) using a truss network. Truss distances between 15 landmarks of 66 specimens were measured. Size adjustment transformations were assessed by dividing characters (truss distances) by centroid size of specimen. Multivariate analysis of variance (MANOVA), principal component analysis and discrimination analysis were performed to investigate distinction and patterns of morphological variations between populations and sexes. The MANOVA (Wilks test) indicated a significant difference for mean vectors between populations ( $\Lambda = 0.136$ ;  $F = 47.76$ ;  $P = 0.001$ ) and sexes ( $\Lambda = 0.120$ ;  $F = 45.32$ ;  $P < 0.001$ ). Discrimination analysis correctly classified 97% and 89.4% samples to their original groups for population and sex, respectively. Our findings support the use of the truss network to study morphological variation among populations as it provides interesting perspectives for the study of biodiversity patterns.

Key word: Morphological discrimination, *Chalcalburnus chalcoides*, Truss network system, Habitat effect.

## Resumen

*Discriminación morfológica entre dos poblaciones del alburno del Danubio, Chalcalburnus chalcoides (Actinopterygii, Cyprinidae), utilizando una red en celosía.*— Recientemente se han criticado diversos métodos de medición de parámetros corporales, que se utilizaban en la identificación de los linajes, debido a la debilidad y los sesgos inherentes a ellos. Como alternativa, cada vez se está usando más un nuevo sistema de medición morfométrica denominado red en celosía, para la identificación de los linajes. Nosotros hemos estudiado las diferenciaciones morfométricas entre dos poblaciones y en los dos sexos del alburno del Danubio (*Chalcalburnus chalcoides*) utilizando este tipo de red. Se midieron las distancias entre 15 puntos determinados o nudos de la celosía en 66 especímenes. Se evaluaron las transformaciones del ajuste del tamaño dividiendo las características (distancias entre nudos) por el valor del centroide del espécimen. Se llevaron a cabo análisis de varianza multivariante (AMOVA), análisis de componentes principales y análisis de discriminación para investigar la distinción y los patrones de las variaciones morfológicas entre poblaciones y sexos. El AMOVA (test de Wilks) indicaba una diferencia significativa para los vectores medios entre poblaciones ( $\Lambda = 0,136$ ;  $F = 7,76$ ;  $P < 0,001$ ) y sexos ( $\Lambda = 0,120$ ;  $F = 45,32$ ;  $P < 0,001$ ). El análisis de discriminación clasificó correctamente el 97% y el 89,4% de las muestras en sus grupos originales de población y sexo, respectivamente. Nuestros resultados respaldan el uso de las redes en celosía para estudiar la variación morfológica entre poblaciones, ya que proporcionan perspectivas muy interesantes para el estudio de los patrones de diversidad.

Palabras clave: Discriminación morfológica, *Chalcalburnus chalcoides*, Sistema de red en celosía, Efecto del hábitat.

*(Received: 10 IV 2007; Conditional acceptance: 3 VII 07; Final acceptance: 16 X 07)*

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## Introduction

Several techniques have been proposed for stock identification, an interdisciplinary field that involves the recognition of self-sustaining components within natural populations. It is a central theme in fisheries science and management (Cadrin et al., 2004). Stock identification can be viewed as a prerequisite for any fishery analysis, just as population structure is considered a basic element of conservation biology (Thorpe et al., 1995).

Stock identification methods have developed in parallel with the advancement of morphometric techniques. The earliest analyses of morphometric variables for stock identification were univariate comparisons, but these were soon followed by bivariate analyses of relative growth to detect ontogenetic changes and geographic variation among fish stocks. As the field of multivariate morphometrics grew, a set of multivariate methods was applied to quantify variation in growth and form among stocks (Cadrin, 2000).

More recent advances have been facilitated by image processing techniques, more comprehensive and precise data collection, more efficient quantification of shape, and new analytical tools, landmark-based techniques of geometric morphometrics (Bookstein, 1991; Rohlf, 1990; Rohlf & Marcus, 1993). These techniques pose no restrictions on the directions of variation and localization of changes in shape; furthermore, they are very effective in capturing information about the shape of an organism (Cavalcanti et al., 1999). Image analysis systems played a major role in the development of morphometric techniques, boosting the utility of morphometric research in fish stock identification (Cadrin & Friedland, 1999).

Morphometry based on truss network data has been used for stock identification (Bronte & Moore, 2007; Shao et al., 2007), species discrimination (Palma & Andrade, 2002), ontogeny (Hard et al., 1999; Debowski et al., 1999) and functional morphology (Dean et al., 2006).

In this study morphometric differentiation between two populations and sexes of shemaya fishes (*Chalcalburnus chalcoides*: Cyprinidae) were investigated using the truss network (Strauss & Bookstein, 1982). The shemaya, *Chalcalburnus chalcoides* (Guldenstadf, 1772), is widely distributed in the river systems of the Black, Caspian and Aral Seas (Bogutskaya, 1997). The species is benthoplagic and it lives in fresh and brackish waters. The populations that live in lakes migrate upstream for spawning from the beginning of May to the end of July (Slastenenko, 1959). There are few investigations about morphologic aspects of shemaya fishes in the south of the Caspian Sea (Abdurakhmanov, 1975; Coad, 1999; Rahmani et al., in press). Little is yet known about the environmental biology of this species (Tarkan et al., 2005; Kiabi et al., 1999).

## Materials and methods

### Study area and sampling

The shemaya (66 specimens) were collected by cast-netting in May 2005 in the estuaries of the Haraz

and Shirud Rivers (fig. 1). During sampling physical factors of water were measured *in situ*.

Locations of sampling were at longitude 52° 21'–51° 21' and latitude 33° 26'–36° 42' from Haraz River and at longitude 50° 48'–50° 49' and latitude 36° 44'–36° 51' from Shirud River. Both rivers are supplied by surface and subterranean waters.

### Data collection

Two-dimensional Cartesian coordinates of 15 landmarks were recorded on the right view of each specimen. The locations of the landmarks were chosen according to two criteria: reliability in terms of correspondence between specimens and the ability to best describe the geometry of the form under study and from some reference. The raw data set therefore corresponded to 66 configurations of 15 (x, y) coordinates (fig. 2). Data were digitized from pictures with 2,304 × 1,704 pixel dimensions using tpsDig version 1.4 (Rohlf, 2004). The fitness of the data set for partial warp analysis was tested using tpsSmall version 1.2 (Rohlf, 2003) to ensure that the distribution of the specimens (in terms of Procrustes distance between the specimens) in Kendal shape space was highly correlated with that of the projected specimens in tangent space (Rohlf, 1996).

Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form. Cells and truss characters are referenced according to the scheme of Strauss & Bookstein (1982); for example, the distance between landmarks 1 and 2 is a truss character in first quadrilateral or cell (landmarks 1, 2, 3, and 4) (fig. 2). Measurements of specimens are made by collecting x–y coordinate data for relevant morphological features. One hundred and four characters were extracted by measuring distances between landmarks. An important stage in the data preparation for morphometric analyses is to eliminate any size effect in the data set when comparing fish of different sizes. Variation should be attributable to body shape differences, and not related to the relative size of the fish. Therefore, transformation of absolute measurements to size-independent shape variables is the first step of the analyses (Reist, 1985). Size adjustment transformations were carried out by division of characters (truss distances) by centroid size of specimen. Centroid size is the square root of the sum of squared distances from the landmarks to the centroid of the landmarks.

### Data analysis

Multivariate techniques were used to analyse patterns of differentiation between samples and assess similarities.

Multivariate analysis of variance (MANOVA) was performed to test for significant differences between the two populations and sexes.

The principal component analysis was employed for the multivariate description of morphometric data. The eigenvectors and eigenvalues were obtained

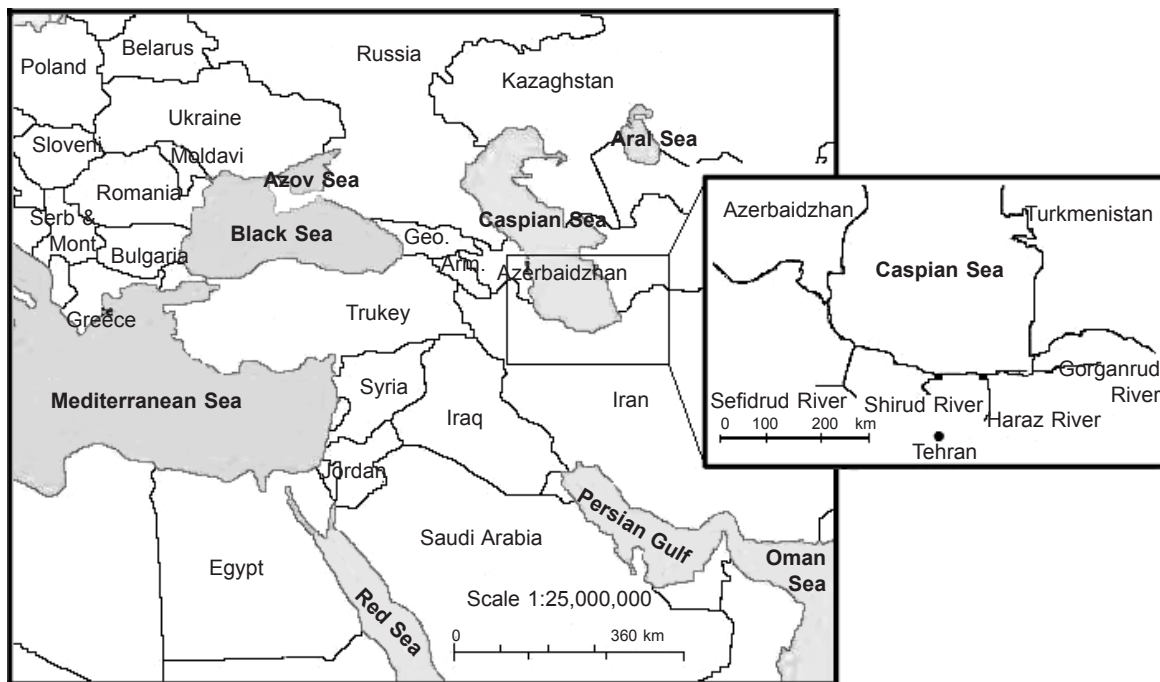


Fig. 1. Map of the study area and sampling site (square).

*Fig. 1. Mapa del área de estudio y del área de muestreo (recuadro).*

from the covariance matrix, which allowed the representation of the largest part of the variance of original variables in a low number of factors. This enabled the evaluation of the relation between the two species or sexes by means of proximity in the space defined by components. Characters (truss distances) that had higher correlations with components (more than  $|0.6|$ ) were depicted over landmarks for illustration locations of more variable truss distances.

Canonical discrimination analysis (CVA) was performed to discriminate groups (populations and sexes). The distribution pattern of specimens based on first and second discriminate functions is shown in figure 6. CVA grouping results were examined by randomization tests. Randomization tests were performed with 100 random grouping data sets. These randomization tests involve determining the significance level of a test statistic calculated for a set of data by comparing the observed value of the statistic with the distribution of values that is obtained by randomly partitioning and reordering the data, and calculating the desired statistic for each replicated sample (Manly, 1991; Crowley, 1992; Solow, 1990).

## Results

The MANOVA (Wilks test) indicated a significant difference for mean vectors between populations

( $\Lambda = 0.136$ ,  $F = 47.76$ ,  $P < 0.001$ ) and sexes ( $\Lambda = 0.120$ ,  $F = 45.32$ ,  $P < 0.001$ ).

Sixteen components accounted for most of the 95% of the total variation. Of these, the first explained 24%, the second 21% and the third 12%, and the other components incorporated 40% of the variance. The projection of specimens in relation to the first and the second principal components (fig. 3) revealed a visual definition of populations and sexes.

Truss distances that have more correlation with the first component are depicted over landmarks in figure 4, and those with the second component are shown in figure 5.

In discrimination analysis, the first function accounted for 58.8% (Eigenvalue = 3.61,  $\Lambda = 0.046$ ,  $P < 0.000$ ) and the second accounted for 30.5% (Eigenvalue = 1.87,  $\Lambda = 0.21$ ,  $P = 0.013$ ) of the between-group variability. Plotting DF1 and DF2 explained 89.3% of the between-group variation and roughly separated the populations and sexes from each other, showing morphologic differentiation among populations (fig. 6).

In the discrimination function analysis, the overall random assignment of individuals into their original population was high (93.9%). The proportions of samples correctly classified to their original groups for population and sex were 97% and 89.4% respectively. The randomization test correctly classified between 47.2 and 69%, with a mean of 57.6%. This shows that classification results can not be estimated randomly.

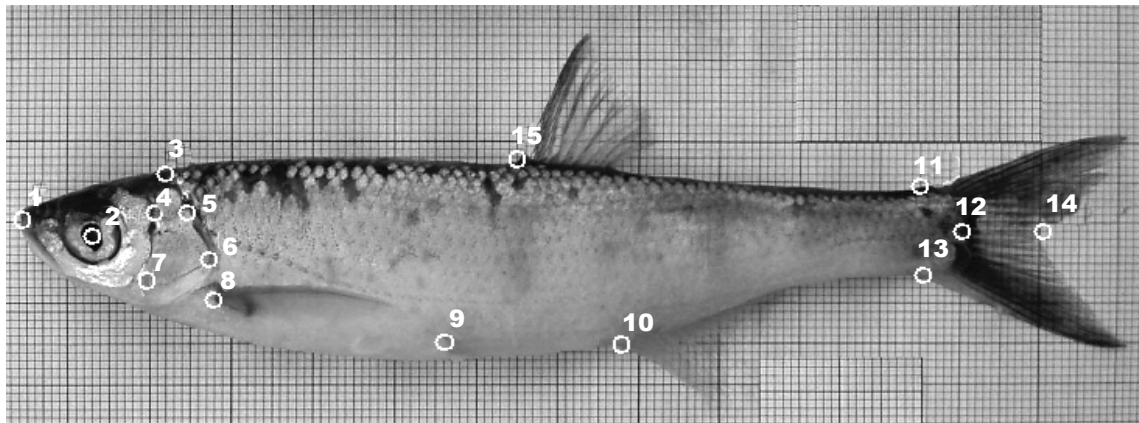


Fig. 2. Locations of the 15 landmarks on the left view of the fish.

*Fig. 2. Localización de los 15 puntos clave en el lateral izquierdo del pez.*

## Discussion

Results showed populations and sexes separated by three forms of multivariate analysis, MANOVA, PCA and CVA.

A clear sexual dimorphism was detected in this study. First principle component scattered most male specimens from the two populations on the left side (with negative scores) of the component scatter plot (fig. 3) and female specimens were scattered on the opposite side (with positive scores). High correlated variables with the first component that were depicted over landmarks (fig. 4) show that female specimens have a larger abdomen body part (high positive correlated trusses between landmarks 2 to 8 with 9–10) and male specimens have longer posterior part of body (high negative correlated trusses between landmarks 11–14 with 9 and 10). Truss–network analysis revealed a sexual dimorphism in shape, in particular for the abdominal region, which appeared to be larger in the females of both populations. This sexual abdominal shape dimorphism, which has been reported for several groups of animals (Adams & Funk, 1997), has been hypothesized to be due to a positive correlation between fecundity and female abdomen size. Hence it may be the result of selection to increase fecundity (Wickman & Karlsson, 1989; Adams & Funk, 1997).

Figure 5 shows that anterior part landmarks (landmarks 1 to 8) in both sexes of Shirud samples were smaller (high negative correlation with PCII) while peduncle landmarks (landmarks 3, 5, 6, 8 and 15 with 11, 12 and 13) were more widely spread (high positive correlation with PCII). This deformation caused Shirud samples to be slender compared to samples of both sexes in the Haraz River (fig. 5). Such shape differences are perhaps the consequence of isolation and fragmentation

of the two populations. The Haraz River has a muddy estuary with a low slope and water velocity and high turbidity. The Shirud River has a sandy bottom with high water velocity and high transparency. Concerning biological and environmental conditions in the Shirud, its fish population has a more slender body than that in the Haraz River as it exerts greater resistance against the water flow while swimming. Differences between sexes in shape were clearer in Shirud River specimens than in Haraz River specimens. It may be due to differences in of water velocity in the two rivers; at a higher water velocity the number of males with large abdomen decreases because they have greater resistance to the water flow. Females have a large abdomen for fecundity regardless of water velocity. Therefore, in higher water flow (as in the Shirud River) the males are clearly thinner than females. The two Shemeya populations studied here are anadromous and have a common origin. The difference in shape is likely an environmental effect, but further genetic studies are needed to confirm this.

The Truss System can be successfully used to investigate stock separation within a species. In the long term it can provide a better and more direct comparison of the morphological evolution of stocks while using the same set of measurements. Traditional morphometric measurements of these specimens in PCA showed overlapping of the two populations. The first axis had a high correlation with length characters (total, standard and fork length) and the second axis with width characters (body, head and tail stem width). The discrimination analysis classified 68% specimens in correct groups (Rahmani et al., in press). In summary, truss measurements appear to be able to classify and determine patterns of shape variations remarkably better than traditional morphometric measurements.

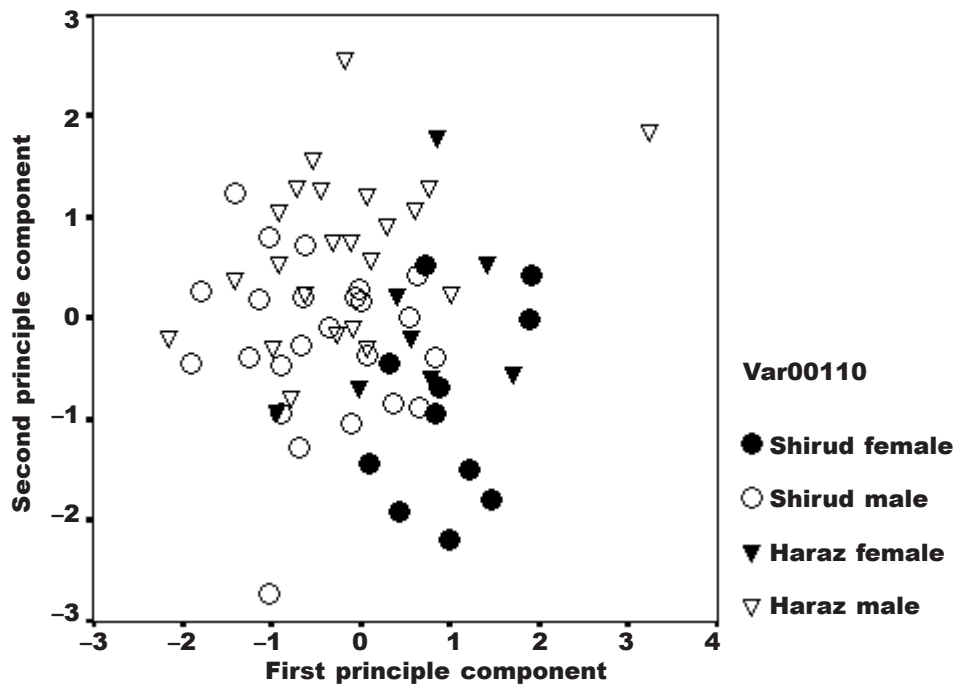


Fig. 3. Scatterplots for individual scores on the principal components of truss net distances.

*Fig. 3. Diagrama de dispersión de los valores individuales de las componentes principales de las distancias de la red en celosía.*

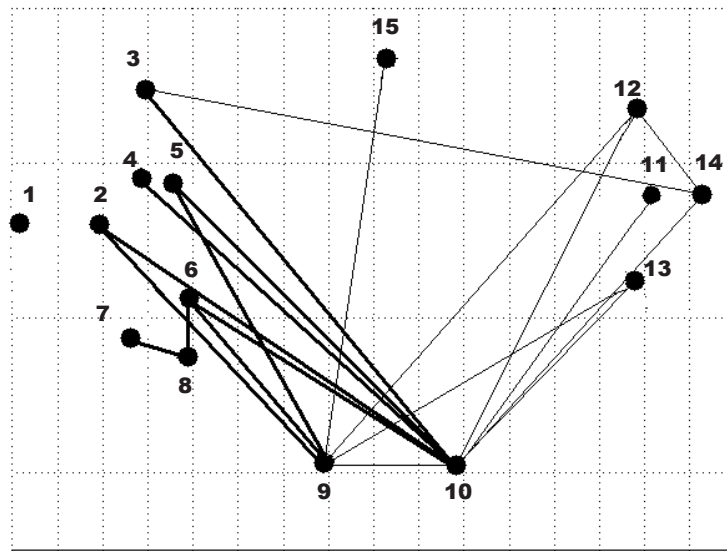


Fig. 4. Principal component loading for first component analyses of truss measurement: thick lines indicate dimensions with high positive loadings and thin lines indicate negative loading with this component.

*Fig. 4. Cargas de la componente principal para los análisis de la primera componente principal en las mediciones de la celosía: las líneas gruesas corresponden a las dimensiones con cargas positivas altas, y las líneas finas a una carga negativa con respecto a dicha componente.*

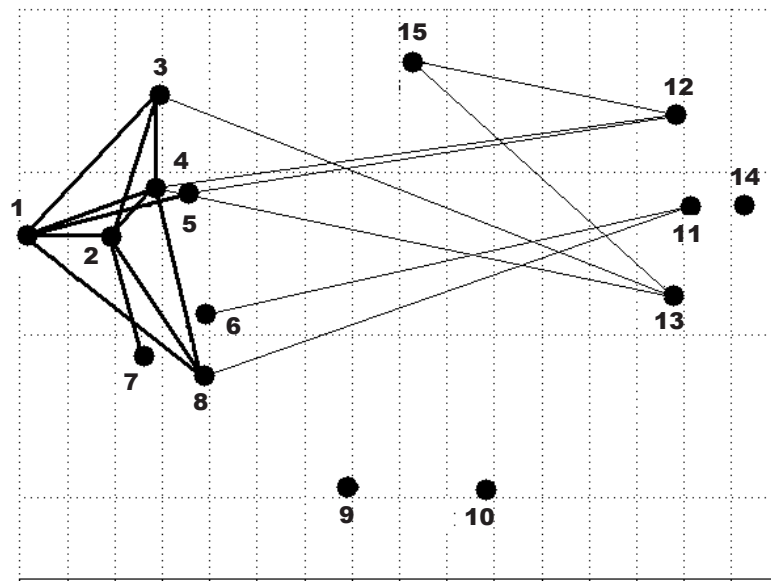


Fig. 5. Principal component loading for second component analyses of truss measurement: thick lines indicate dimensions with high positive loadings and thin lines indicate negative loading with this component.

Fig. 5. Cargas de la componente principal para los análisis de la segunda componente principal en las mediciones de la celosía: las líneas gruesas corresponden a las dimensiones con cargas positivas altas, y las líneas finas a una carga negativa con respecto a dicha componente.

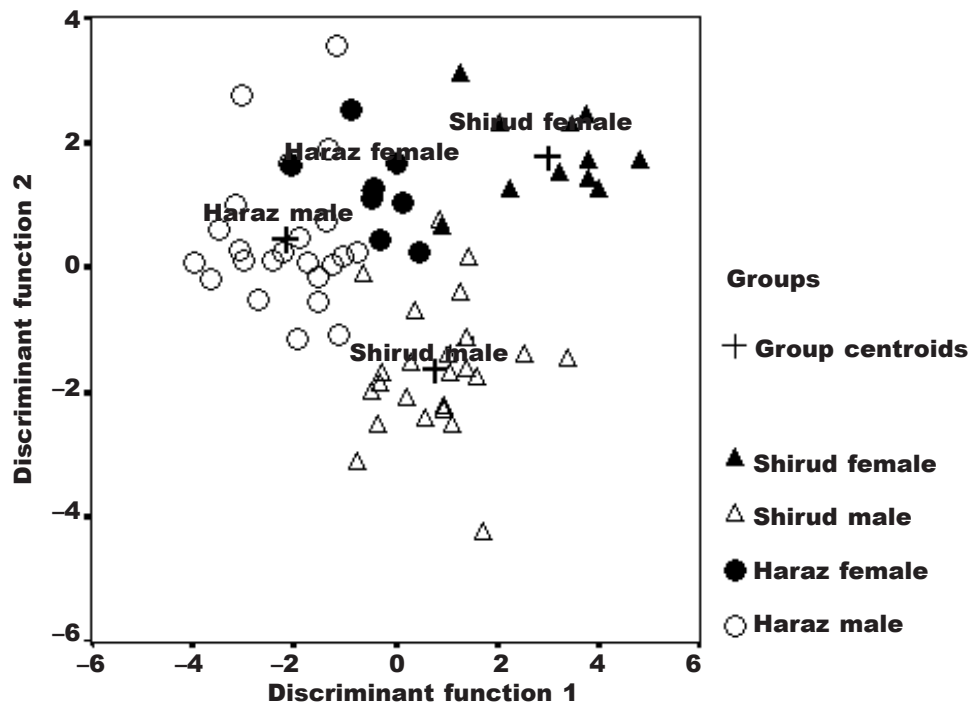


Fig. 6. Canonical variation scores of truss characters.

Fig. 6. Resultados de la variación canónica de los caracteres de la celosía.

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