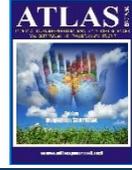




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THE USE OF GEOMETRIC MORPHOMETRY TECHNIQUES IN THE DIFFERENTIATION OF SPECIES AND SUBSPECIES

TÜR VE ALTTÜRLERİN AYRIMINDA GEOMETRİK MORFOMETRİ YÖNTEMLERİNİN KULLANILMASI

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ABSTRACT

Geometric morphometry is a shape-based classification tool. It uses only digital photographs and software. Using geometric morphometry methods in zoology, botany, anatomy areas where morphology is very important can make our work very easy. Namely; sometimes we find it difficult to distinguish species that are very similar to one another. Geometric morphometry works very well in the following situations: 1. In the separation of different species, which are difficult to distinguish. 2. Identification of newly formed subspecies 3. In the distinction of bees race and to get pure bee race (Gençer 1999), (Nazzi 1992). 4. Comparing the anatomical structures. eg in distinguishing between schizophrenic and normal brain (Waleed 2000). 5. Where geographically isolated species and subspecies have been identified, 6. Can be used to classify and distinguish between radiological, histological and electron microscopic images.

The advantages of geometric morphometrine are: 1. We can say that it is much cheaper than molecular genetics and biochemical methods, and even there is no cost at all. 2. Quick result is obtained with this method. 3. Numerous samples allow you to work at the same time. 4. Uses "multivariate statistics" from the latest and advanced statistical methods. 5. Provide assessment of fossil specimens which DNA is not isolated.

In this study, the geometric morphometry method is described step by step until the finest detail. The method is supported by an example while being explained. Our goal in doing this study is for researchers to learn this method and to spread it.

Keywords: Geometric morphometry methods, multivariate statistic, biometry software

ÖZET

Geometrik morfometri, dijital fotoğrafları ve yazılımları kullanan şekil tabanlı bir sınıflandırma aracıdır. Zooloji, botanik, morfolojinin çok önemli olduğu anatomi alanlarında geometrik morfometri yöntemlerini kullanarak çalışmalarımıza yeni bir boyut kazandırabiliriz. Taksonomik çalışmalarda birbirine çok benzeyen türlerin ayırt edilmesi bunlardan biridir. Geometrik morfometri, şu durumlarda araştırmacılara büyük faydalar sağlamaktadır: 1. Ayırımı zor olan farklı türlerin ayrılmasında. 2. Yeni oluşan alt türlerin tanımlanmasında 3. Arı ırkının ayırımında ve saf arı ırkının elde edilmesinde (Gençer 1999) (Nazzi 1992). 4. Anatomik yapıların karşılaştırılması. Örneğin şizofren ve normal beyin arasında ayırımı yapmakta (Waleed 2000). 5. Coğrafi olarak izole olmuş tür ve alt türlerin hangi bölgeye ait olduklarının belirlenmesinde 6. Radyolojik, histolojik ve elektron mikroskopik görüntüleri sınıflandırmak ve ayırmakta kullanılabilir.

Geometrik morfometrin avantajları şunlardır: 1. Moleküler genetik ve biyokimyasal yöntemlerden çok daha ucuz hatta hiç bir maliyetin olmadığını söyleyebiliriz. 2. Bu yöntemle hızlı sonuç elde edilir. 3. Çok sayıda örneğin, aynı anda çalışmanıza izin verir. 4. En son ve ileri istatistiksel yöntemlerden "çok değişkenli istatistikleri" kullanır. 5. DNA'nın izole edilmediği fosil örneklerinin değerlendirilmesini sağlamaktadır.

Bu çalışmada geometrik morfometri yöntemi en ince ayrıntısına kadar bir örnek ile adım adım anlatılmıştır. Bu çalışmadaki amacımız araştırmacıların bu yöntemi öğrenmeleri ve bu yöntemin ilgili araştırmacılar arasında yaygınlaşarak bilime katkı sağlanmasıdır.

Anahtar Kelimeler: Geometrik morfometri yöntemleri, çok değişkenli istatistik, biyometri yazılımı

1. INTRODUCTION

Geometric morphometry is used to distinguish between species, to identify sexually related dimorphisms, to identify new species as a result of geographical isolation, to control colony breeding and to numerical taxonomy. (Tatsuta et al. 2004).

This method, now called geometric morphometry, has been called biometrics in the past. In the years 1950 and 1960, metric measurements of assets were carried out. These measurements were evaluated with variance and chi square tests. After 1970, metric measurements were evaluated with multivariate statistical methods. In these years, the name has started to be called multivariate morphometry. In those years measurements were made manually. For this reason, the method was troublesome. In addition, statistical analysis was also difficult to perform. Because of these reasons, in those years that method was not widely used in scientific studies. The method used in those years is called traditional morphometry. Traditional morphometry approach is applied by many researchers. Some studies conducted by Nagamitsu et al. (1998), Sueli and Alves (2002), Lehmann et al. (2005), Arizaga et al. (2006), Francoy et al. (2006), Meixner et al. (2007), and Özkan et al. (2009) After the 1980s, with the widespread use of computers, morphometry methods also developed.

The digitalization of the photographs facilitated the morphometry work. now dozens of pictures could be stored on the computer. Now the measurements of these images were carried out in computer environment. The measurements were carried out with landmark marking. distance and angle values between landmarks can be easily calculated with this method. this work can actually be regarded as a somewhat modernized version of traditional morphometry. the measurement results obtained were evaluated in statistical package programs such as SPSS. The fact that the data in the traditional morphometry are scalars caused some limitations. There is no direction in scalar data for this reason it does not give any healthy information about the form. For this reason the landmarks in the new morphometry applications began to be expressed in x, y coordinates. The x, y coordinates gave more realistic information about the shape in the two-dimensional plane. This new form of expression led to the emergence of geometric morphometry. Now the landmarks were evaluated together. This allowed landmarks to be on top in the most optimistic way. this method is expressed as superimposition. Superimposition measurement also removes the angular rotation defects of the object. The x, y coordinates recalculated after superimposition are now ready for statistical analysis. The X, Y format has become compatible with the matrix operations.

From the data in matrix form: discriminant analysis, canonical variates analysis, anova, factor analysis are performed. With multivariate statistical analyzes, it is determined whether there is a difference between the groups studied.

In this study, we was aimed the discrimination of some species of the genus *Ammophila* (W. Kirby, 1798) by using geometric morphometry. By using geometric morphometry methods and software, we can distinguish between *Ammophila heydeni*, *Ammophila sabulosa* and *Ammophyla terminata* We separated species from each other only by using wings. (Yüksel and Tüzün 2011).

2. MATERIAL AND METHOD

The first stage in geometric morphometry studies; the anatomical structure to be compared is to obtain photographic images. We used the front wing photographs of the digger wild bees in this study. The wings were removed in an appropriate manner. It was placed between the lam and the coverslip. It was glued with Canadian balsam. Preparations prepared were dried.

Photos were taken on a Carl Zeis Digital microscope. Photos saved in high resolution, JPG format are now suitable for Geometric Morphometry work.

A biometric measurement list was prepared from photographs uploaded to TPSutil software. (Rohlf 2006). A biometric measurement list was uploaded to TPSdig software. TPSdig software used this list to display the photographs to landmarks being marked. After all landmark markings have been made the Landmark measurement file was saved in TPS format. (Rohlf 2003). CoordGen6f standardized landmark data in TPS format file with superimposition method (Sheet 2001). After the standardization process the discrimination function analysis was performed by using CVA Gen6o (Yüksel and Tüzün 2011).

2.1. Geometric Morphometry Analyses Procedure

Even though a lot of software is used in geometric morphometry studies, TPS and IMP series software which are widely used are the most popular ones. The process of taking photos until the graphics are obtained can be summarized as follows:

1. Recording wing photographs to PC in JPG format
2. Creating a list of photographs to be measured in TPSutil software
3. Performing sequence and landmark measurements in the TPSdig software (Rohlf 2003).
4. Standardization of landmark data of TPS format obtained with the measurement with CoordGen6f. (Sheets 2001).
5. Conducting discriminant function analysis with CVAGEN6o or performing factor analysis (by Principal Component method) with PCA Gen6p (Sheets 2002).
6. Obtaining 2D or 3D graphics from discriminant functions or factor components.

2.2. Creating a list of photos to be measured in TPSutil software

If the wing photographs are to be measured in which category (Type, species, subspecies), the folder where the name of the category is given is recorded. The wing paintings in the folder are *Ammophila heydeni*_M045.jpg, ... *Ammophila sabulosa*_N112.jpg.

The TPSUtil software is run and the "Select tps file from images" is selected by checking the Select operation option.

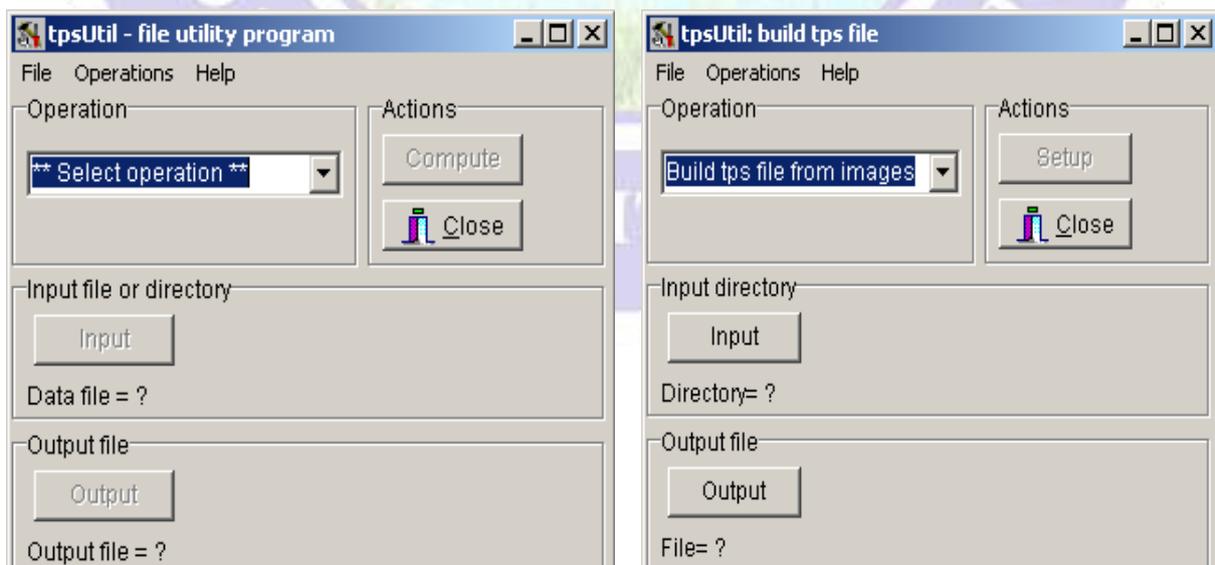


Figure 1 Creating a measurement list with the TpsUtil program.

Then click the "Input" button that is activated and click the "open" option. Then the output button is clicked and a name is given to the TPS file under which all landmark measurements will be recorded under the same folder. After this process is activated, click the "Setup" button to check the list.

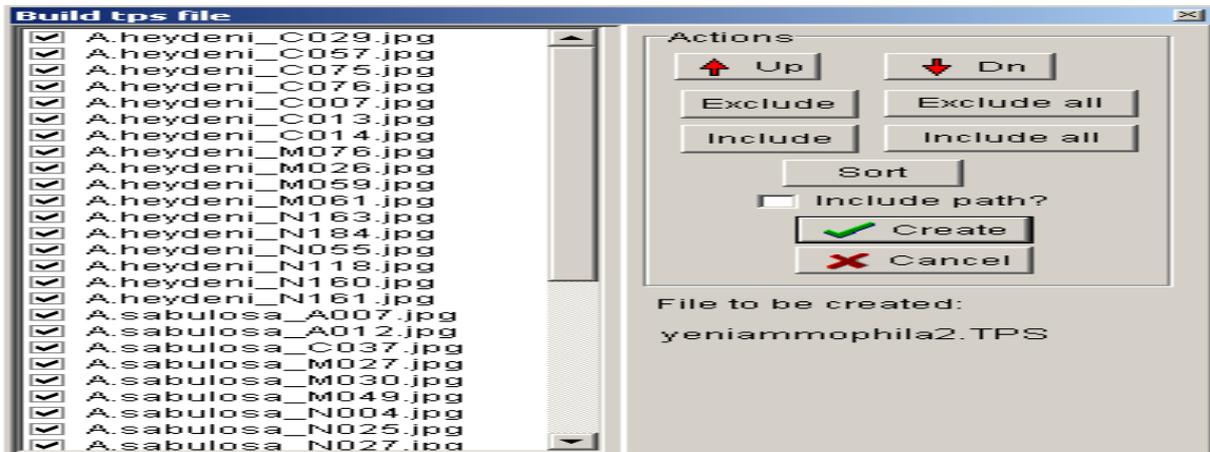


Figure 2 List created in TpsUtil program

Click on the "Create" button in the opened window to create the TPS file.

Performing rank and landmark measurements by executing the listener in TPSdig software.

The TPS file created in the previous step is opened with the TPSDig2 software. (File, Input Source, File)

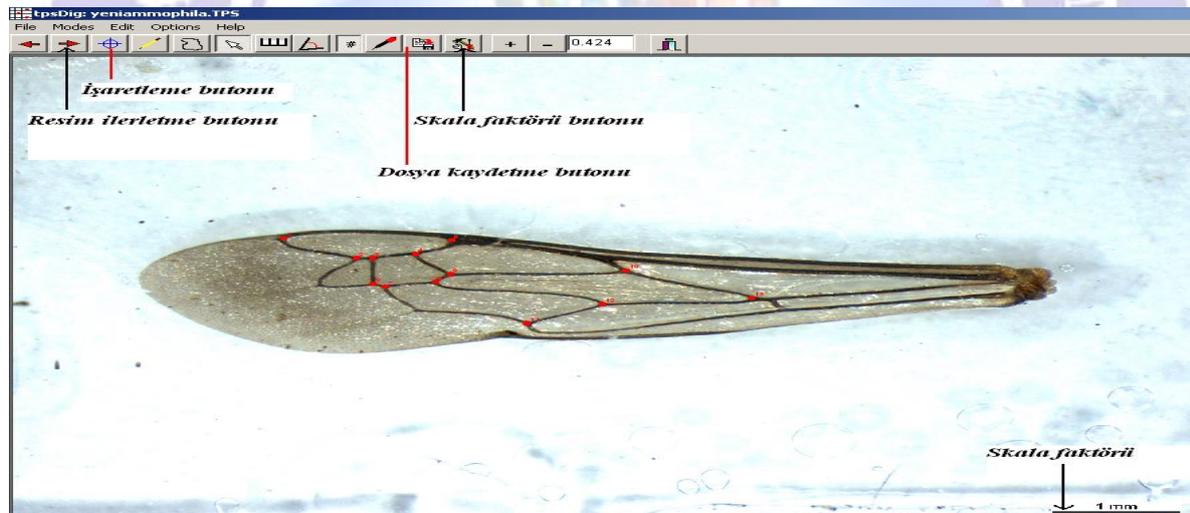


Figure 3 TPSDig program interface.

When the TPS file is opened, the wing image is displayed according to the list in the TPS file. Click on the scale factor button to take the measurement of the scale at the lower right corner of the above picture. Click "Set Scale" for this operation. With the cursor, the start and end of the scale are selected, and the "OK" button is clicked. In this way the picture scale factor is determined. Clicking on the Mark button will mark the landmarks that are selected. Click on the Save button to complete the measurement for the image on the screen. The image advance button is clicked to the next wing image. The above operations are repeated until the latest wing picture is reached.

2.3. Standardization of landmark data of TPS format obtained as a result of measurement with CoordGen6f

The measured TPS file In the CoordGen6f software, click the Load TPS File (with ruler) button to open the TPS file we created in the previous step. Then click on the Carry Out Rescaling button to standardize the lengths of the scale factors.

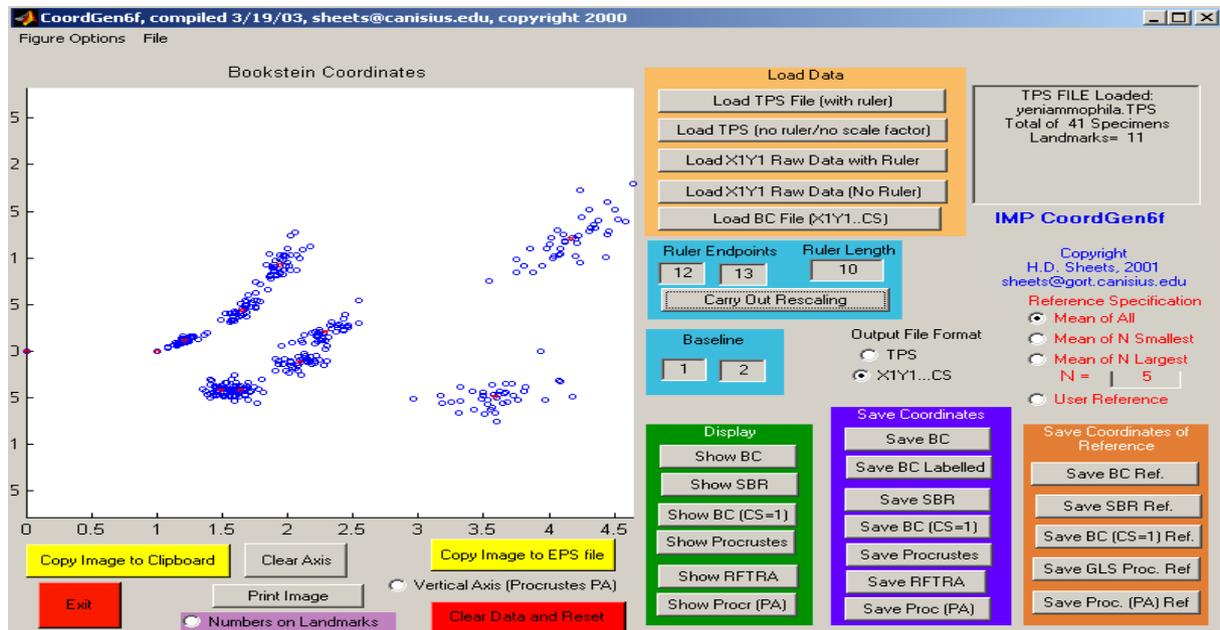


Figure 4 Coordgen6f software interface

Then click on the "Show Procrustes" button and the landmarks Procrustes landmark standardization or Superposition will be done.

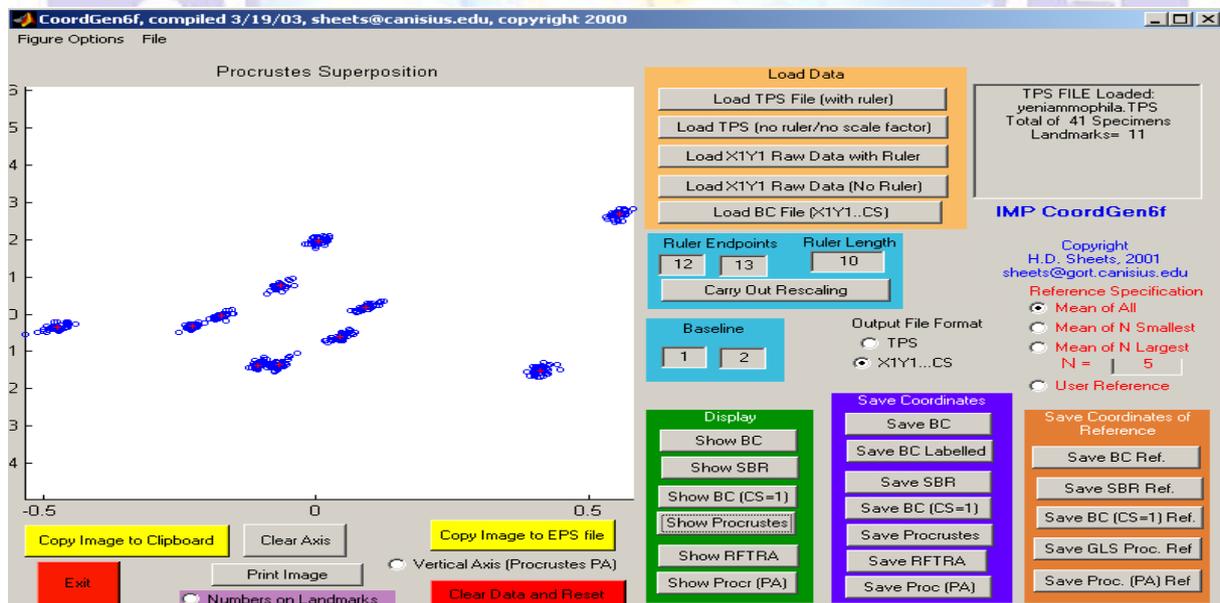


Figure 5 CoordGen6f software interface and view of landmarks

As you can see from the above, all the wing landmarks are overlaid. Then click "Save Procrustes" button to get the standardized measurement file. If the extension of the file is specified as .txt, the editing process is easier.

2.4. Performing discriminant function analysis with CVAGen60 or performing factor analysis (by Principal Component method) with PCAGen6p.

In the standardized data file created in the previous phase, the measured values of the species come from the sub-line. It is necessary to create a "group file" indicating the groups to which these values belong. There is no need to use the group file in PCA analysis. With the Show Labels feature groups can be observed in a graphical environment. PCA analysis is equivalent to Factor Analysis performed in SPSS. As in Factor Analysis, there is no need to enter the groups that the data belong to in the analysis. However, in CVA analysis, it is absolutely necessary to create a group file. CVA analysis is the counterpart of the Discriminant Function Analysis in SPSS. In the DFA analysis, the groups to which the data indicated in the previous section belongs must be specified before analysis. The standardized data file looks like this. It appears that the group file shows which group the measurement data belongs to on each line.

The screenshot shows a text editor window titled 'Komut İstemi - edit' with the file path 'D:\yeni_ipm\ammophila.txt'. The content is a list of standardized measurements for 33 different species, each represented by a line of 11 numerical values. The values are arranged in a grid-like format with some negative signs.

Species	1	2	3	4	5	6	7	8	9	10	11
1	-0.50117	0.059312	-0.225607	0.00723388	-0.173372	0.0198483	-0.0490766	0.069918	0.0814	0.0814	0.0814
2	-0.462869	0.0611899	-0.249578	-0.00358684	-0.16174	0.0104819	-0.0271858	0.0814	0.0814	0.0814	0.0814
3	-0.464533	0.0745377	-0.221603	0.0163729	-0.17416	0.0286749	-0.051538	0.0857876	0.0814	0.0814	0.0814
4	-0.536875	0.0573942	-0.238648	0.0111809	-0.172216	0.0350707	-0.0475645	0.08113	0.0814	0.0814	0.0814
5	-0.48809	0.0514412	-0.227171	0.0149558	-0.15897	0.0403008	-0.0490766	0.087564	0.0814	0.0814	0.0814
6	-0.498836	0.0528627	-0.224409	0.0149382	-0.17167	0.0332969	-0.0547931	0.082609	0.0814	0.0814	0.0814
7	-0.470689	0.0576433	-0.226017	0.0193128	-0.158464	0.0429426	-0.0538836	0.08684	0.0814	0.0814	0.0814
8	-0.50923	0.0571477	-0.24581	0.0102698	-0.172003	0.035292	-0.0529413	0.0842951	0.0814	0.0814	0.0814
9	-0.447806	0.0524178	-0.228237	0.00880302	-0.17167	0.0304913	-0.0349539	0.09564	0.0814	0.0814	0.0814
10	-0.446757	0.0548409	-0.230402	0.0111809	-0.17167	0.0281517	-0.0538825	0.081963	0.0814	0.0814	0.0814
11	-0.485287	0.0581683	-0.228495	0.0122408	-0.170023	0.0276425	-0.0491011	0.08906	0.0814	0.0814	0.0814
12	-0.472122	0.0554819	-0.231787	0.00829963	-0.17167	0.0298623	-0.0475148	0.08697	0.0814	0.0814	0.0814
13	-0.467388	0.0513111	-0.240055	0.0105574	-0.182182	0.0236694	-0.04799	0.0868957	0.0814	0.0814	0.0814
14	-0.465368	0.0570078	-0.234442	0.00708818	-0.162238	0.0295455	-0.0490766	0.0958	0.0814	0.0814	0.0814
15	-0.472692	0.0569219	-0.216951	0.0166214	-0.173942	0.0301733	-0.0316432	0.10414	0.0814	0.0814	0.0814
16	-0.446683	0.0716402	-0.21001	0.0241797	-0.189044	0.0287264	-0.0547998	0.086650	0.0814	0.0814	0.0814
17	-0.570969	0.054075	-0.245115	0.0111809	-0.166039	0.0341734	-0.0490766	0.088848	0.0814	0.0814	0.0814
18	-0.450941	0.0650525	-0.223867	0.00750785	-0.172067	0.0291477	-0.0490108	0.0869	0.0814	0.0814	0.0814
19	-0.455191	0.0631973	-0.234451	0.00743168	-0.167497	0.0287264	-0.0541071	0.0890	0.0814	0.0814	0.0814
20	-0.433611	0.0593311	-0.228495	0.00264103	-0.172435	0.0233336	-0.0503315	0.0869	0.0814	0.0814	0.0814
21	-0.472692	0.0576929	-0.229004	0.0071116	-0.155775	0.0295128	-0.0243463	0.09668	0.0814	0.0814	0.0814
22	-0.486298	0.0572479	-0.228495	0.00737316	-0.148268	0.0317407	-0.0545835	0.0814	0.0814	0.0814	0.0814

Figure 6 Standardized measurements

The screenshot shows a text editor window titled 'Komut İstemi - edit' with the file path 'D:\yeni_ipm\ipm\ammophilagrup.txt'. The content is a list of group membership values for 33 different species, each represented by a line of 11 numerical values. The values are arranged in a grid-like format with some negative signs.

Species	1	2	3	4	5	6	7	8	9	10	11
1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1

Figure 7 Group file for the standardized data set.

When the group file is created, the group file must match the correct types in the group membership dataset, because the number of measurements in the dataset (the number of types) and the number of group members in the group file are equal at the beginning of what needs to be taken care of (when both file numbers are equal). In line skip reduction situations, the group file must be reorganized. There should be no "line space" at the beginning, middle, and end of the group file. As is known, there is a gap in the computer language gap. In such cases the program will fail. This type of error is usually found in the last line. After entering the last line of the group member, enter a space character at the end of the space bar and end group membership instead of entering the line with the enter.

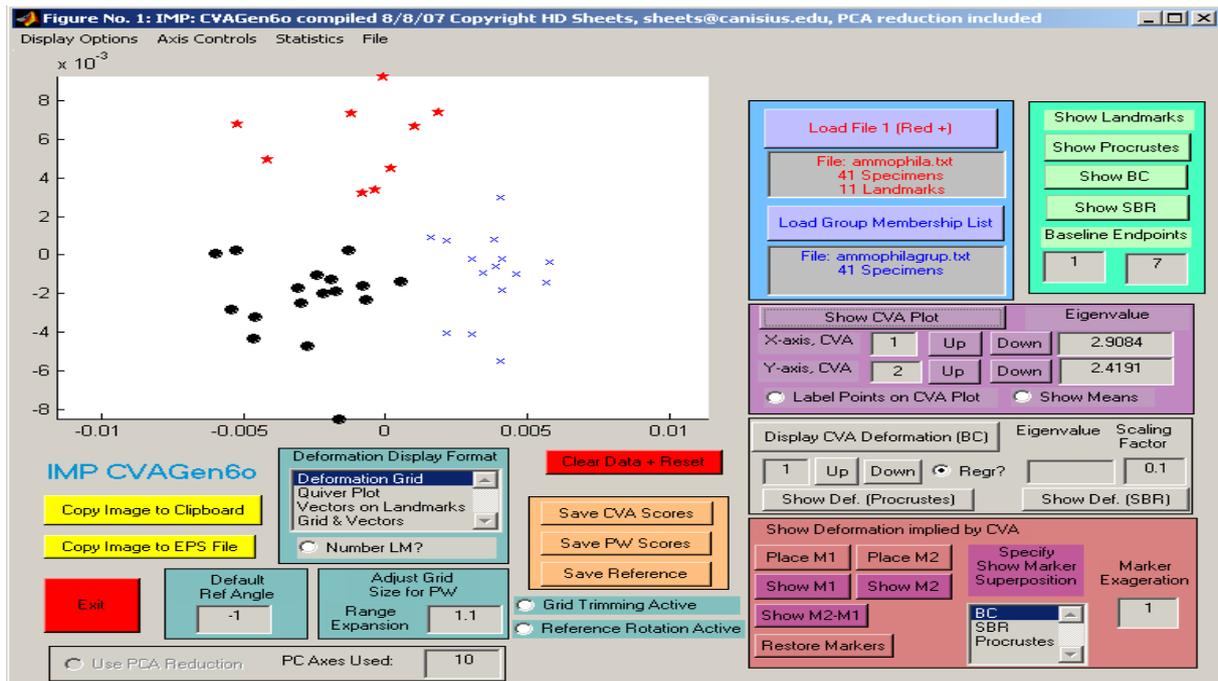


Figure 8 CV analysis performed in the CVAGen60 program

The CVAGen60 program is run before starting the CV analysis. Click the Load file button to open the standardized measurement file shown in the picture. Then click on the Load group membership button and the group file shown in the picture is loaded. Click on the Show CVA plot button to create a CVA graph using the 1st function on the X axis and the 2nd function on the Y axis. Generally, the first and second functions are used to generate the graph.

3. DISCUSSION

Morphometrics is very important in biology because it allows quantitative descriptions of organisms. Quantitative approach allowed scientists to compare shapes of different organisms much better and they no longer had to rely on word descriptions that usually had the problem of being interpreted differently by each scientist (Gelsvartas 2005).

Traditional morphometry can answer the following questions: 1. Are two specimens different? How are they different? 2. Are two (or more) samples different? How are they different? 3. With what factors are the differences correlated? 4. Do the differences correspond to a particular model or hypothesis? 5. How have the differences evolved? But Geometric morphometrics (both landmark-based and outline) is powerful and popular because information regarding the spatial relationship among landmarks on the organism is contained within the data. This gives the ability to draw evocative diagrams of morphological transformations or differences, offering an immediate visualization of shape and the spatial localization of shape variation. Such graphical representation is easier to intuitively understand than a table of numbers (Webster and Sheets 2010).

For this reason, in this study, we have included the latest and advanced biometrics method, Geometric morphometry theory and applications. Geometric morphometry has a distinct place in biological sciences, as it is a school in itself and supports both taxonomy studies. By studying this article only, we think that the researchers will gain knowledge and skills in the theory and practice of this subject.

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