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Full Length Research Paper

Significance of wing morphometry in distinguishing some of the hymenoptera species

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In this study geometric morphometric analysis of some Hymenoptera species was undertaken. The aim was to discriminate *maxillosus, flavipennis* and *pruinosus* species of the *Sphex* genus from each other by applying geometric morphometric methods. Species were identified by making use of the morphometric wing measurement data from different families in Hymenoptera group. In this study, the possibilities of applying morphometrics methods on taxonomy studies were explored. Moreover, the success rates of both methods were compared using geometric morphometry techniques.

Key words: Hymenoptera, species identification, taxonomy, geometric morphometrics.

INTRODUCTION

Morphology of wing geometry in different species of *Sphex* genus (*Sphex maxillosus, Sphex flavipennis and Sphex pruinosus)* was studied. Morphologic characters are mostly benefited from purified races' classification, distinguishing from each other, improvement and purity's controlling. Moritz assessed the wing angles and cubical index characters on the samples he obtained from Western Europe and differentiate the level of purity and hybridity of the purified races of Carniyol and Western Europe (Moritz, 1992). "Dupraw (1965)" identified species by making use of the wing angle and index characters of purified races of Europe. "Güler and Bek (2002)" accurately identified and classified pure races in Turkey by utilizing wing vein angles with discriminant analysis method.

In the current study, angles between wing veins of the species belonging to different Hymenoptera families as well as vein length ratios were calculated. It was aimed that diagnosis of expected species could be made from saved values. Firstly, by means of computer software, the measurement data were recorded in the database. These data recorded in the database were then compared with wing landmark values obtained from randomly selected museum samples. It was found that the comparison results were mostly helpful in species estimation. Moreover, at the end of the study it was discovered that similarities had coherence at the species level; and the relevance of evolutionary connection to the wing morphometry should be examined.

MATERIALS AND METHODS

Identification of the species

Wing geometric morphology in *S. maxillosus, S. flavipennis* and *S. pruinosus* were studied. Although these three species resemble each other in terms of appearances, they are distinct in terms of their length ratio of angle of wing veins. Standard deviation averages of each species for all possible angles and ratios were calculated. Angle and ratio values that have significance in the distinction of this species are presented in Table 1.

(X, y) Landmark data transform algorithm:

1. The intersection points on the wing veins were identified. These points were numbered from 1 to 20 (Figure 1) and 1-24 (Figure 3).
2. The distance between these points were identified.
3. The vein length ratios were calculated according to the achieved distance values (a/b = ratio).

All the possible comparisons and ratios were calculated according to this method. The achieved values were recorded. Moreover, the angle values between the points were calculated and recorded. Thus, a table for ratios and angles were formed for all the species.

Example:

R2.3 / 5.6 = the distance between 2 and 3/ the distance between 5 and 6, R12.17/17.18 = the distance between 12 and 17/ the distance between 17 and 18, ANGLE 349 = The angle of number 4 on the triangle that is formed between the points 3, 4 and 9.

4. The tables showing the values of each species were analyzed

Table 1. Statistical differences between the species of *Sphex* genus (Classification Results).

			Predicted Group Membership			Total
		Species	*S. flavipennis*	*S. maxillosus*	*S. pruinosus*	
Original	**Count**	*Sphex flavipennis*	25	0	0	25
		Sphex maxillosus	0	18	0	18
		Sphex pruinosus	0	0	7	7
	%	*Sphex flavipennis*	100,0	,0	,0	100,0
		Sphex maxillosus	,0	100,0	,0	100,0
		Sphex pruinosus	,0	,0	100,0	100,0
Cross-validated	**Count**	*Sphex flavipennis*	21	4	0	25
		Sphex maxillosus	3	9	6	18
		Sphex pruinosus	0	5	2	7
	%	*Sphex flavipennis*	84,0	16,0	,0	100,0
		Sphex maxillosus	16,7	50,0	33,3	100,0
		Sphex pruinosus	,0	71,4	28,6	100,0

a) Cross validation was done only for those cases in the analysis. In cross validation, each case was classified by the functions derived from all cases other than that case.
b) 100, 0% of original grouped cases correctly classified.
c) 64, 0% of cross-validated grouped cases correctly classified.

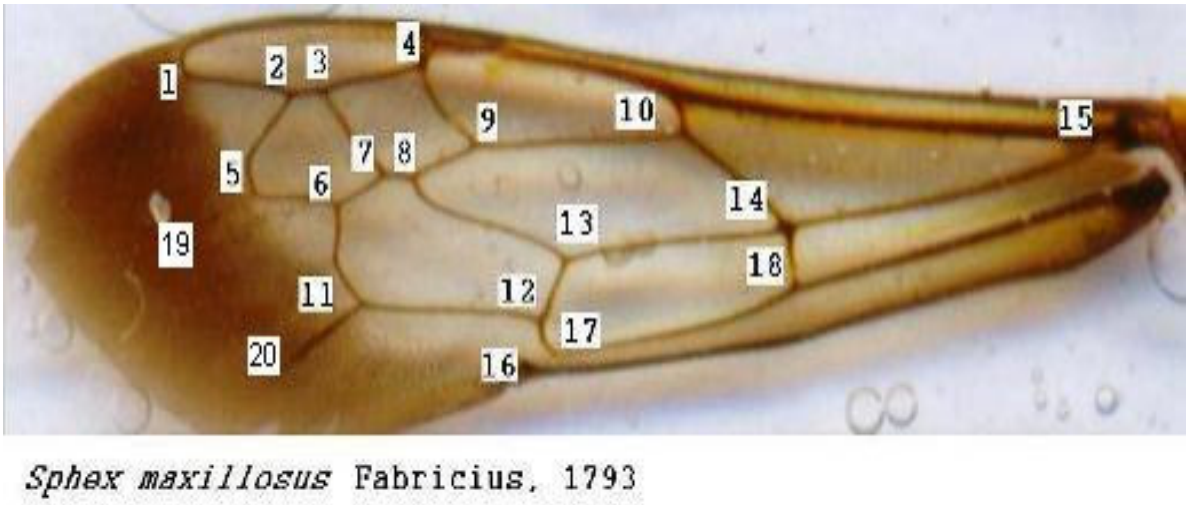

Figure 1. Calculated landmark points used in wing measurement (1,2,3,4 = (RS) + (r-rs), 2 -5 = (3r-m), 3- 7 = (2r - m), 4- 9 = (Rs), 5-6-7-8 = (M), 9 -10 = (Rs + M), 11 -6 = (2m - cu), 8 -13 = (1m - cu), 13 -14 = (cu), 14 - 10 = (M), 14 - 15 = (M + Cu), 12- 13 = (Cu), 12 -17 = (2cu - a), 18 -15 = (A)).

statistically for comparing each different species (Using stepwise discriminant function analysis).

Forewings of *S. maxillosus, S. flavipennis* and *S. pruinosus* species were used. The values given in Supplemental 1 were calculated separately for the species according to wing landmark patterns shown in Figure 1. Microscopic slides of wings were fixed with Canada balsam and they were scanned with a 2400 dpi HP scanner. Pictures of wings were saved in computer as digital images. Measurements were taken using a computer and all possible angle and index values were calculated. For each species, these values were not accepted as a distinguishing character if these values were very close to each other or intricate. If intervals of these calculated values (average + - standard deviation) did not intersect with other species, they were then considered as a distinguished character in species differentiation (Supplemental 1).

Classification analysis of the measurement results (Table 1-2, Figure 4) were done on SPSS program (Gnandesikan R, 1990).

Geometric morphometry method

Preparation of (X, Y) Landmark coordinate data from the wing photos

Forewing photos were taken using Leica documentation system. In the photos, Scale factor values were shown on the ruler. Wing photos were inserted in the same folder in computer and their names

Table 2. Characteristics of morphometric differences between the species of *Sphex* genus ordered by importance.

Step	Entered	Wilks' Lambda							
		Statistic	df1	df2	df3	Exact F			
						Statistic	df1	df2	Sig.
1	R(2-3/5-6)	,383	1	2	47,000	37,929	2	47,000	,000
2	R(12-17/17-18)	,242	2	2	47,000	23,766	4	92,000	,000
3	R(4-9/9-10)	,184	3	2	47,000	19,961	6	90,000	,000
4	ANGLE-3,4,9	,136	4	2	47,000	18,876	8	88,000	,000
5	R(11-12/12-13)	,107	5	2	47,000	17,745	10	86,000	,000

At each step, the variable that minimizes the overall Wilks' Lambda is entered. a) Maximum number of steps is 150. b) Minimum partial F to enter is 3.84. c) Maximum partial F to remove is 2.71. d) F level, tolerance, or VIN insufficient for further computation, R: Ratio.

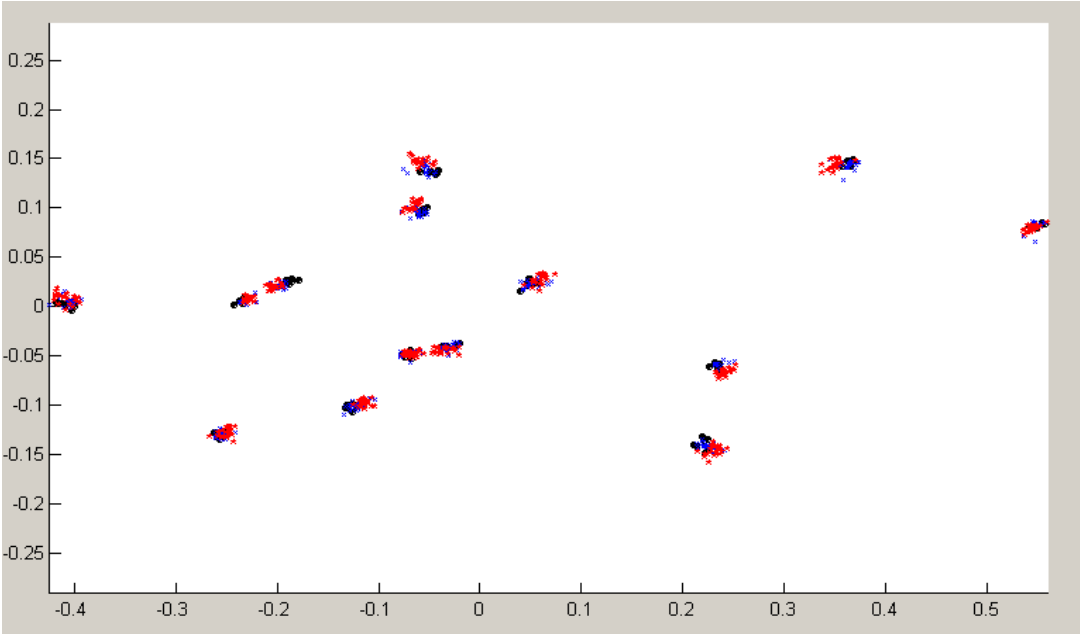

Figure 2. Graphical distribution of the 14 landmarks according to groups in PCAGen6p program.

were ordered in groups. (Such as, *S. maxillosus* 001.jpg, *S. maxillosus* 020.jpg). The TPS file was then formed using TpsUtil software (Rohlf, 2006a). The file formed by targeting the wing photo folder and using the "build TPS from image" option was saved in the wing photo folder. The TPS file that was formed in TpsUtil software was then opened using TpsDig2 software (Rohlf, 2006b) and proceeded with wing landmark labeling.

Analysis of wing Landmark data (in TPS format)

At the end of the process, the TPS file that contain all the data was loaded on TpsRelw program (Rohlf 2006c) for Relative Warp Analysis (Table 5). For Principal Component Analysis, (Figure 7), PCAGen6p software (Sheets, 2002) was utilized. Since this software was incapable of direct TPS file processing, data transformation was undertaken using Co-ordGen6f software (Sheets, 2002). Landmark data file that has been converted to BC format is not meaningful alone for the PCA analysis. This program needs a group file in ASCII format as well. The group file was formed in Edit (Dos 6.22) program. As seen below, 14 landmarks were shown on the wing photo (Figure 2). Individual groups were labeled with different colors. In the PCA analysis, among all the PCA scores calculated for building the cluster graph, first and second axes which possess high characteristic value (Eigen value) and variance were utilized. For the Canonical Varieties Analysis CVAGen6o software (Sheets, 2002) was used. Data input in this program is the same as that in PCAGen6p. Since CVA results were satisfactory, the data was not further subjected to PCA deformation. The method descrybed above was applied to both sections of the study.

Wing geometric morphometrics based species estimation

Wing landmark values of the saved wing pictures were added to the database. 24 landmark locations were marked on the wing (Figure 3).

When comparing the landmark values between the species found

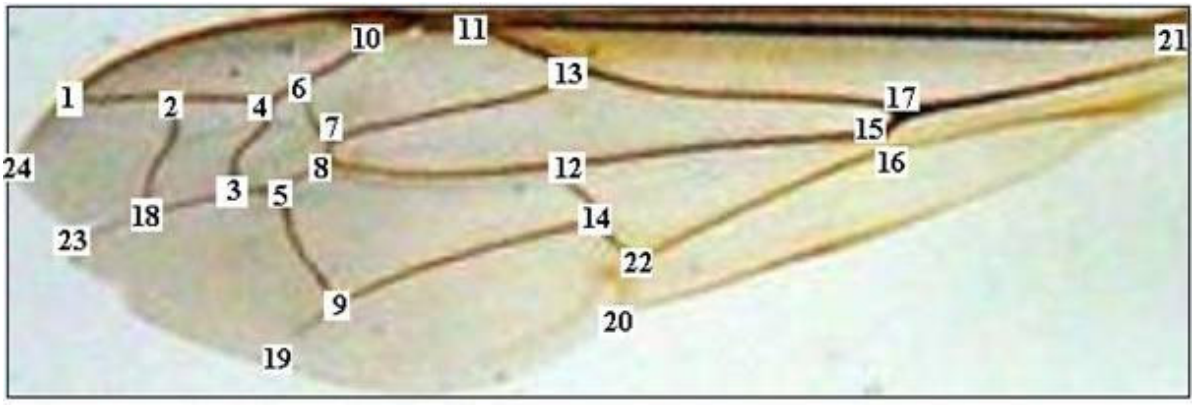

Figure 3. Wing landmark points (1,2,4,6 = (RS) + (r-rs), 2 - 18 = (3r-m), 3- 4 = (2r - m), 6- 7 = (Rs), 18- 3 - 5 - 7 = (M), 7 - 13 = (Rs + M), 5 - 9 = (2m - cu), 8 -12 = (1m - cu), 12 -15 = (cu), 13 - 17 = (M), 15 -21 = (M + Cu), 12- 15 = (Cu), 20 - 22 = (2cu - a), 22 -21 = (A)).

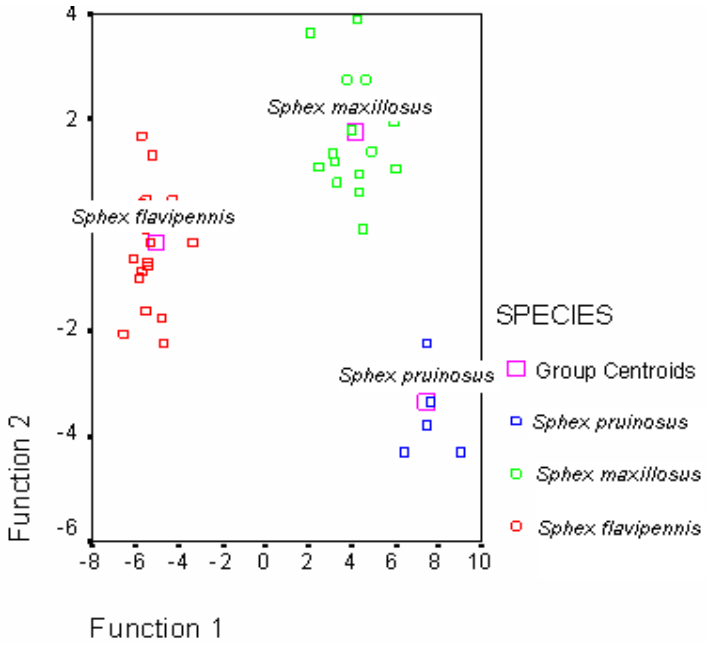

Figure 4. Chart of classification results.

in the database and the species to be identified, angle values between the landmarks and the distance between the landmarks were used. The software gives results according to the total difference between angle and distance values.

Total Angle variation = |Angle1 $^{[\text{unknown species}]}$ – Angle1 $^{[\text{species found in the database}]}$ | + |Angle2 $^{[\text{unknown species}]}$ – Angle2 [species found in the database] | + ……+ |Angle12 $^{[\text{unknown species}]}$ – Angle12 $^{[\text{species found in the database}]}$ |

Total Length variation = |1-(Length 1$^{[\text{unknown species}]}$ / Length 1$^{[\text{species found in the database}]}$) | + | 1- (Length 2$^{[\text{unknown species}]}$ / Length2 $^{[\text{species found in the database}]}$) | + ……+ |1- (Length 12$^{[\text{unknown species}]}$ / Length 12$^{[\text{species found in the database}]}$)|

The greater the total values were, the greater was the difference and the possibility that there is a different species was increased. With the decrease in the total value (sum of differences), the similarity increased and this supported species estimation.

The distance between landmarks was calculated according to landmark x, y coordinates, and the angles were calculated according to distance values in Cosinus theorem. Due to the distance calculations according to the differences in x_1:y_1, x_2:y_2…x_{21}:y_{21} coordinates, the same angle and distance values were obtained in the oblique, reverse, horizontal and vertical scans of photographs of the same wing pattern. This feature makes the measurements easier.

RESULTS AND DISCUSSION

The species *S. pruinosus, S. maxillosus and S. flavipen-*

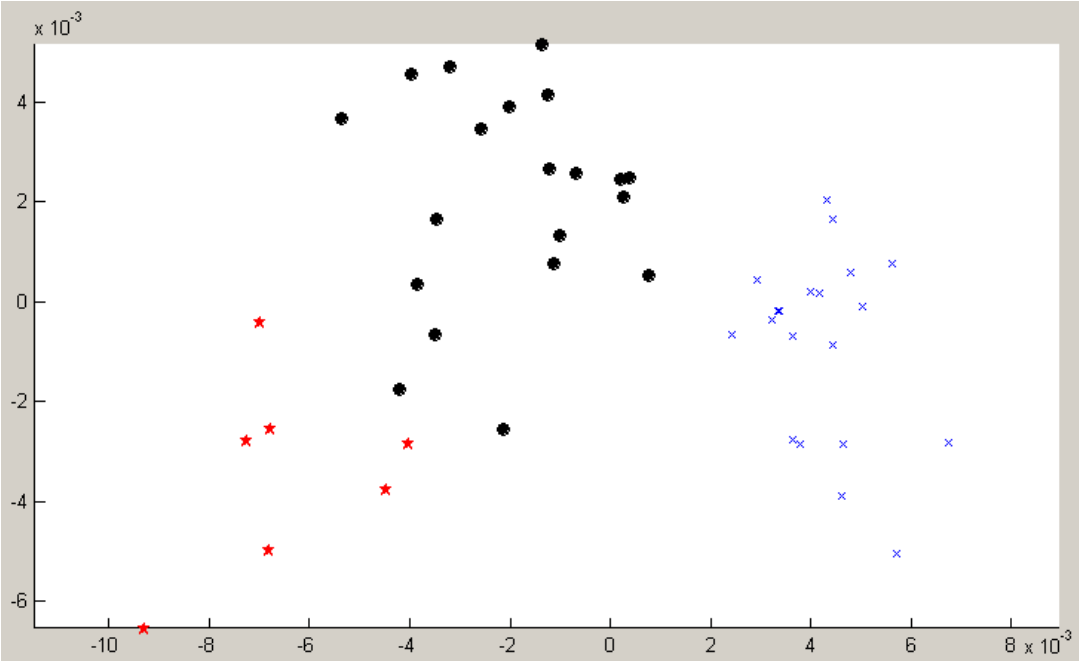

Figure 5. Canonical Varieties analysis (Axis 1 Lambda = 0.0573 chisq = 92.9270 df = 48 p = 0.000108006 X - Axis CVA 7.5688 Y - Axis CVA 1.0364) *Sphex flavipennis, Sphex maxillosus and Sphex pruinosus* were labeled with *o, x* and **,* respectively.

nis could be distinguished depending on their wing structure. Some of the angle and length ratio values among the intersection points of wing veins shown in Figure 1 could be used in differentiating *S. pruinosus, S. maxillosus and S. flavipennis* from each other. The ratio of length of body parts to each other is more important than the length values in terms of taxonomy (Mayr, 1969). Therefore, angle and length values among the wing veins were used. According to the canonical discriminant function analysis made with respect to these values, species were separated from each other quite successfully in geometric morphometric terms (Figure 4). According to the results (Table 2) of the Stepwise discriminant function analysis made by using these values, Ratio (2-3/5-6), Ratio (12-17/17-18), Ratio (4-9/9 -10), Angle (3-4-9) and Ratio (11-12/12-13) appeared to be particularly important in differentiating these species. Characteristics of morphometric differences between the species of *Sphex* genus ordered by importance are shown in Table 2. All calculated values were presented in Supplemental 1.

Geometric morphometry results

According to the CVA results of and the 1st and 2nd axis values, *Sphex flavipennis, Sphex pruinosus ve Sphex maxillosus* were clustered as shown in Figure 5. The proximity of the clusters of *S. flavipennis and S. pruinosus* members were noteworthy. The cluster of the members of *S. maxillosus* appeared to be clearly separated. As observed in Table 3 and Table 4, the best success in clustering in terms of group memberships was observed in *S. maxillosus* species. CVA analysis (or Discriminant Function Analysis-DFA-) has also been utilized in first method. Function 1 in DFA corresponds to Axis X in CVA. As seen in Figure 4, DFA or CVA results of the transformed data appeared to be more satisfactory. In the first method (data transformation method) all three *Sphex* species could be separated from each other more efficiently.

In PCAGen6 and CVAGen6 software, it is not possible to undertake clustering analysis. These programs more likely verify the accuracy of the previously predicted group memberships. Landmark data recorded in TPS or BC format and used in geometric morphometry methods, like the linear data, is not appropriate for use in statistics software designed for general purposes. However, Relative Warp analysis offers a solution for this problem. In this analysis, basic components are ordered according to their characteristic value (Eigen value) and variance values, a method similar to factor analysis. If there are various methods to determine the number of factors, usually 1st, 2nd and 3rd factors are used. In this study, 1st and 2nd factors were used (Figure 6). Relative Warp Analysis results showed groups similar to those found in CVA. Yet, there are no limitations in the statistical analysis of transformed data. It is possible to obtain satisfactory results using any software that is capable of K means clustering analysis.

According to the clustering results obtained using the

Table 3. Group memberships formed according to the CVA results.

Specimen	Ordinal group	Group code	CVA Group	Specimen	Ordinal group	Group code	CVA group
1	1	1	1	25	2	2	2
2	1	1	1	26	2	2	2
3	1	1	1	27	2	2	2
4	1	1	1	28	2	2	2
5	1	1	1	29	2	2	2
6	1	1	1	30	2	2	2
7	1	1	1	31	2	2	2
8	1	1	1	32	2	2	2
9	1	1	1	33	2	2	2
10	1	1	3	34	2	2	2
11	1	1	1	35	2	2	2
12	1	1	1	36	2	2	2
13	1	1	1	37	2	2	2
14	1	1	1	38	2	2	2
15	1	1	1	39	2	2	2
16	1	1	1	40	2	2	2
17	1	1	1	41	3	3	3
18	1	1	1	42	3	3	1
19	1	1	1	43	3	3	3
20	1	1	1	44	3	3	3
21	2	2	2	45	3	3	3
22	2	2	2	46	3	3	3
23	2	2	2	47	3	3	3
24	2	2	2				

Table 4. Clustering success rates according to the CVA results.

Species	Sample Size	Number of Correctly Clustered	Percentage of Correctly Clustered
Sphex flavipennis	20	19	95%
Sphex maxillosus	20	20	100%
Sphex pruinosus	7	6	85%

1st and 2nd basic components obtained in Principle Component Analysis of clustering results, *S. flavipennis* and *S. pruinosus* individuals were successfully separated from each other. However, *S. maxillosus* individuals were observed in both groups (Figure 7).

Wing geometric morphometrics based species estimating

"The similarity coefficient "was calculated by using the total discrepancy of the ratio of wing veins to each other and the total discrepancy of the angles among the veins in the formula below.

Similarity coefficient = (1 / A x R) x K

A = Sum of differences in wing angles; R = sum of differences among the ratios of wing veins; and K = constant number.

Geometric Morphometry results of various Hymenoptera species

Based on 21 different landmarks, groups that were shown in different colors did not exhibit homogeneity (Figure 8). According to the CVA results, the groups were not clearly separated, and most groups were mixed (Figure 9, Table 7). The basic reason for this is that different species of Hymenoptera families exhibit significant differences. High variance values detected in the groups, and the average values calculated for each variable are not specific for each group. Therefore, the group memberships of the species that stand away from the center

Table 5. RWA statistical results.

Variances at each landmark for aligned specimens				Consensus configuration		Eigen values for each principal warp:	
Landmark	S²x	S²y	S²				
1	5.41E-05	3.64E-05	9.05E-05	-0.38603	0.12888		
2	2.1E-05	5.35E-06	2.64E-05	-0.21717	0.07589		
3	4.97E-05	6.12E-06	5.58E-05	-0.18223	0.08011		Eigen value
4	4.31E-05	2.5E-05	6.81E-05	-0.02966	0.1132	1	2.73E+02
5	5.18E-05	5.07E-05	0.000103	-0.00913	0.15246	2	2.25E+02
6	3.57E-05	1.33E-05	0.000049	-0.27961	-0.04641	3	1.24E+02
7	7.12E-05	9.82E-06	8.1E-05	-0.14401	-0.05886	4	5.89E+01
8	3.22E-05	7.84E-06	0.00004	-0.07987	-0.02515	5	4.46E+01
9	5.63E-05	1.05E-05	6.68E-05	-0.04389	-0.0308	6	2.25E+01
10	8.34E-05	1.25E-05	9.59E-05	0.06079	0.00723	7	1.33E+01
11	5.07E-05	3.61E-05	8.68E-05	0.17245	-0.20492	8	1.01E+01
12	3.17E-05	2.72E-05	5.89E-05	0.20883	-0.13272	9	7.07E+00
13	4.01E-05	1.12E-05	5.13E-05	0.54526	-0.0884	10	3.38E+00
14	8.58E-05	1.98E-05	0.000106	0.38429	0.02949	11	1.18E+00

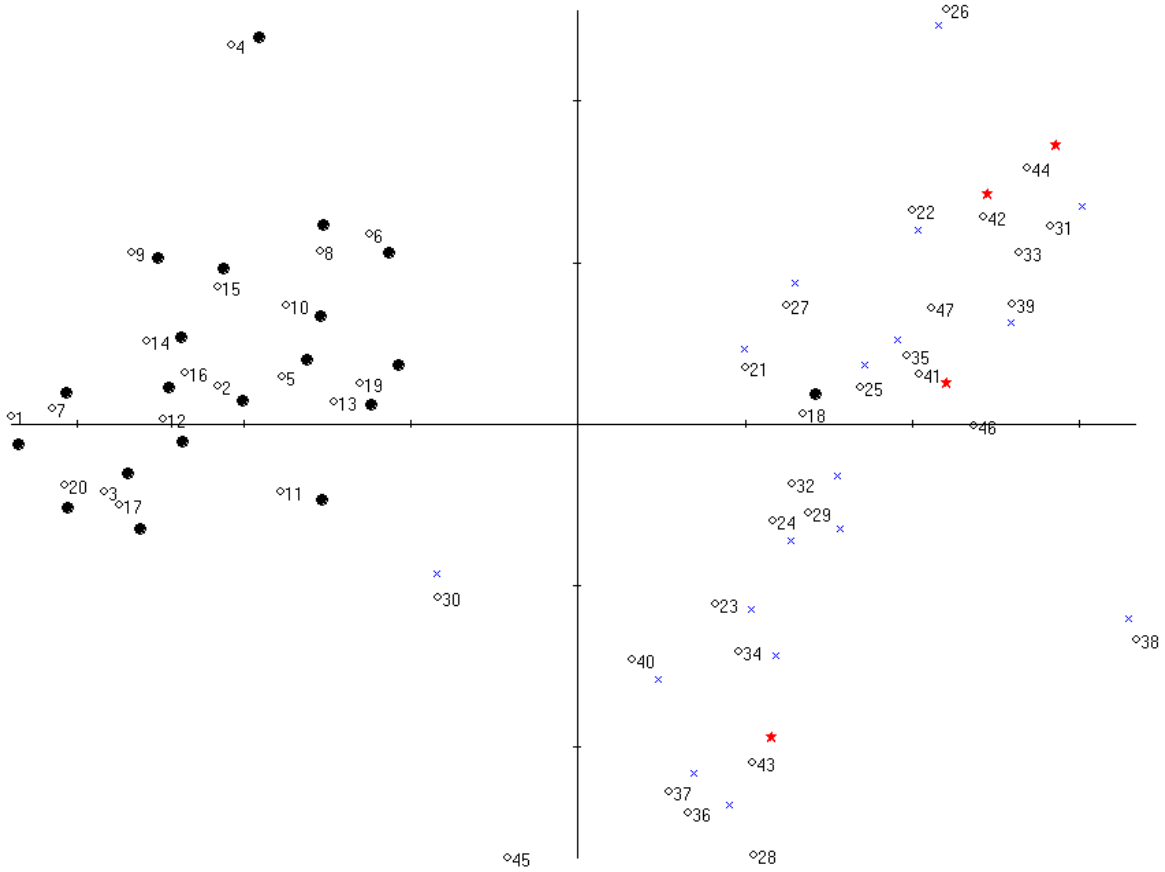

Figure 6. Relative Warp Analysis clustering graph (*Sphex flavipennis x, Sphex maxillosus o and Sphex pruinosus* were marked with *.)

of the cluster are not clear. Thus, as an alternative, the 1st method that is based on comparison of transformed morphometric data has been used (Table 6). In the 1st method, data are not required to be normally distributed.

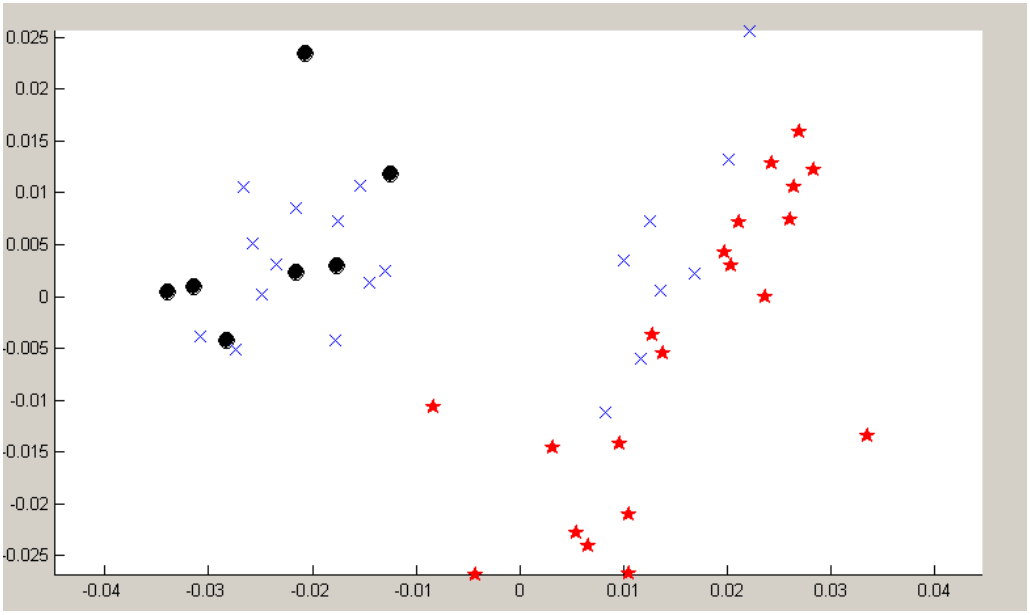

Figure 7. Clustering according to Principle Component Analysis. (Axis X variance explained: 0.4294, Axis Y variance explained: 0.1507. (*Sphex flavipennis, Sphex maxillosus and Sphex pruinosus* were labeled with **, x* and *o,* respectively).

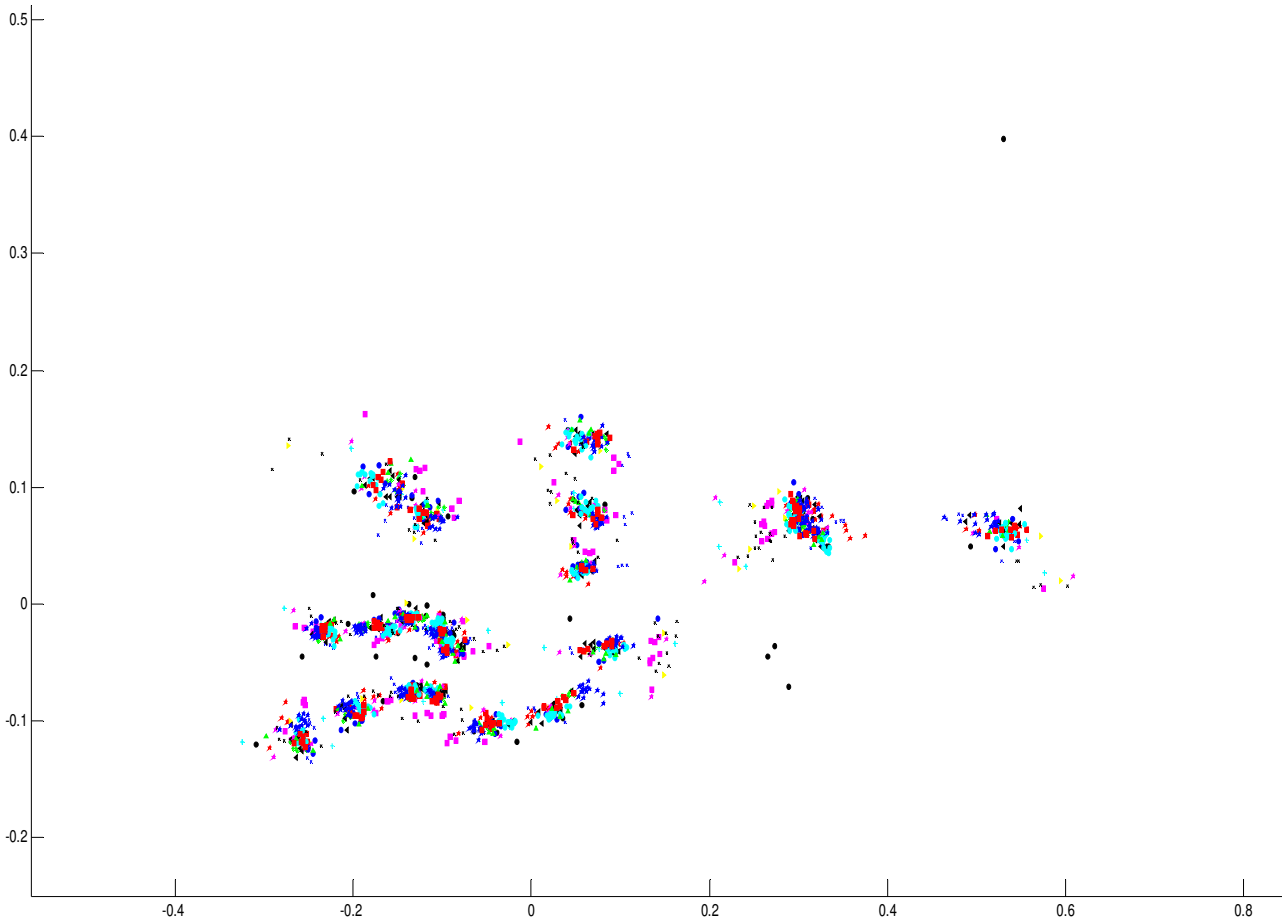

Figure 8. Graphical distribution of the 24 landmarks according to groups in PCAGen6p program.

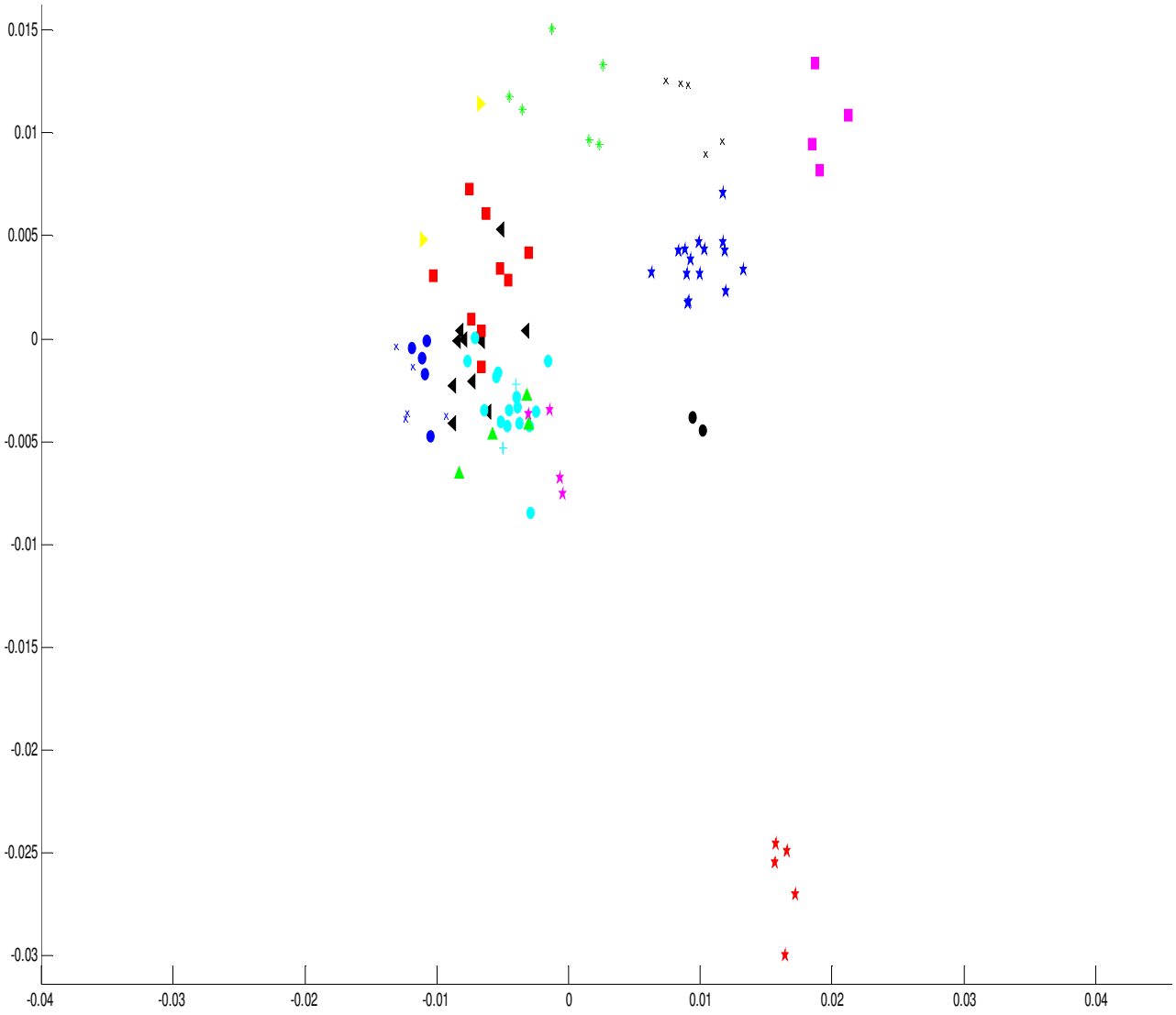

Figure 9. CVA results clustering graph.

This method should be used for data clusters that belong to groups with significant variance.

Conclusion

The difference between the similar species are identified according to the ratio and the angle of the wings. (difference refers to the different species with similar structures, which look like the same species) If the table of ratio and angle are recorded in the database, species can even be identified through the material method defined.

The question that was mostly intended to address in this study was whether geometric morphometrics measurements and the statistical analysis based on these measurements could help taxonomic studies in species and subspecies determination.

In the first part of the study, it was clearly seen in Figure 4, in classification graphics, as a result of the statistical analysis of wing geometric morphometrics measurements and calculations of the species of *Sphex* genus, that the wing structure would make the largest contribution in the classification of these species in taxonomic terms. When the classification results (Figure 4) were compared, groups of the same species appeared to be intertwined. Groups of different *Sphex* species, on the other hand, were clearly separated from each other. The reason of this successful grouping was that the utilization of 77 different ratio and angle values for forewing data for each species. In differentiation of species belonging to different Hymenoptera families, wing angle ratio values were used with a similar method. Wing angle ratio values were compared for the same and different species and similarities in morphometric terms were observed in samples of the same species.

The similarity coefficient above was formulated in order

Table 6. Identification of species according to wing geometric morphometrics values.

Pre diagnosed species	Species estimated by the program	Sum of differences in wing angles (A)	Sum of differences among the ratios of wing veins (R)	Result (Similarity coefficient)	Families that the estimated species belong to
Vespa orientalis	***Vespa orientalis***	**19.873**	**2.111**	**23.8**	**Vespidae**
	Vespa crabro	51.714	2.856	6.8	Vespidae
	Vespa bicolor	55.926	3.368	5.3	Vespidae
	Vespa basalis	65.014	3.772	4.1	Vespidae
	Eumenes coronatus detensus	75.230	5.290	2.5	Eumenidae
	Dolichovespula maculata	87.123	3.389	3.4	Vespidae
Sphex rufocinctus	***Sphex rufocinctus***	**60.036**	**0.495**	**33.6**	**Sphecidae**
	Sphex maxillatus	87.458	2.459	4.6	Sphecidae
	Myzina tripunctata	78.030	2.001	6.4	
	Dahlbomia atra	89.260	1.020	11.0	Sphecidae
Eumenes dubius cyranaius	***Eumenes dubius cyranaius***	**16.048**	**0.171**	**364.4**	**Eumenidae**
	Eumenes coronatus detensus	33.398	3.193	9.4	Eumenidae
	Eumenes pomiformis	60.840	4.483	3.7	Eumenidae
	Rhyncium aculatum	79.067	1.210	10.5	
	Euodynerus cunuctensis	87.000	1.190	9.7	Eumenidae
	Dolichovespula adulterina	95.013	1.825	5.8	Vespidae
	Vespula vulgaris	98.665	0.681	14.9	Vespidae
Polistes dominulus munchi	***Polistes dominulus munchi***	**29.503**	**0.224**	**151.3**	**Vespidae**
	Polistes sulcifer	30.037	0.484	68.8	Vespidae
	Polistes atrimandibularis	30.229	1.084	30.5	Vespidae
	Polistes biglumis alpium	30.465	2.873	11.4	Vespidae
	Vespula rufa	30.858	1.658	19.5	Vespidae
	Vespula vulgaris	36.124	1.010	27.4	Vespidae
	Polistes glominus	36.922	0.852	31.8	Vespidae
	Polistes gallicus	38.039	0.563	46.7	Vespidae
	Vespula germanica	39.776	1.863	13.5	Vespidae
Katomenes sizhalii	***Katomenes sizhalii***	**16.140**	**0.173**	**358.1**	**Eumenidae**
	Katomenes d. dimitraticus	23.456	3.659	11.7	Eumenidae
	Eumenes mediterranus	50.861	2.444	8.0	Eumenidae
Eumenes mediterranus	*Katomenes d. dimitraticus*	48.931	7.969	2.6	Eumenidae
	Eumenes mediterranus	**57.569**	**0.189**	**91.9**	**Eumenidae**
	Apoica flassima	60.167	2.897	5.7	
	Eumenes coronatus detensus	63.762	0.955	16.4	Eumenidae
	Eumenes pomiformis	65.539	2.643	5.8	Eumenidae
	Rhyncium aculatum	94.950	4.461	2.4	Eumenidae

to calculate the similarity. According to the similarity coefficient calculation, different species were separated from each other successfully (Table 6).

This research shows that when suitable measurement and statistical methods were used, the geometric morphometrics studies will contribute in taxonomy research in determining the species and subspecies.

It appeared in this study that the method of transformation of landmark data (x,y) to one dimensional data, when evaluated using the help of data transformation and geometric morphometry methods, was more successful than the geometric morphometry methods alone.

Table 7. Results from CVA/Manova.

Axis	Lambda	chisq	Degrees of freedom	p-value
1	0	1467.416	df = 570	p < 2.22045e-016
2	0	1236.224	df = 518	p < 2.22045e-016
3	0	1030.538	df = 468	p < 2.22045e-016
4	0	849.1385	df = 420	p < 2.22045e-016
5	0	692.299	df = 374	p < 2.22045e-016
6	0.0002	569.3226	df = 330	p = 5.44009e-015
7	0.0011	456.4093	df = 288	p = 9.25315e-010
8	0.005	355.4369	df = 248	p = 8.83638e-006
9	0.0184	267.7416	df = 210	p = 0.00432007

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