Systematical studies on Anthidiini

Hymenoptera Megachilidae

A geometric morphometric approach

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Abstract The classificatory schema in some groups of Megachilidae reorganized by means of shape differences in terms of the inter- and intra-specific and inter- and intra-generic and subgeneric deformations. The relative warps UPGMA trees and PCA graphs obtained from 30 landmarks were shown. Both Anthidium cingulatum Latreille and A. florentinum Fabricius should better classify in the same subgenus Anthidium s. str. In the other case under the results of shape variation our results supported the hypothesis that Paraanthidium as a subgenus of Anthidium Fabricius. On the other hand Icteranthidium Michener and Paranathidium Michener showed enough dissimilarity to be kept as separate genera. Our analysis also supported the monophyletic origin of Megachilini which was clustered as a separate group by all methods used.

Key words Megachilidae Anthidiini systematics geometric morphometrics UPGMA generalized procrustes analysis relative warps

1 INTRODUCTION

Resembling most of the other groups in entomology there is still no consensus among researchers about the classification of some groups in Megachilidae systematics Warncke 1980 Özbel and Zander 1993 Gogola 1999 Michener 2000. Possible reasons for this confusion may be the lack of sufficient morphological characters or the existence of artificial intermediate taxa. However these problems were partially solved by means of changes made in the basic characters leading to changes in the place of some genera Michener 2000.

The tribe Anthidiini also experiences similar systematical confusion mentioned above. The tribe can be differed from the others in terms of the following features Shortness of stigma and pterostigma the existence of yellow white or red maculations on integument having cleft claws in females or having basal articulation the existence of the second recurrent vein in the distal of the second submarginal cell the lack of dorsal lamella of metapleurum the lack of preapical transverse flange or carina in T6 of the males

Banaszak and Romasenko 1998 Michener 2000. The earliest study on the phylogeny was carried out by Mülle 1996. Despite the whole variation in the male genitalia Warncke 1980 classified all the genera of Anthidium which are not parasitic in the genus Anthidium Fabricius 1804. However the taxa which were considered as subgenera in this study were treated as genera level by Tkalc 1966 Banaszak and Romasenko 1998 Gogola 1991 and 1999. Özbel and Zander 1993 regarded Paraanthidium Friese 1898 as a subgenus of Anthidium Fabricius 1804 and Paranathidium Michener 1948 as a subgenus of Pseudoanthidium Friese 1898. Michener and Griswold 1994 and Michener 2000 viewed Paraanthidium Friese 1898 and Archianthidium Mavromustakis 1939 as subgenera belonging to the genus Trachusa Panzer 1804 and he regarded the Paranathidium Michener 1948 as the synonymous of the Pseudoanthidium Friese 1898.

The lack of consensus in the systematical studies directed several authors toward the search for alternative approaches Mayr and Ashlock 1991. The DNA based techniques and cladistic methodology in time partially solved some of these problems. The use of

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geometric morphometrics in understanding the process underlying the biodiversity is a relatively new methodology. After the big revolutionary change in the 1990’s this approach began to represent an important new paradigm for the statistical study of “shape” in biology and other fields of science. Rohlfs 1999a, Rohlfs 2002. In the last ten years geometric morphometric citations per year have increased considerably Lynch 2004. Especially the landmark-based morphometrics has gained significant support among scientists including anatomists Pavlinov 2001, Hennessy and Stringer 2002, Lockwood et al. 2002 and entomologists Alibert et al. 2001, Kligenberg 2003. Unlike the analytical approaches the geometric one is aimed at comparison of the shapes themselves Pavlinov 2001. Its high level of explanation power on the shape and size variation and co-variation among the organisms provided an alternative solution especially for problematic groups in systematics and ecology Aytekin et al. 2003.

The principles of these methods are based on capturing the two- or three-dimensional Cartesian coordinates of landmarks which are basically the homologous points among the individuals that have been previously assigned the same names Bookstein 1991. Differences among individual configurations of landmarks can be translated to several mathematical functions which fit the differences Alibert et al. 2001. Then the Procrustes distance, the square root of the sum of the squared distances between homologous landmarks can be used as a metric for comparing shapes Rohlfs 1999a. The registered landmark configurations k landmarks and m dimensions can be represented as points in a shape space which is of lower dimensionality than the figure space since location rotation and scale differences have been removed O’Higgins and Jones 1999. For two-dimensional data the space of Procrustes registered specimens is therefore of dimensionality km-4 Bookstein 1991, O’Higgins and Jones 1999, Rohlfs 1999a. This space is known as Kendall’s shape space Kendall 1984 which is however non-Euclidian. But it possibly can allow the study of well known multivariate analysis Sneath and Sokal 1973 like obtaining a principal components analysis PCA or unweighted pair-group method using arithmetic averages UPGMA phenogram if it is superimposed orthogonally or stereographically in the tangent plane to Kendall’s shape space Dryden and Mardia 1998. In addition as these methods preserve the geometrical relationships among landmarks they also provide a powerful vision for visualizing deformations in the shape of the original specimen in means of displacement vectors or deformation grids as warps Rohlfs 1993 Alibert et al. 2001. Some structures like the insect wings with the least degrees of freedom are one of the most appropriate structures for such studies Pavlinov 2001. Together with warps PCA of Procrustes residuals and UPGMA phenograms can provide an excellent combination of techniques for the two purposes of such studies as we made for Anthidiini here First to detect and then to describe the differences among taxa Lockwood et al. 2002.

2 MATERIALS AND METHODS

2.1 Study area and sampling

92 specimens were used from the Megachilidae collection in Hacettepe University which were sampled between 1999 and 2003 from different eco-regions of Middle Anatolia Fig. 1. The study area totally 178.080 km² has generally a steppe character with Astragalus sp., Acantholimon sp., Centaurea sp., Echium sp. and Anchusa sp. There are mountains and hills altitude ca. 1 800 m that covered mainly by Pinus nigra Arn. ssp. pallasianum Lamb Holmboe different species of Quercus sp., Picea sp. and Juniperus sp. with Cedrus libani A. Rich Davis 1968 – 1985. Agriculture and urbanization with high industry is also common in most places where collections were made. Right front wings right hind wings and head from frontal view of the females of 24 Anthidium cingulatum Latreille 1809, 12 Anthidium florentinum Fabricius 1775, 15 Ieteranthidium laterale Latreille 1809, 15 Megachile maritima Kirby 1802, 16 Paraanthidium interruptum Fabricius 1781, and 10 Paraanthidium lituratum Panzer 1801 were prepared for morphometric analysis. Megachile maritima which is from Megachilini was used as control-group for the analysis not as reference. Megachilini were preferred because the tribe Osmiini was understood to be paraphyletic with respect to Megachilini Engel 1999. Diagnose of the species and nomenclature followed that proposed by Banaszak and Ramasenko 1998 and Warncke 1980. All the specimens were screened at the time they were collected for the presence of known ecto- and/or endo-parasites to shield the morphometric data from possible traumatic variations Mayr and Ashlock 1991 Aytekin et al. 2002.

The body parts were then removed from each specimen with forceps and mounted in entellane on labelled slides. All slides were photographed using a Leica MZ-7.5 t stereo zoom dissection microscope with a DC-300 digital camera system digitizied and archived with special information codes added. From the 92 specimens some of them were eliminated because of the problems during preparation procedure and finally 92 specimens for front wings 91 one specimen from P. interruptum is ignored specimens for hind wings and
89 (two specimens from *I. laterale* and one specimen from *P. lituratum* are ignored) specimens for heads were used for the morphometric analysis. All specimens were scored by one experimenter (BM).

![Fig. 1 Study area and distribution of the collection points of the examined species](image)

**Fig. 1** Study area and distribution of the collection points of the examined species.

### 2.2 Data acquisition and shape analysis

Two-dimensional Cartesian coordinates of 16 landmarks from front wings (Fig. 2), 6 landmarks from hind wings (Fig. 3) and 8 landmarks from the heads (Fig. 4) were digitized by tps-DIG1.40 (Rohlf, 2004a) after utilizing in tps-UTIL1.28 (Rohlf, 2004b) software. The landmark configurations obtained were than scaled, translated and rotated against the consensus configuration by GLS Procrustes superimposition method (Bookstein, 1991; Rohlf, 1993, 1999b; Alibert *et al*., 2001) which is a "generalized superimposition minimizing the partial Procrustes distance over all shapes in the sample, using a least squares fitting function" (Zelditch *et al*., 2004). The coordinates were then analyzed by tps-RELW1.34 (Rohlf, 2004c) which are all free access computer softwares that are available on the Internet (http://life.bio.sunysb.edu/morph/). Eigenvalues for each principal warp, consensus configurations of the populations and relative warps were conducted. The variability in the shape space was assessed using the scores obtained by each individual on the first two relative warps. To better visualize the shape variation we only considered the consensus configuration for each species. Thus, the consensus configurations for wings and heads were subjected to relative warps analysis.

The landmarks obtained from tps-DIG were than used in the software Morphologika® (O'Higgins and Jones, 1999) to perform principal components analysis (PCA) of Procrustes registered landmark data in the tangent space to Kendall's shape space (Dryden and Mardia, 1998). The principal components of the partial warp scores were later used for SAHN clustering (Sequential, Agglomerative, Hierarchical and Nested clustering method) to obtain an UPGMA phenogram by Nsys-Pr2.1® (Rohlf, 2000). $(16 \times 2) + (6 \times 2) + (8 \times 2) = 60$ characters which handled from GPA transformed landmark locations were used as variables for this analysis. Euclid distance was preferred for the pooled interval data to obtain a similarity matrix.

In order to reduce the measurement error ($\xi_i$) all
specimens were digitized twice by Arnqvist and Mårtensson 1998. The second session of measurement was conducted after the specimens had been removed and replaced under the microscope in order to take the positioning error into account by Arqvist and Mårtensson 1998; Alibert et al. 2001. No analogous systems were used during the whole procedure.

3 RESULTS

3.1 Relative warps

By using an orthogonal alignment projection method data obtained from front wings hind wings and heads were analyzed by means of the first two relative warps. For the front wings relative positions of the average configurations of the species A. cingulatum A. floreintum and P. interruptum were clustered together where P. lituratum and I. laterale formed another group while M. maritima separated from rest of all in the shape space defined by the first two relative warps x = 1 y = 2 a = 0 Fig. 5. The deformation grids of the front wing landmark configuration showed that the out-group M. maritima have a typical shape difference than the other taxa Fig. 6. Eigenvalue was calculated as 4.788 for the first relative warp. Whereas Anthidiun spp. and Paraanthidiun interruptum have similar front wing shapes when they are superimposed on the mean shape configuration. Same methodology also used for the hind wings and heads. We have got similar configuration for Anthidiun spp. and Paraanthidiun interruptum on the hinwings. Relative positions of the average configurations of the species A. cingulatum A. floreintum and P. interruptum were linearily arranged where P. lituratum and M. maritima formed another group while I. laterale separated from rest of all in the shape space defined by the first two relative warps x = 1 y = 2 a = 0 Fig. 7. The deformation of the hindwings for A. floreintum and P. interruptum were more similar especially on the landmarks 5 and 6 Fig. 8. Eigenvalue was calculated as 2.107 for the first relative warp. This is also determined on the head shape. Relative positions of the average configurations of the species A. cingulatum A. floreintum I. laterale and P. interruptum were clustered together where P. lituratum and M. maritima separated from rest of all in the shape space defined by the first two relative warps x = 1 y = 2 a = 0 Fig. 9. The similar orientation of the landmarks 1 3 4 5 and 6 showed typically for Anthidiun spp. and Paraanthidiun Fig. 10. Eigenvalue was calculated as 2.330 for the first relative warp.

Fig. 5 Relative positions of the average configurations of the species I. cingulatum A. floreintum M. maritima I. laterale P. interruptum P. lituratum for Anthidiun and Megachiliun in the shape space defined by the first two relative warps x = 1 y = 2 a = 0. Data from front wings. Filled points indicate the species.

Fig. 6 Deformation grids of the six species of Anthidiun and Megachiliun used in the present study superimposed on the reference configuration x = 1 y = 2 a = 0 in relative warps. Data from front wings. Open circles with numbers indicate the landmarks.
and heads we reached similar results. The species were
ordered along the first two principal components similar
to front wings Fig. 11 and hind wings Fig. 12 data. The PCA of head data gave relatively sparse
distribution than the ones obtained from the wing Fig.
13.

3.3 UPGMA

The UPGMA phenogram formed by SAHN clustering obtained from the data of whole landmarks
60 characters used in the study showed distinct group patterns for all taxa Sneath and Sokal 1973. 80 specimens were randomly used for the cluster formation.

The control-group *M. maritima* clustered separately from the rest of the taxa. *I. laterale* and *P. lituratum* also clustered as a distinct group which indicates not only the species difference but also enough for identification of the genus level. *A. florentinum* *A. cingulatum* and *P. interruptum* showed enough distinct patterns for being different species group but showed a
mixed structure for a different genus category level
Fig. 14.

4 DISCUSSION

The research field of geometric morphometrics is a
diversity of morphological structures which may be
uncertain individual variation differences among
discrete groups like taxa sexes ages insect castes
biomorphs ecotones and others Pavlinov 2001 or
sometimes fluctuating asymmetry Klingenberg 2003.
Therefore we hypothesized that the classificatory schema in some groups of Megachilidae would be reorganized by means of shape differences in terms of the inter and
intra-specific and inter and intra-generic and subgeneric
deformations.
Fig. 10  Deformation grids of the six species of Anthidiini and Megachilini used in the present study superimposed on the reference configuration. $x = 1$, $y = 2$, $a = 0$ in relative warps. Data from heads. Open circles with numbers indicate the landmarks.

Fig. 11  Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 16 landmarks digitized from the front wings.
Fig. 12  Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 6 landmarks digitized from the hind wings.

Fig. 13  Distribution of the six different species of Anthidiini and Megachilini. Principal component analysis conducted for the 8 landmarks digitized from the heads.
The results obtained from the relative warps of a total of 30 landmarks from three main body parts showed that *A. cingulatum* and *A. florentinum* and *P. interruptum* did not show enough differences in the bending energies of the grid deformations. Therefore it is better not to classify those in separate genera. The inter-specific variations were basically in landmarks 10 and 11 in front wings. This information can indicate a close relatedness but clearly need additional data especially from DNA sequence. The general inter-specific variations were in landmarks 2, 3 and 5 in hind wings while 4, 5 and 6 in head shape.

The patterns of covariance in the landmark coordinates after Procrustes superimposition were studied with PCA by Dryden and Mardia [1998] Klingenberg [2003]. The first extracted two PC’s from our analysis had a clear geometric explanation in Anthidium. Especially the data obtained from the wings supported our hypothesis. Relatively sparse distribution pattern from the head data would possibly due to the landmark types measurement error and most degrees of freedom Readers unfamiliar with the techniques may be directed to Bookstein [1991] and Zelditch et al. [2004] for details. The UPGMA phenogram also clearly gave the same schema.

These results are however different from those proposed by Warmke [1980]. Both *A. cingulatum* and *A. florentinum* showed separate species character but should better classify in same subgenus as *Anthidium* s. str. In the other cases *P. interruptum* should better not classified as a separate genus. Under the results of shape variation we would propose *Paraanthidium* as a subgenus of *Anthidium* Fabricius, 1804. On the other hand *Iteranthidium* and *Paraanthidium* showed enough dissimilarity to be kept as separate genera. This classification supported the approach of Özbek and Zanden [1993]. Our analysis also proved the monophyletic origin of Megachilini which was clustered as a separate group by all methods used.

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摘要:
利用采集于土耳其安纳托利亚中部的切叶蜂科4个种116个标本，选取虫体上的18个特征点（其中前翅8个，后翅4个，头部6个），利用UPGMA等数值分类方法对不同属和种的亲缘关系进行了探讨。据此对一些种的归属和一些属的划分提出了一些与传统分类观点相同或不同的见解，但各种分析方法的结果都支持切叶蜂族起源的单系性。

关键词：切叶蜂科;黄斑蜂族;系统分类学;几何形态测量;

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