Elliptic Fourier Analysis of Male Genitalia in *Bombus* (S. Lato) Latreille

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Abstract.- There are several unaddressed problems in the taxonomy of bumblebees which motivate researchers to look for alternative methods. The morphological characters of the male genitalia are reliable and also distinctive for bumblebee taxonomy as in most of the other insect groups. However, it is always used to identify species of bumblebees but not used in its higher classification. Maybe because of the complex morphology, quantitative analysis such as geometric morphometrics on the male genitalia have been neglected so far. The structure of this organ is not proper for 2D-landmark based morphometrics. Therefore, we aimed to make an Elliptic Fourier shape Analysis (EFA) to explore the shape differences of male genitalia among the members of four different subgenera (*Megabombus* Dalla Torre, *Melanobombus* Dalla Torre, *Subterraneobombus* Vogt and *Thorachobombus* Dalla Torre) of *Bombus* (s. lato) Latreille distributed in Turkey. Nineteen species were used for analysis and their Elliptic Fourier Descriptors (EFDs) for dorsal view were described. Ordination methods, such as Canonical Variate Analysis (CVA) and Principle Component Analysis (PCA), were used to discriminate the different subgenera based on the male genitalia shape. As a result of these analyses all the methods gave us promising results in discriminating the subgeneric boundaries between previously assigned groups. This is the first study in which male genitalia of bumblebees were defined quantitatively. The results suggest an alternative methodology in establishing higher classification of bumblebees.

Keywords: Bumblebees, subgenera, male genitalia, morphometrics, Elliptic Fourier analysis.

INTRODUCTION

Bumblebees (Apidae: Hymenoptera) are a group of well-known social insects and a member of true bees that contains approximately 250 species all over the world (Williams and Osborne, 2009). Due to their significant morphology and role in pollination (Sheikh *et al*., 2014), taxonomical studies on bumblebees have been dated back to Linnaeus; all species were treated under the genus *Apis* Linnaeus. Afterwards, Latreille grouped all the bumblebee species in a separate genus, which was termed as *Bombus* at the beginning of the 19th century (Williams *et al*., 2008). These classification still largely accepted by the authors. However subgeneric classification within this genus is highly controversial (Barkan and Aytekin, 2013). These discussions were also related with the methodologies used to estimate the taxa. Since the 19th century, many of the methods lost their validity (Goulson, 2010). In the first attempt, the taxonomical characters were based on the coat colour patterns (Dalla Torre, 1880) and nine subgenera were identified (Alford, 1975). Secondly,

Radoszkowski (1884) proposed another classification based on male genitalia (Ito, 1985). Vogt (1911) modified Radoszkowski's system. Richards (1968) classified all bumblebees within 35 subgenera. After this study, three more subgenera were added (Willams, 1998). However, the system was not convenient because of its sophisticated structure. These situations lead the division of *Bombus* into 15 subgenera based on phylogenetic analysis (Williams *et al*., 2008), which is broadly an accepted system for bumblebee taxonomy. This system was based on morphological characters of the male genitalia, too.

In spite of the importance and significance (Williams, 1985, 1991, 1994; Williams *et al*., 2008) of the male genitalia within subgeneric classification of bumblebees, no studies have been done on comparing the shape differences of this character among species or subgenus. At that point geometric morphometrics is an inspiring methodology, which includes landmark-based and outline-based analyses to quantify variation of shape and their covariations with other variables (Claude, 2008; Holwell and Herberstein, 2010; Adams *et al*., 2013). Especially

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the outline methods have been efficiently applied to the analysis of various biological shapes in animals (Daegling and Jungers, 2000; Loy *et al*., 2000; Monti *et al*., 2001; Bertin *et al*., 2002; Dommergues *et al*., 2003) in which any sufficient number of homologous landmarks cannot be found. Elliptic Fourier descriptors (EFDs) (Kuhl and Giardina, 1982) is a kind of outline methods that describe any type of shape with a closed two-dimensional contour (Iwata and Ukai, 2002). All x, y coordinates of the two-dimensional contour are converted into Fourier coefficients by which a vector chain code is created (Rohlf and Archie, 1984). The results of Fourier analysis studies can be summarized and visualized using principal component analysis (PCA). These kinds of studies give advantages to the researches for analyzing small shape variations, which cannot be observed with the naked eye, and for classifying the materials without evaluating their size (Yoshioka *et al*., 2004). PCA scores are also utilizable as quantitative characters in different analyzing methods (Yoshioka *et al*., 2004). Elliptic Fourier analysis has been utilized successfully to analyze shape variation in a number of studies on insect genitalia (Arnqvist and Thornhill, 1998; Monti *et al*., 2001; Holwell, 2008), but have not been applied to bumblebee taxonomy, yet.

In this study we tried to use EFA to see if it is working on the male genitalia of the *Bombus* (s. lato) species. Nineteen species which belong to four subgenera (*Megabombus* Dalla Torre, *Melanobombus* Dalla Torre, *Subterraneobombus* Vogt and *Thorachobombus* Dalla Torre) were used to explore the shape differences between male genitalia within subgenus level in bumblebees. These subgenera were chosen because they are the four biggest taxa distributed in Turkey. Because of the fact that this is the first study for the literature using EFA in bumblebee taxonomy it was important to see if it could be an additional tool for bumblebee taxonomy.

MATERIALS AND METHODS

Specimens used in the study

Within the scope of this study, 19 specimens belonging to *Bombus* (s. lato) species (Table I) were analyzed. At subgenus level*,* the structure of

gonostylus, volsellae and penis valve are especially more characteristic than the other parts of the genitalia in males (Smith, 1970). So that, only volsella, gonostylus and gonocoxite parts of the male genitalia were used in the analyses. 2D photographs of the material were taken from the Natural History Museum collection, London.

Table I.- *Bombus* species used in analysis and abbreviations used in following figures.

Bombus species	Abbreviations used in following figures
Bombus (Megabombus) argillaceus (Scopoli, 1805)	mg_arg
Bombus (Megabombus) hortorum (L., 1761)	mg_hor
Bombus (Megabombus) portschinsky Radoszkowski, 1883	mg_por
Bombus (Melanobombus) erzurumensis Özbek, 1990	ml_erz
Bombus (Melanobombus) incertus Morawitz, 1881	ml_inc
Bombus (Melanobombus) lapidarius (L., 1761)	ml_lap
Bombus (Subterraneobombus) fragrans (Pallas, 1771)	sb_fra
Bombus (Subterraneobombus) melanurus Lepeletier 1836	sb_mel
Bombus (Subterraneobombus) subterraneus Lepeletier 1836	sb_sub
Bombus (Thoracobombus) armeniacus Radoszkowski, 1877	th_arm
Bombus (Thoracobombus) laesus Morawitz, 1875	th_lae
Bombus (Thoracobombus) mesomelas Gerstaecker, 1869	th_mes
Bombus (Thoracobombus) mlokosievitzii Radoszkowski, 1877	th_mlo
Bombus (Thoracobombus) muscorum (L., 1758)	th_mus
Bombus (Thoracobombus) pascuorum (Scopoli, 1763)	th_pas
Bombus (Thoracobombus) persicus Radoszkowski, 1881	th_per
Bombus (Thoracobombus) pomorum (Panzer, 1805)	th_pom
Bombus (Thoracobombus) ruderarius (Muller, 1776)	th_rud
Bombus (Thoracobombus) sylvarum (L., 1761)	th_syl

Elliptic Fourier analysis of outlines and statistical analysis

Elliptic Fourier analysis were conducted with a software package for quantitive evaluation of biological shapes based on EFDs (SHAPE ver. 1.3; Iwata and Ukai, 2002). It includes sub-programs which process image, recording contour, EFDs'
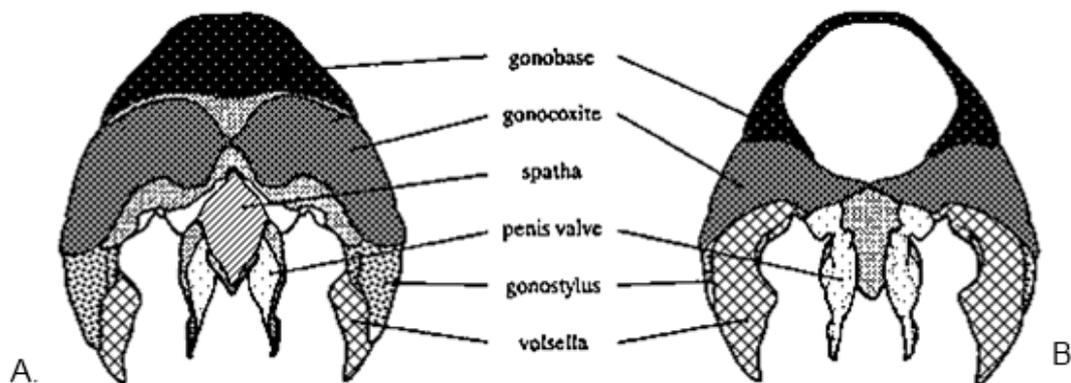
Fig. 1. *Bombus* male genitalia schematic structure (from Williams, 1991; with the permission of Dr. Williams, 19.12.2013). A, from dorsal view; B, from ventral view.

derivation, EFDs' principal component analysis and shape variations' visualization (Iwata and Ukai, 2002).

The digitized male genitalia were prepared for the elliptic Fourier analysis with Paint.NET (Brewster, 2009), a free software package. From the dorsal view of the genitalia (Fig. 1), the right parts of gonocoxa, gonostylus and volsella were used for the analysis. These parts were converted into monochromatic and .bitmap format for simplifying the chain coding process.

The PCA scores of the analysis were summarized with scatterplot matrices that were carried out by using R software (R Core Team, 2013). The principal component analysis results were evaluated by Canonical Variance Analysis using PAST ver. 2.10 (Hammer *et al*., 2001).

RESULTS

A minimum number of 30 harmonics (Fig. 2) were considered sufficient for capturing shape information in 19 *Bombus* species' male genitalia. Results of the principal component analysis show that 93.13% of the total shape variation was captured within the first six components (Table II).

Effect of each principal component on shape variation is given in the Figure 3. The illustrations PC1-6 show the overall genitalia parts which are volsella, gonostylus and gonocoxite.

The illustrations on the top are superimposed outlines corresponding to the mean and extreme values of the principal components. The -2StD, mean and +2StD illustrations are superimposed outlines corresponding to the mean and extreme values of the principal components.
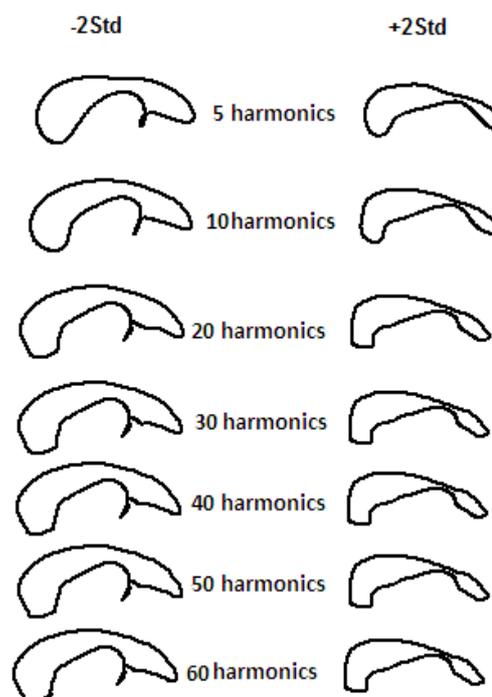

Fig. 2. Increasing number of harmonics and their descriptive on the overall genitalia parts (volsella, gonostylus and gonocoxite).

Table II.- **Contribution of principal components to shape variation.**

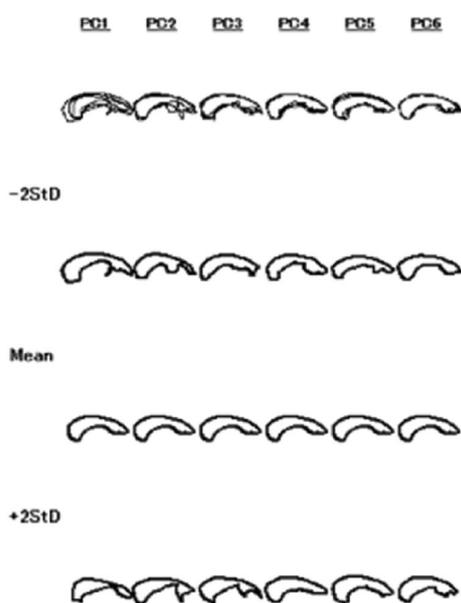| Component	Eigenvalue	Cumulative (%)	p<0.05
PC1	2,17E-02	55,5432	*
PC2	6,27E-03	71,5859	*
PC3	3,04E-03	79,3657	*
PC4	2,28E-03	85,1942	*
PC5	1,78E-03	89,7519	*
PC6	1,32E-03	93,128	*

Fig. 3. Effect of each principal component (PC1-6) on shape variation.

PCA based on the data from the shape of volsella, gonostylus and gonocoxite is summarized in a scatterplot matrix shown in Figure 4.

PCA scores are evaluated by canonical variates analysis. The diagram showing the separation of different subgenera is depicted in Figure 5.

DISCUSSION

Subgeneric classification, which is an intermediate step to species identification of the bumble bees has been used from the 19th century (Williams, 1985). The currently using system which was suggested by Williams *et al.* (2008) was created with the aims that it has to be monophyletic, consists of fewer taxa, diagnosable from morphology and named for principal behavioural and ecological groups. Subgenus taxonomy of *Bombus* (s. lato) Latreille mainly depends on male genitalia morphology because of the reliable and distinctive characters of this organ (Shapiro and Porter, 1989; Williams, 1985, 1991, 1994; Williams *et al.*, 2008).

Detailed morphological characters are potentially useful in species recognition and diagnosis (Gonzalez *et al.*, 2013); but with the fact that the decisions are connected with the experience of taxonomist and that is for they are not as objective as they have to. Using semi-automatic taxonomical system integrated with taxonomist's experience, mathematics and statistics (Aytekin *et al.*, 2007; De Meulemeester *et al.*, 2009; Wappler *et al.*, 2012; Barkan and Aytekin, 2013) can give us a simplified and stable classification.

In this way, evaluating the characters of the male genitalia of *Bombus* species by a different methodology can provide us a new point of view. EFA is an alternative method for shape analysis of biological structures (Kuhl and Giardina, 1982), especially when there are not homologous points to summarize shape (Monti *et al.*, 2001; Tatsuta *et al.*, 2004; Kergoat and Alvarez, 2008). So we applied this method on such an irregular shape of male genitalia.

Since EFA can be summarized by PCA (Rohlf and Archie, 1984), firstly PCA socres were summarized. Figure 4 displays the scatterplot matrix which was generated with PCA results representing the nineteen species of four subgenera (*Megabombus* Dalla Torre, *Melanobombus* Dalla Torre, *Subterraneobombus* Vogt and *Thorachobombus* Dalla Torre) and the distribution of individuals along the PC1 and PC2, PC2 and PC3, PC1 and PC3. All species formed distinct groups suggesting that EFA is capable of separating and identifying these species. The boxes on the upper right hand side of the whole scatterplot are mirror images of the plots on the lower left hand. The diagram summarizing the similarities of individuals, but the aim of the study was discrimination of the taxa in subgenera level. Since
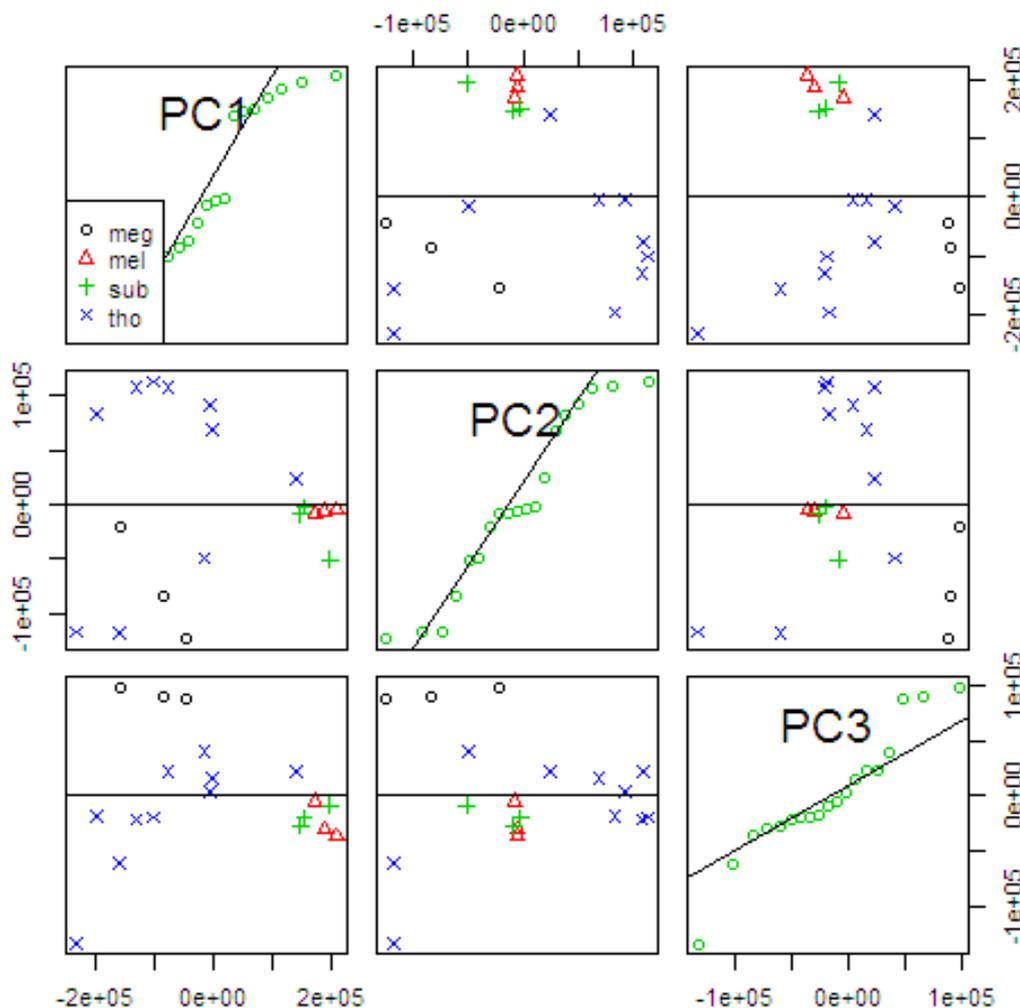
Fig. 4. Principal component analysis results of 19 different *Bombus* species of male genitalia (Circle: *Megabombus* Dalla Torre; Triangle: *Melanobombus* Dalla Torre; Plus: *Subterraneobombus* Vogt; X: *Thoracobombus* Dalla Torre).

PCA scores are also used as quantitative characters for CVA (Yoshioka *et al*., 2004), we used these results of PCA scores to perform CVA. As it seen in Figure 5, the discrimination of the four subgenera is distinguished. According to the PCA and CVA graphs, species clustering together were mostly close species (Cameron *et al*., 2007) with similar genitalia morphology.

Based on these results we could suggest that, the shape of the male genitalia in bumblebees have significant differences among subgenera and these deformations can be explained and grouped mathematically by using EFA. According to the subgeneric system of *Bombus* (Williams *et al*., 2008), we can claim that our methodology is promising.

Altogether, these results may help future outline based studies on insect taxonomy and systematics. Especially, evaluating different parts of male genitalia separately could provide more reliable results to resolve the systematics of the taxa.
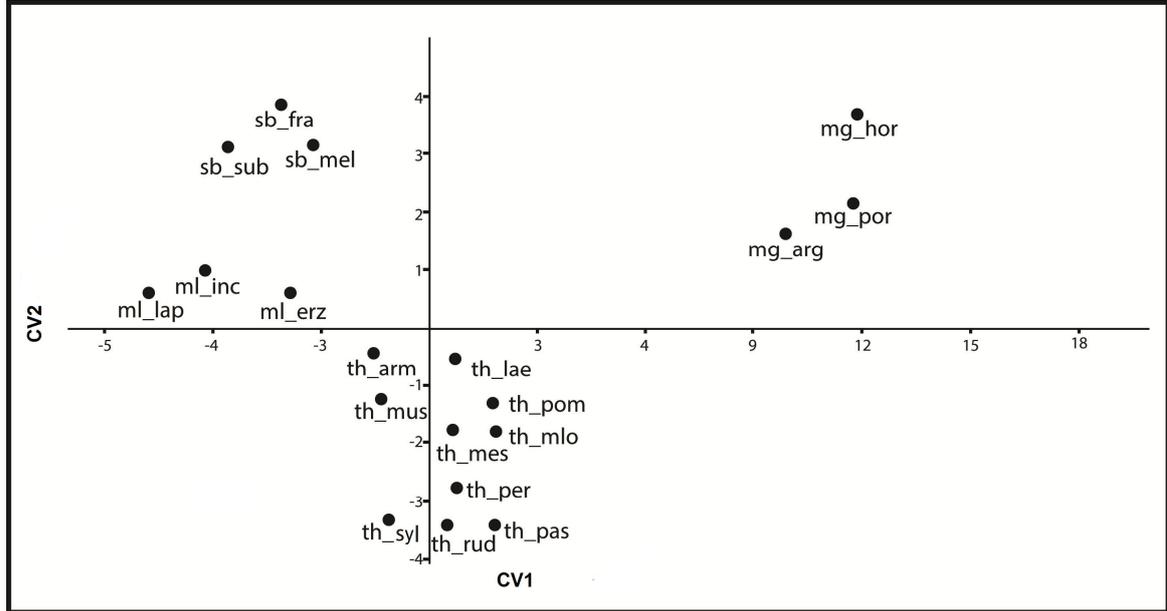
Fig. 5. Canonical variates analyses result graphics of the bumblebee species.
mg_arg, *Bombus (Megabombus) argillaceus* (Scopoli, 1805); mg_hor, *Bombus (Megabombus) hortorum* (L., 1761); mg_por, *Bombus (Megabombus) portschinsky* Radoszkowski, 1883; ml_erz, *Bombus (Melanobombus) erzurumensis* Özbek, 1990; ml_inc, *Bombus (Melanobombus) incertu*s Morawitz, 1881; ml_lap, *Bombus (Melanobombus) lapidarius* (L., 1761); sb_fra, *Bombus (Subterraneobombus) fragrans* (Pallas, 1771); sb_mel, *Bombus (Subterraneobombus) melanurus* Lepeletier 1836; sb_sub, *Bombus (Subterraneobombus) subterraneus* Lepeletier 1836; th_arm, *Bombus (Thoracobombus) armeniacus* Radoszkowski, 1877; th_lae, *Bombus (Thoracobombus) laesus* Morawitz, 1875; th_mes, *Bombus (Thoracobombus) mesomelas* Gerstaecker, 1869; th_mlo, *Bombus (Thoracobombus) mlokosievitzii* Radoszkowski, 1877; th_mus, *Bombus (Thoracobombus) muscorum* (L., 1758); th_pas, *Bombus (Thoracobombus) pascuorum* (Scopoli, 1763); th_per, *Bombus (Thoracobombus) persicus* Radoszkowski, 1881; th_pom, *Bombus (Thoracobombus) pomorum* (Panzer, 1805); th_rud, *Bombus (Thoracobombus) ruderarius* (Muller, 1776); th_syl, *Bombus (Thoracobombus) sylvarum* (L., 1761).
meg, *Megabombus* Dalla Torre; mel, *Melanobombus* Dalla Torre; sub, *Subterraneobombus* Vogt; tho, *Thoracobombus* Dalla Torre.

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