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Elliptic Fourier Analysis of the Wing Outline Shape of Five Species of Antlion (Neuroptera: Myrmeleontidae: Myrmeleontini)

Qing-Bin Zhan and Xin-Li Wang*

Department of Entomology, China Agricultural University, Beijing 100193, China

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Qing-Bin Zhan and Xin-Li Wang (2012) Elliptic Fourier analysis of the wing outline shape of five species of antlion (Neuroptera: Myrmeleontidae: Myrmeleontini). *Zoological Studies* **51**(3): 399-405. We analyzed interspecific wing outline shape variations of 5 species of antlion: *Myrmeleon bore* (Tjeder, 1941), *M. immanis* Walker, 1853, *M. fuscus* Yang, 1999, *Euroleon coreanus* Okamoto, 1926, and *E. flavicorpus* Wang, 2009. In total, 98 forewings and 98 hindwings from the 5 species were sampled and subjected to an elliptic Fourier analysis. Twenty 1st Fourier harmonics were summarized via a principal component analysis and the 1st 8 principal components of shape variation were considered for statistical tests (multivariate analysis of variance, canonical variate analysis, and cluster analysis). *Euroleon coreanus* and *E. flavicorpus* were recognized as a group, while *M. bore*, *M. immanis*, and *M. fuscus* comprised a separate group. Results of the analysis of wing outline shapes of the 5 species agree with the current taxonomic system.
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Key words: Elliptic Fourier analysis, Canonical variate analysis, Comparison, Contour.

Antlions are insect members of the order Neuroptera. Most antlions live beneath the sand or dry soil and molt twice and thus have 3 instars. All species in the tribe Myrmeleontini construct pitfall traps to capture unwary wanderers (Stange 2002). The larvae are so dependent on this lifestyle that they have lost the ability to walk forward (Stange 1998). The widespread tribe Myrmeleontini includes 9 genera and more than 200 species. *Myrmeleon* is the largest genus of the family Myrmeleontidae. *Euroleon* is related to *Myrmeleon*, and both genera belong to the subtribe Myrmeleontina. The genus *Myrmeleon* was established by Linnaeus in 1767. The genus *Euroleon* was erected by Esben-Petersen (1918) for the single European species, *Myrmeleon europeaus* Mclach, 1873; this name is now a junior synonym of *E. nostras* (Geoffroy in Fourcroy 1758).

Traditionally the 2 genera were distinguished by the wing spot and the positions of the 6th main vein (CuP+1A) and 5th main vein (CuA) (Krivokhatskiy and Zakharenko 1995, Stange 2004). The wing venation is also widely used at the species level. In spite of these indications, the delimitation of some species of different genera or within a single genus is still fuzzy. It would be meaningful to try and reinforce species' differences using additional markers.

Wings of adults of Myrmeleontidae present abundant variety among genera and species, not only in venation but also in the outline of the shape of the wing. Distinct venation as diagnostic characters is widely used in taxonomic descriptions of the Myrmeleontidae. Nevertheless, differences in the outline of the wing shape were seldom applied for identification and taxonomy of the Myrmeleontidae, because no analytical methods were available to quantify variations in wing outline shapes of this family. Herein, we attempted to translate this morphological trait into quantitative

*To whom correspondence and reprint requests should be addressed. E-mail:wangxl@cau.edu.cn

data and compare the obtained results with more traditional methods.

The aim of the work was to estimate overall wing shape variations and compare wing shapes of 5 species, and then evaluate whether phylogenetic information can be obtained from the wing outline shape of members of the Myrmeleontidae.

MATERIALS AND METHODS

All analyzed specimens (Table 1) were deposited in the Insect Collection of China Agricultural University (ICCAU), Beijing, China. The 5 analyzed species were identified using the works of Bao et al. (2009) and Ao et al. (2009).

Elliptic Fourier analysis

Elliptic Fourier descriptors (EFDs) can describe any type of closed contour and were effectively applied to evaluate various biological shapes in animals and plants (Ferson et al. 1985, White et al. 1988, Diaz et al. 1989, Laurie et al. 1997, Iwata et al. 1998 2000 2002, Yoshioka et al. 2004). The elliptic Fourier analysis decomposes the contour shape into a series harmonics which are described by 4 Fourier coefficients (Rohlf and Archie 1984). Kaesler and Waters (1972) were one of the 1st groups to apply Fourier descriptors to study morphological shapes in systematics, and many others followed. The elliptic Fourier analysis proved to be a powerful tool for comparing shapes at different taxonomic levels, including the intraspecific level (Monti et al. 2001, Andrade et al. 2008 2010).

Wing outline digitization

A pinned specimen with wings expanded was placed on a pedestal; the forewing and hindwing of each sample were put on a platform to keep them at the same level. A digital image of each wing was created from directly overhead with a Nikon 4500 digital camera (Nikon Co., Tokyo, Japan). Wing images were converted to black-and-white contour bitmaps in Adobe Photoshop CS3 (Adobe Systems incorporated, California, USA) and imported into the software, SHAPE (Iwata and Ukai 2002), which includes 3 sub-programs, Chaincoder, Chc2Nef, and PrinComp, to carry out the elliptic Fourier analysis.

Elliptic Fourier description of the wing outline shape

Digital photos of the wing outline shape were used to obtain coordinates of the outlines using the Chaincoder program. Then the Chc2Nef program accepted the transformed Chaincoder files and calculated the normalized EFDs. The normalization transforms original coefficients so that they become invariant with respect to the size, rotation, and starting point. The procedure uses the orientation and size of the 1st harmonic to perform normalization. The decision as to how many harmonics must be selected to summarize the information is to some extent arbitrary; this number of harmonics can also be decided empirically (Rohlf and Archie 1984, Andrade et al. 2008). In this study, 20 1st harmonics were considered to be sufficient for correctly depicting the wing outlines. Coefficients of these 1st 20 harmonics were selected for a principal component (PC) analysis (PCA) of the coefficients of the EFDs. This analysis was performed with the PrinComp program. Scores of observations of the derived PCs were used for subsequent various analyses of the wing outline shape.

Statistical analysis

To estimate the measurement error of intraspecific variations, we produced 3 replications of 9 specimens for each species. For each species, each hindwing of an individual was imaged and edited 3 times. Then an analysis of variance (ANOVA) was used to partition the total

Table 1. Number of specimens and sampling localities

Species	No.	Sampling localities
Myrmeleon bore	30	Henan, Hebei, Beijing
Myrmeleon immanis	18	Henan, Hebei, Shaanxi
Myrmeleon fuscus	9	Guangdong, Guangxi, Fujian
Euroleon coreanus	29	Henan, Hebei, Shaanxi, Beijing
Euroleon flavicorpus	12	Shanxi, Ningxia

variance of the 1st PC for each species into within- and between-individual variations. The percentage measurement error was calculated using the formula, $ME\% = S^2_{with} / (S^2_{with} + S^2_{between}) \times 100$ as indicated in Yezerinac et al. (1992).

Differences in wing outline shapes between species were evaluated using a canonical variate analysis (CVA). We selected the 1st 8 PCs as variables to carry out the CVA using the program, PAST (Hammer et al. 2001). The matrix of pairwise Mahalanobis distances between species was used to carry out a cluster analysis in the program, NTSYSpc 2.1e (Rohlf 2000). An unweighted pair-group method with arithmetic means (UPGMA) was chosen to produce a phenogram. Tests of differences of wing outline shapes among species were made using a multivariate ANOVA (MANOVA). Wilk's lambda criterion was used in the MANOVA. Pairwise comparisons were performed using Hotelling's tests, with Bonferroni's corrections.

RESULTS

Wing outline shape variations and measurement errors

Forewing outline shape variations were described by the 1st 8 PCs, and these accounted for 93% of the total variance. Shape variations along the 3 1st PCs is presented in figure 1. The 1st PC (64.0% percent of the total variance) was related to variations in the width of the middle part of the wing. The 2nd PC (8.5% of the total variance) was characterized mainly by the change in the proximal part of the wing. At 1st glance, the PC1 vs. PC2 scatterplot (Fig. 2) shows overlapping areas among most species, but the 2 genera were well differentiated. On the 1st PC, the percentage measurement error reached 5.8%

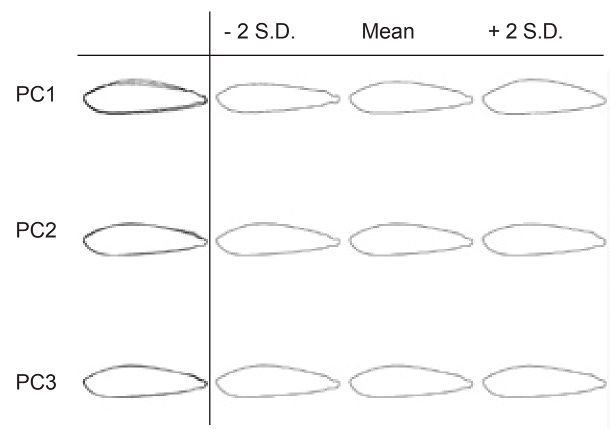

Fig. 1. Reconstructed contour of forewing shape variations described by the 1st 3 principal components.

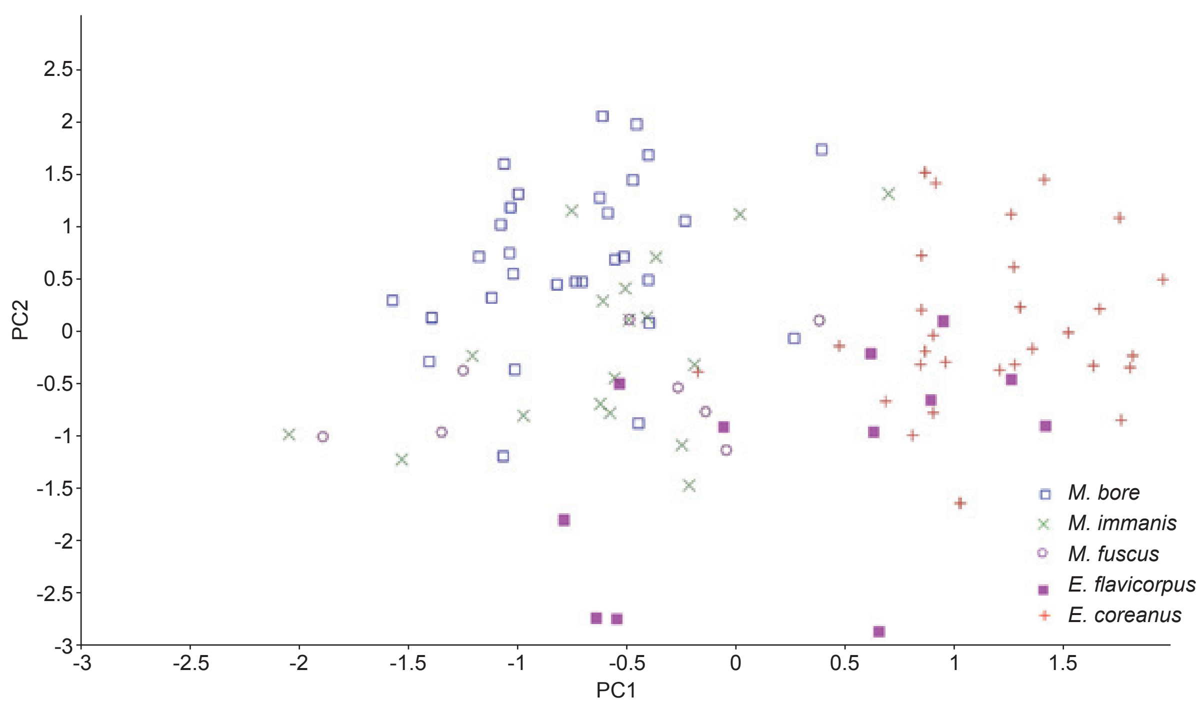

Fig. 2. Principal component (PC)1 vs. PC2 plot of the forewing outlines of 5 antlion species.

of the intraspecific variance for *M. bore*, 2.3% of the intraspecific variance for *M. immanis*, 2.0% of the intraspecific variance for *M. fuscus*, 3.4% of the intraspecific variance for *E. coreanus*, and 2.7% of the total variance for *E. flavicorpus*.

Hindwing outline shape variations were also described by the 1st 8 PCs, which accounted for 92% of the total variance. Variations of hindwing outlines are described along the 1st 3 PCs in figure 3. The proportions of the 1st 3 PCs were 55.95%, 13.02%, and 9.58%, respectively. The 1st component was a good measure of the width/length ratio of the wing and also expressed changes in width in the middle part of the wing. The 2nd PC was related to the curvature of the wing tip. PC1 was related to intergeneric variations, while the 2nd axis was influenced by interspecific differences within a genus (Fig. 4). The main shape variation in the intergeneric difference lay in the aspect ratio. Changes in the curvature of the wing tip more concerned differences between species of the same genus. On the 1st PC, the percentage measurement error reached 0.33% of the intraspecific variance for *M. bore*, 6.3% of the intraspecific variance for *M. immanis*, 2.8% of the intraspecific variance for *M. fuscus*, 2.4% of the intraspecific variance for *E. coreanus*, and 2.0% of the total variance for *E. flavicorpus*.

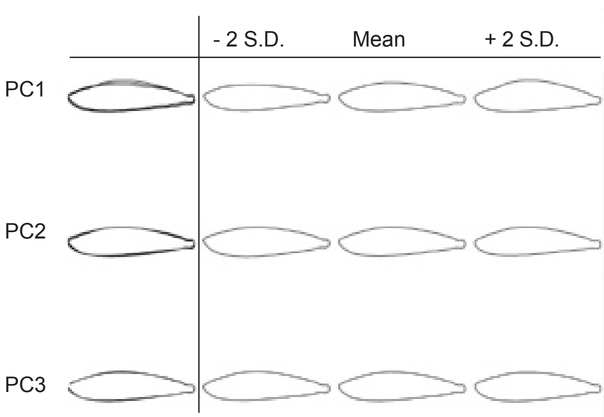

Fig. 3. Reconstructed contour of hindwing shape variations described by the 1st 3 principal components.

Phylogenetic information of wing outline

Examination of the ordination of observations on the CVA scatterplots (Figs. 5, 6) shows that the 1st canonical axis is concerned with differences among genera, while the 2nd axis is concerned with differences between species within genera

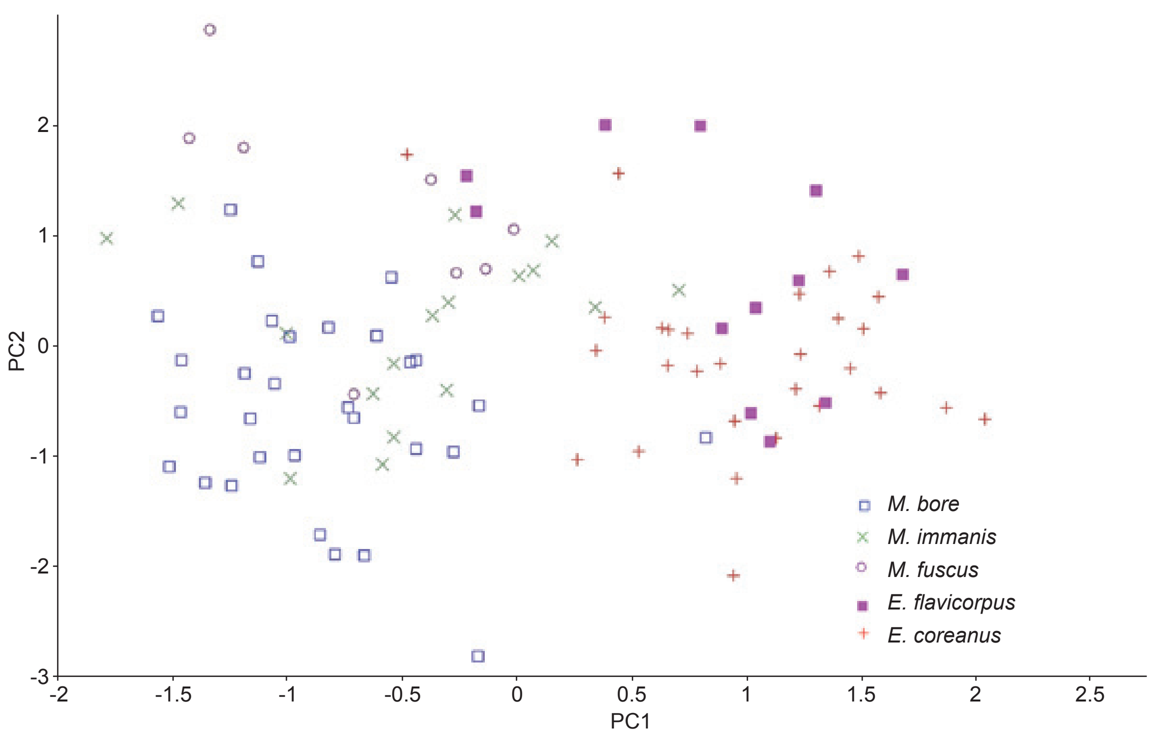

Fig. 4. Principal component (PC)1 vs. PC2 plot of the hindwing outlines of 5 antlion species.

for both the hind- and forewings. This result is somewhat similar to the exploration of shape variations along the PC for the hindwing. The UPGMA dendogram (Fig. 7) shows 2 main clusters, and the separation between the clusters corresponds to differentiation among genera. This result therefore agrees with the current taxonomic system of the Myrmeleontidae.

Table 2 shows the results of tests for differences in wing outline shape among the 5

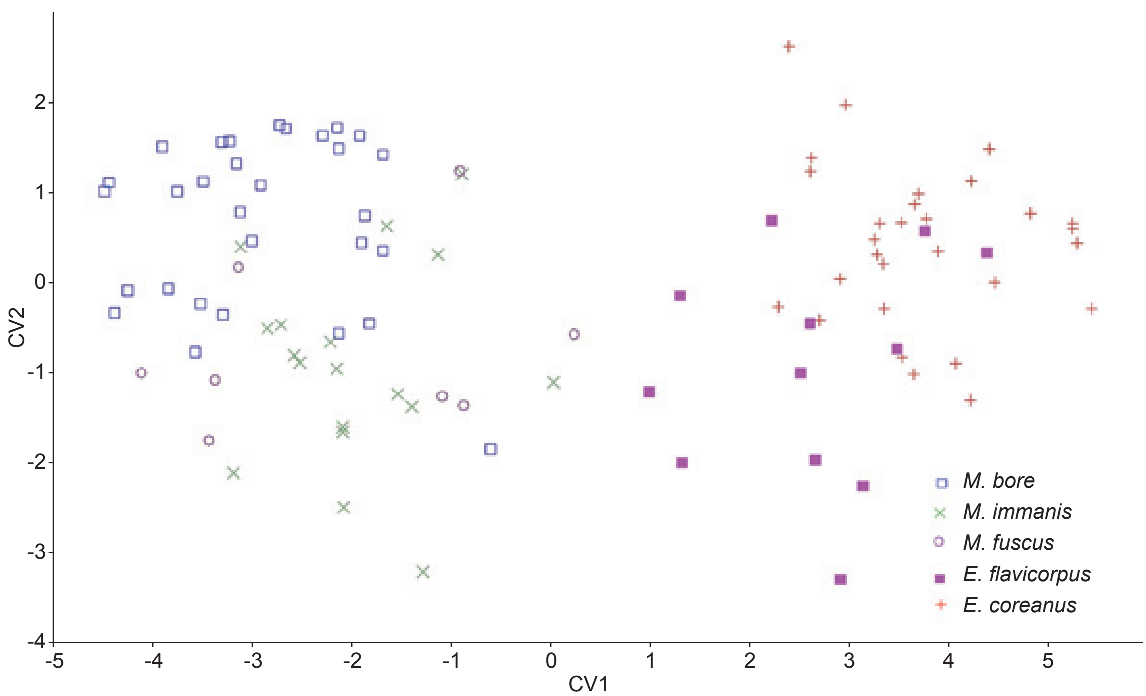

Fig. 5. Canonical variate analysis plot of the forewing outlines of 5 antlion species.

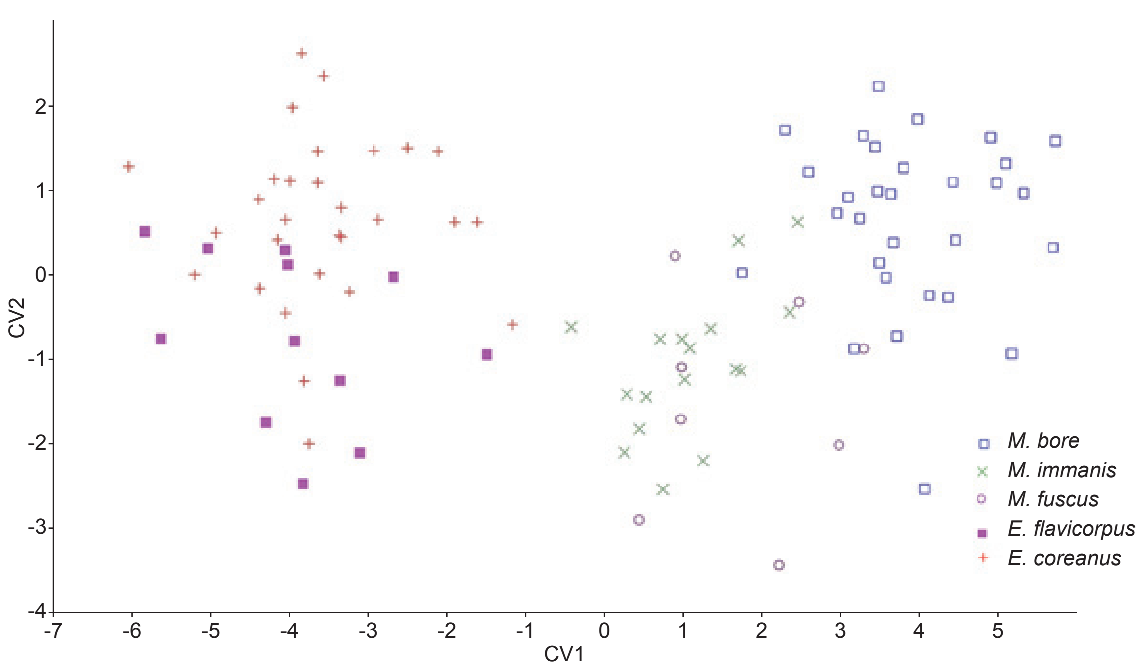

Fig. 6. Canonical variate analysis plot of the hindwing outlines of 5 antlion species.

species: for the forewing Wilk's lambda = 0.0043, F = 13.26, p = 1.6E-41 and for the hindwing Wilk's lambda = 0.04723, F = 12.68, p = 3.374E-41. Pairwise differences were always significant between species belonging to different genera, but were sometime not significant for species belonging to the same genus for both the hind- and forewings.

DISCUSSION

In this study, we show that intraspecific variations were much greater than the measurement error (always < 7%), which indicates that the elliptic Fourier analysis can be informative for depicting the wing shape outline above the individual level.

Ordination of individuals on the CVA and PCA showed a relatively similar pattern with strong intergeneric differences, while differences between species within the genus were more subtle and more difficult to depict. However, only the forewings of the pair, *M. fuscus*- *M. immanis*, did not significantly differ; and only the hindwings of the pair, *M. bore*- *M. fuscus*, did not differ, meaning that in general, interspecific differences can even be seen at the species level. Ordination of species on the CVA and the UPGMA showed that wing outline shape variations of the 5 species agreed with the current taxonomic system and demonstrated that the wing outline shape is a valuable taxonomic tool.

Although our study did not attempt to depict the functional content of wing-shape differences, it did show that for adult antlions, there can be some adaptive constraints. In their study, Scharf et al. (2009) found that phenotypic plasticity and variations in antlion adults were explained along a climatic gradient, showing that wing size and shape were affected by various factors, such as elevation, foraging, behavior, flying mechanism, and pollen load. Since there is taxonomic content to the antlion's wing outline shape variations, our study shows that the elliptic Fourier analysis can produce definitions of new characters for

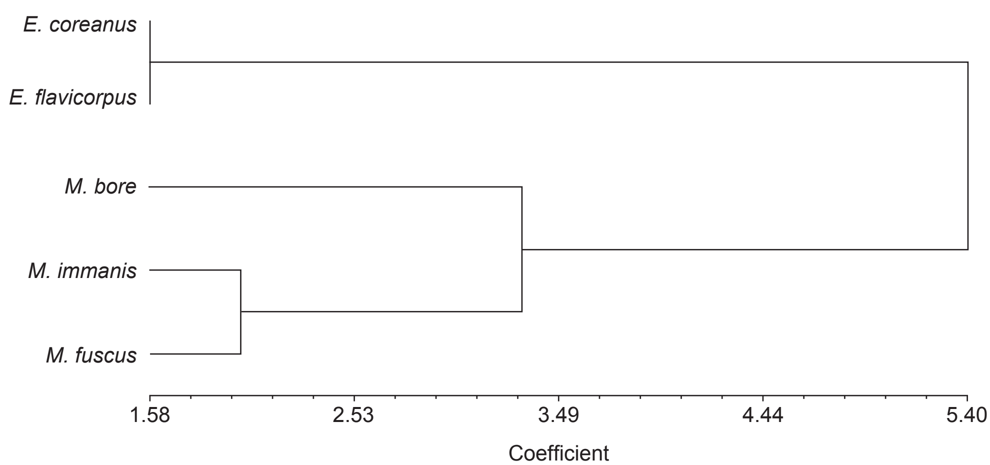

Fig. 7. UPGMA phenogram of the hindwing outline shapes of 5 antlion species.

Table 2. p values derived from the MANOVA

	Euroleon coreanus	*Myrmeleon bore*	*E. flavicorpus*	*M. immanis*	*M. fuscus*
E. coreanus	-	5.6104×10^{-24}	0.286072	1.23455×10^{-12}	2.7692×10^{-9}
M. bore	1.7801×10^{-26}	-	1.04095×10^{-14}	1.45242×10^{-8}	0.000161
E. flavicorpus	0.03224	1.0894×10^{-12}	-	9.71592×10^{-7}	0.000468
M. immanis	8.70783×10^{-18}	0.00042	4.70682×10^{-8}	-	0.785159
M. fuscus	1.4680×10^{-9}	0.23452	0.0061569	0.238379	-

Values for the hindwing and forewing are respectively presented on the upper and lower diagonals.

distinguishing antlions, and also may be useful for studying fossil wings.

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