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Description of sexual dimorphism in hind wing morphology of cantharid beetles based on a geometric morphometric analysis

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Abstract
Insect wing morphology has been used in many studies in clarifying the relationship between the closely related taxa or populations, but less focused on the discrimination of the male and female within species. This study is conducted to determine differences in the hind wing morphology between sexes of three cantharid species, including *Lycocerus orientalis* (Gorham, 1889), *Prothemus purpuripennis* (Gorham, 1889) and *Themus impressipennis* (Fairmaire, 1886), using the method of geometric morphometrics. The results show that shape variations of the hind wings between male and female are different in one species from anther as shown in the principal component analysis. Discriminant function analysis shows that there are significant differences in the hind wing shape variations between sexes for each species, which demonstrates the presence of sexual dimorphism within the species of cantharid beetles.

Keywords: Cantharidae, hind wing morphology, sexual dimorphism, geometric morphometric analysis

1. Introduction
Sexual dimorphism is defined as the variation in morphology between individuals of different sexes that belongs to the same species. It has captured the interest of entomologists since normally the differences between sexes among insects unremarkable or the individuals are very small; thus finding discrimination in morphological characters will permit easy identification of sexes [1].
Wing morphology is of a primary importance to entomologists interested in systematics [2]. Since 1970's, with the introduction of geometric morphometrics, the wing shape morphology has been extensively studied in the field of entomology to clarify the relationship between closely related taxa and helps in identifying population within and between species of insects [3-13]. However, studies about sexual shape dimorphism in wing morphology have been less accounted [2, 14].
Geometric morphometrics (GM) is a relative recent method in quantifying biological form. Landmark-based GM analysis uses a set of landmarks to describe the object or specimen, and it will preserve the physical integrity of the form [15], making it possible to find and analyze shape variations in the organisms within and between populations [16]. Moreover, geometric morphometric tools present the advantage of laying results that not only have a high statistical power but also have easily visualized results, thus it allows better comparison of the shapes of different organisms and would no longer rely on word descriptions that usually encounter problem of being interpreted differently by scientists [17].
In Cantharidae, hind wing morphology has already verified, useful in distinguishing genera and species in our recent study[18], here we are going to test if there is any significant difference between sexes within species or not and describe their variations using geometric morphometric analysis.

2. Materials and methods
Three species in different genera of Cantharinae are chosen as the studied subject, they are *Lycocerus orientalis* (Gorham, 1889), *Prothemus purpuripennis* (Gorham, 1889) and *Themus impressipennis* (Fairmaire, 1886). The number of the specimens of each sex for each species is listed in the Table 1 (174 wings in total). These materials of the representative species are

deposited in the Museum of Hebei University, Baoding, China (MHBU) and Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZAS) respectively. The studied methods follow that of Guan *et al.* [18] by analyses of Principal Component Analysis (PCA) and Discriminant Function Analysis (DA) using the softwares TpsUtil [19], TpsDig2.16 [20] and MorphoJ [21]. The veins nomenclature of the hind wing of Cantharidae follows that of Kukalová-Peck and Lawrence [22]. The coordinates of the landmarks (13 landmarks in total, Table 2) are digitized by the as shown in Fig. 1.

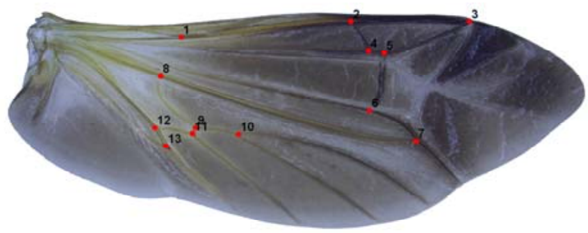

Fig 1: Hind wing of male of *Lycocerus orientalis* (Gorham, 1889) showing digitizing landmarks.

Table 1: The number of specimens of each species used in the GM analysis

Species name	Number of the specimens	
	Male	Female
Lycocerus orientalis (Gorham, 1889)	25	30
Prothemus purpuripennis (Gorham, 1889)	27	29
Themus impressipennis (Fairmaire, 1886)	31	32

Table 2: Landmarks of hind wing used in the GM analysis

No.	junctions of veins	No.	junctions of veins
1	ScP (Subcosta Posterior) and RA	8	MP_{1+2} and MP_{3+4}
2	RA (Radius Anterior) and RA_{3+4}	9	MP_{3+4} and CuA_1 (Cubitus Anterior)
3	RA_{1+2} and RA_{3+4}	10	MP_4 and MP_3
4	RA_{3+4} and r3 (radial crossvein)	11	CuA_1 and CuA_2
5	RA_{3+4} and r4	12	CuA and CuA_{1+2}
6	r4 and RP (Radius Posterior)	13	AA (Anal Anterior) and CuA_{3+4}
7	RP and MP_{1+2} (Media Posterior)		

3. Results

The shape variation for hind wings in *Lycocerus orientalis* is shown by the first two principal components of PCA (Fig. 2A). The thin plate spline visualization shows that the junctions of MP_4 and MP_3 (No. 10) and CuA and CuA_{1+2} (No. 12) are more variable in female than in the male, r4 and RP (No. 6) and RP and MP_{1+2} (No. 7) are varied in different directions between sexes. In *Prothemus purpuripennis* (Fig. 3A), the median area, which is surrounded by the junctions of MP_{3+4} and CuA_1 (No. 9), MP_4 and MP_3 (No. 10), CuA_1 and CuA_2 (No. 11) and AA and CuA_{3+4} (No. 13), contributes most in the shape variations between sexes, of which the junction of MP_4 and MP_3 is more variable in female than in male, conversely for others; also ScP and RA (No. 1) is more variable in female than in male. In *Themus impressipennis* (Fig. 4A), the junctions of CuA and CuA_{1+2} (No. 12) and AA and CuA_{3+4} (No. 13) are more variable in female than in male.

The DA plots of shape differences (Figs. 2B, 3B, 4B) show that male and female occupies a different area respectively for each species. Mahalanobis distances (Table 3) between both sexes of each species are highly significant in all pairwise comparisons ($P<0.05$), and Procrustes distances (Table 4) are similar.

The centroid size (Fig. 5A, Table 5) is significantly different between sexes in *Lycocerus orientalis* ($P<0.05$), while the differences are insignificant in the other two species ($P>0.05$).

Table 3: Difference in the hind wing shapes between both sexes of each species. Mahalanobis distances (above) & *P*-values (below).

Male Female	*Lycocerus orientalis*	*Prothemus purpuripennis*	*Themus impressipennis*
Lycocerus orientalis	2.3947 <.0001	—	—
Pothemus purpuripennis	—	2.7184 <.0001	—
Themus impressipennis	—	—	2.3376 <.0001

Table 4: Difference in the hind wing shapes between both sexes of each species. Procrustes distances (above) & *P*-values (below).

Male Female	*Lycocerus orientalis*	*Prothemus purpuripennis*	*Themus impressipennis*
Lycocerus orientalis	0.0201 0.0072	—	—
Pothemus purpuripennis	—	0.0177 0.0079	—
Themus impressipennis	—	—	0.0159 <.0001

Table 5: Tukey HSD for the CS between both sexes of each species: mean differences (above); *P*-values (below). Asterisk (*) indicates the mean difference is significant at the 0.05 level.

Male **Female**	***Lycocerus orientalis***	***Prothemus purpuripennis***	***Themus impressipennis***
Lycocerus orientalis	-335.3919703(*) 0.037	—	—
Pothemus purpuripennis	—	220.021687 0.301	—
Themus impressipennis	—	—	15.8455122 1

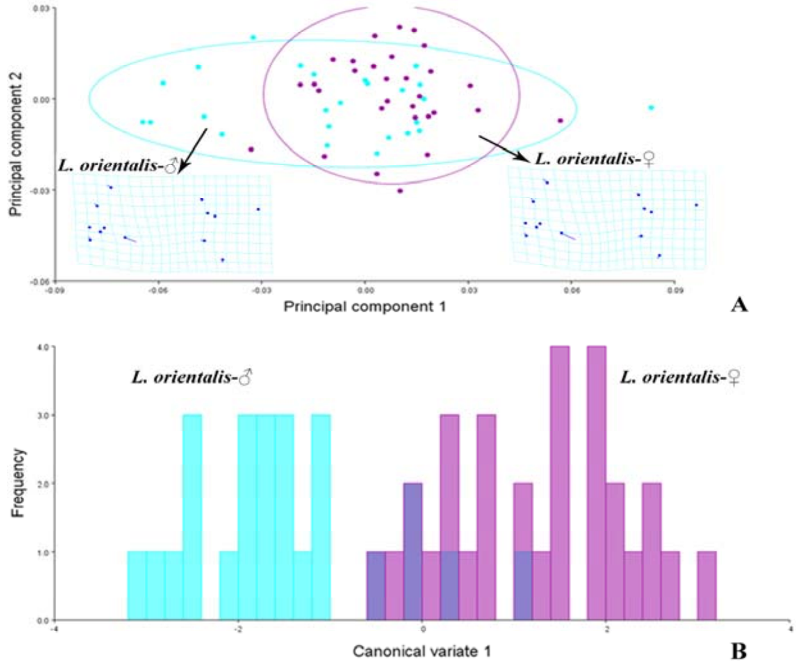

Fig 2: Shape variables of hind wings in both sexes of *Lycocerus orientalis* (Gorham, 1889). (A) principal component analysis (PCA) of hind wing configuration. Plot of PC1 (47.321% of total variation) and PC2 (9.546% variation) showing 90% confidence ellipses of population means. (B) discriminant function analysis (DA) of same matrix, also showing 90% confidence ellipses of population means. The averaged shape of each sex is depicted as deformations using thin plate splines.

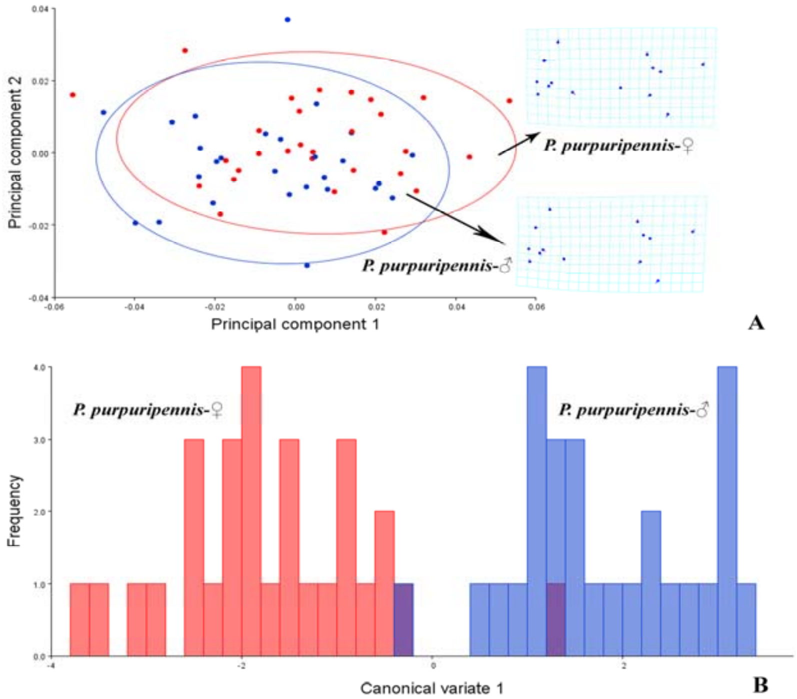

Fig 3: Shape variables of hind wings in both sexes of *Prothemus purpuripennis* (Gorham, 1889). (A) principal component analysis (PCA) of hind wing configuration. Plot of PC1 (35.141% of total variation) and PC2 (11.066% variation) showing 90% confidence ellipses of population means. (B) discriminant function analysis (DA) of same matrix, also showing 90% confidence ellipses of population means. The averaged shape of each sex is depicted as deformations using thin plate splines.

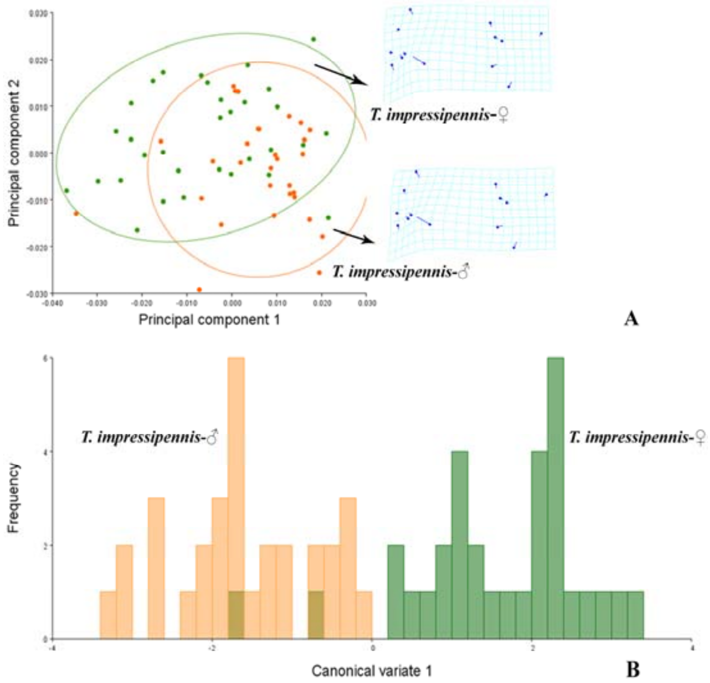

Fig 4: Shape variables of hind wings in both sexes of *Themus impressipennis* (Fairmaire, 1886). (A) principal component analysis (PCA) of hind wing configuration. Plot of PC1 (26.055% of total variation) and PC2 (14.269% variation) showing 90% confidence ellipses of population means. (B) discriminant function analysis (DA) of same matrix, also showing 90% confidence ellipses of population means. The averaged shape of each sex is depicted as deformations using thin plate splines.

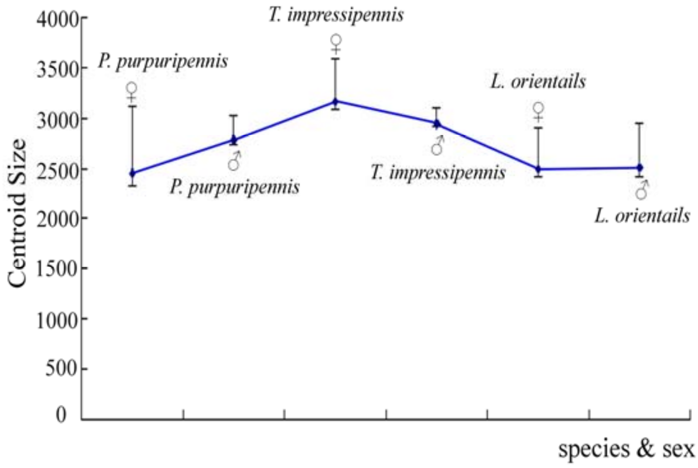

Fig 5: Comparisons of centroid size variables (SD: above; SE: below) between both sexes of *Lycocerus orientalis*, *Prothemus purpuripennis* and *Themus impressipennis*.

4. Discussion

The female and male of *Lycocerus orientalis*, *Prothemus purpuripennis* and *Themus impressipennis* could be successfully discriminated by DA (Figs. 2A, 3A, 4A) respectively, since that Mahalanobis and Procrustes distances for each group are significantly different ($P<0.05$). But the centroid size cannot resolve all the three cases because of insignificant differences ($P>0.05$) in the latter two species. It demonstrates that there is a significant difference in the hind wing shape between the sexes for the species of Cantharinae, but the size is doubtful because of the inconsistent results in the three species.

Furthermore, the PCs (Figs. 2B, 3B, 4B) show that the median area contributes most to the shape variables between sexes for each species, but the variations are associated with different veins junctions in different species. This suggests that the differences of the hind wing shape between sexes are different in one species from another.

Herein we conclude that sexual dimorphism is present in the hind wing shape of Cantharinae species, and the variations between sexes are exclusive in each species.

5. Acknowledgment

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