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Landmark-based geometric morphometric analysis of forewing sexual dimorphism in *Mycalesis ita*

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Abstract

This study presents the phenotypic variation in sexual dimorphism on wing shape of *Mycalesis ita*. Butterfly wings hold highly diverse phenotypes as a result of interactions between adaptive processes, phylogenetic history and developmental constraints. The results of this study revealed that there is no considerable difference in wing morphology within sexes in both sites, though sexual dimorphism of wing shape between sexes is highly pronounced, completely separating the male and female *M.ita*. In addition, it is notable that male morphology is more stable across sites, having 45% portion of its population with uniform morphology compared to 27.5% portion of female population. This further emphasize the differences in the physiology and life history of male and female and suggest that female morphology may undergone or undergoing more changes overtime. Environmental isolation and varying level of gene pool could also one of the factors causing these variations. Hence, this study proved the ability of modern geometric morphometrics to distinguish body shape variations existing within and between populations of *M.ita*.

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Introduction

The genus *Mycalesis,* popularly known as bushbrown butterflies are characterized by a brushy foot. *Mycalesis* almost resembles the species *Orsotriaena medus*, the only difference is the number of spots in the wings.

They are common in the warm regions from Central Asia to Australia, and have a high diversity in South East Asia. Characteristically, these types of butterfly are low flight and prefer shady areas to perch (Braby, 1995). Among 100 species in this genus, 24 are present in the Philippines (Kodandaramaiah *et al*., 2010). One of these is *Mycalesis ita*, which is the most common and abundant in Bukidnon province. Species within this genus are highly cryptic because they are polymorphic as a mechanism to survive wet- and dry-seasonal changes (Brakefield and Frankino, 2009). Without dissection of genitalia, the only physical characters that can distinguish between male from female are the size and color. But these characters are rather relative to individual and environment, and these measurements of dimorphic form often encounter a problem being interpreted differently by scientists (Adams *et al*., 2004).

Sexual dimorphism is one of the most interesting sources of phenotypic variation among organisms, and considered as a very important area of study in the field of evolutionary biology (Benitez *et al*., 2011).

Thus, this study focused on the wing shape morphology using geometric morphometric analysis, which has been extensively applied in the field of entomology to either link or separate closely related taxa and helps in identifying population within and between species of insects. However, studies about sexual shape dimorphism in wing morphology have been less accounted (Gidaszewski *et al*., 2009).

Experts in the field of entomology have gained great interest in the study because they were able to distinguish differences between sexes in insect that is often very small or indistinguishable. It permits the easy identification of sexes that proved to be very useful for understanding the life history and behaviour of the species. Based on these, there is a need to establish a comparison between male and female of *M. ita* using geometric morphometric analysis.

Materials and methods

Collection sites

The specimens were collected from two selected Barangays in the Municipality of Wao, Lanaodel Sur where the species is abundantly observed. An agricultural landscape in Barangay Banga and large forested woodland in Barangay Park area, which were designated by Merckx and Van Dyck (2006) as poor and very suitable habitat for butterfly, respectively (Merckx and Van Dyek, 2006).

The collection of samples from these two unrelated populations was done on September–October 2017.

Collection of samples

A total of 164 specimens were collected from two sites and only undamaged samples were used for geometric morphometric analysis. Eighty (80) specimens were used in the study comprised of 40specimensfrom each site with 20 males and 20 females.

They were anesthetically killed by suffocation in a jar with 90% alcohol. Before image acquisition, descaling of the wings was performed to reveal their venation.

Image acquisition

A DSLR (Cannon EOS 1200D) camera attached to a fixed tripod was used to capture the image of the specimens in a uniform manner, both in distance and angle.

Morphometric and statistical analysis

Geometric Morphometric analysis was performed to determine the morphological differences within and between sexes and populations from the two collection sites.

Fig. 1. Map of the Philippines showing the location of Province of Lanao del Sur, with the locations of the two Barangays: Banga and Park Area.

It enables the precise and detailed analysis of shape change and shape variation in organism on the basis of positions of homologous anatomical landmarks (Rohlf and Marcus, 1993). This method allows generating graphic presentation of results for visual display and comparison of shape changes based on measured distances, angles, and ratios.

A total of 15 landmarks on the forewing positioned at vein origin, intersections and terminals (Fig. 2) were collected and digitized using TpsDig 2.16 (Rohlf, 2010). These landmarks were used to correspond to x, y coordinates in a Cartesian space (Adams *et al.*, 2004). The configurations in the geometric x and y coordinates from the digitized landmarks were first transformed into shape variables prior to executing the statistical analyses of shape variation because the images have shape and non-shape variables resulting from the slight differences in the distance, position and angle of the forewing during the image acquisition, Generalized Procrustes Analysis (GPA) was performed using MorphJ software (Klingenberg, 2011). The Principal Component Analysis (PCA) also known as Relative Warp Analysis was also performed using Morpho J. The generated RW scores were used to determine the different wing shape variation present in the butterfly wing. Scores were subjected to Multivariate Analysis (MANOVA), Canonical Variate Analysis (CVA) and Discriminant Function (DFA) using PAST (Paleontological Statistic) (Hammer *et al.*, 2011) statistic tool to further analyze the variation present between males and females, and also between geographical locations where butterfly specimens were collected.

Results and discussion

Geometric morphometric analysis was performed to investigate the wing shape variation that may be present within and between populations of *M. ita*. Individuals of the same butterfly are capable to live and adapt to variety of habitats, or forced to do so because of habitat degradation (Hill *et al.*, 2001, 2002; Dover *et al.*, 2009).

Table 1. Summary of the MANOVA results of two different populations of *Mycalesis ita*.

	Site 1 (Banga)	Site 2 (Park Area)
Wilks' lambda	0.001107	0.0007163
Pillai trace	0.999	0.9995
P-Values	2.999E-10-1.561E-10	4.245E-11-8.756E-12
Eigenvalue1	902.1	1295
Eigenvalue2	0.0001163	0.0001545

Table 2. Summary of the DFA results of two different populations of *Mycalesis ita*.

	Site 1 (Banga)	Site 2 (Park Area)
P-Value	3.328E-10	6.368E-10
Correctly classified (%)	100 %	100 %

In addition, conditions that the individuals experienced may further change over time, and the rate of change can be abruptly due to natural catastrophic events, regularly or more or less predictable change caused by seasonal variation or progressively due to global warming (Vitousek *et al.*, 1997; Hill *et al.*, 2002; Dover *et al.*, 2009).

In a constant environment, selection could work to favor one optimal wing shape and size. But environment in nature are at a constant change, heterogeneous in both space and time.

It its more advantageous for the species if it allows for flexibility in wing development, or other traits in general, so that they could develop the most optimal flight design in each of the different environments (Bradshaw, 1965; Stearns and Koella, 1986; Stearns, 1989; Schlichting and Pigliucci, 1998).

Table 3. Summary of the MANOVA results for *Mycalesis ita* males and females between two populations.

	Male	Female
Wilks' lambda	0.4058	0.451
Pillai trace	0.5942	0.5491
P-Values	0.9559	0.9816
Eigenvalue1	1.464	1.217
Eigenvalue2	1.198E-06	0.0002596

Table 4. Summary of the DFA results for *Mycalesis ita* males and female between two populations.

	Male		Female	
	P-Value	Correctly classified (%)	P-Value	Correctly classified (%)
Site 1 vs Site 2	0.99	55 %	0.9988	72.5 %

Selection thus operate on the plasticity of traits crucial for the survival and reproduction such as best flight morphology, ensuring that that the development of these traits is flexible enough to allow for a quick response to varying conditions across space and time (Schlichting and Pigliucci, 1998; Sultan, 2003; West-Eberhard, 2003; Sultan, 2004).

There are numerous factors that cause the variation in this species population, such as geographical isolation resulting to limited gene pool, selection pressure, sexual dimorphism and many others.

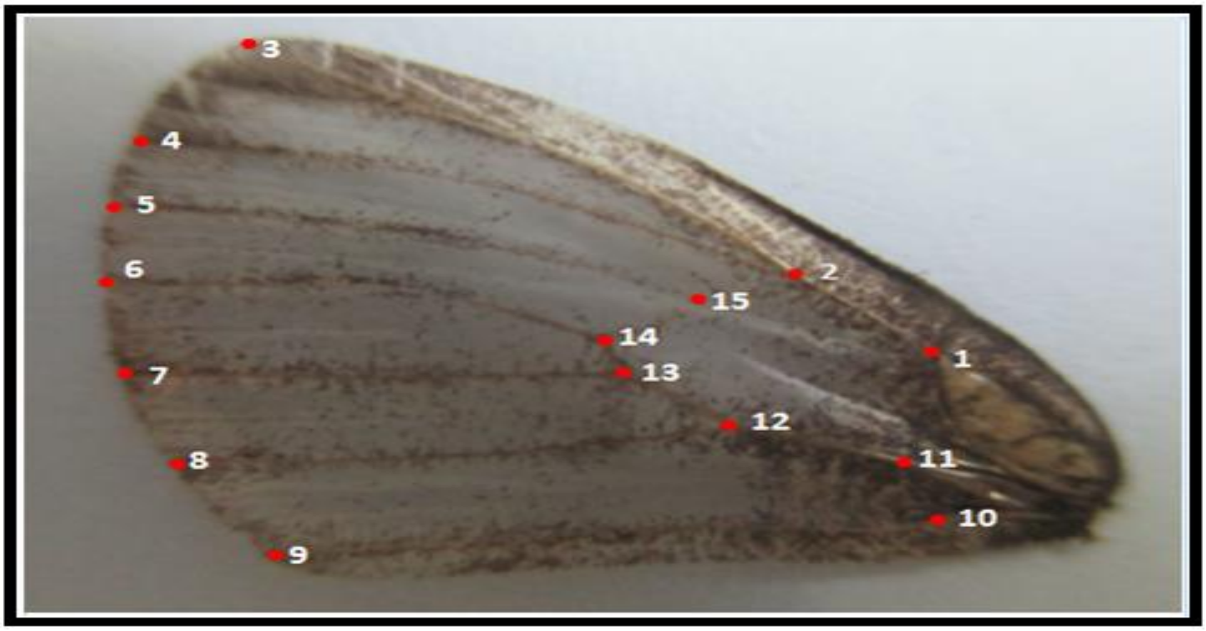

Fig. 2. Image of *Mycalesis ita* right ventral forewing with its 15 homologous anatomical landmarks. 1, 11, 10 vein origins; 2, 15, 14, 13, 12, vein intersections;3, 4, 5, 6, 7, 8, 9, vein terminals based on the criteria of Zelditch (2004).

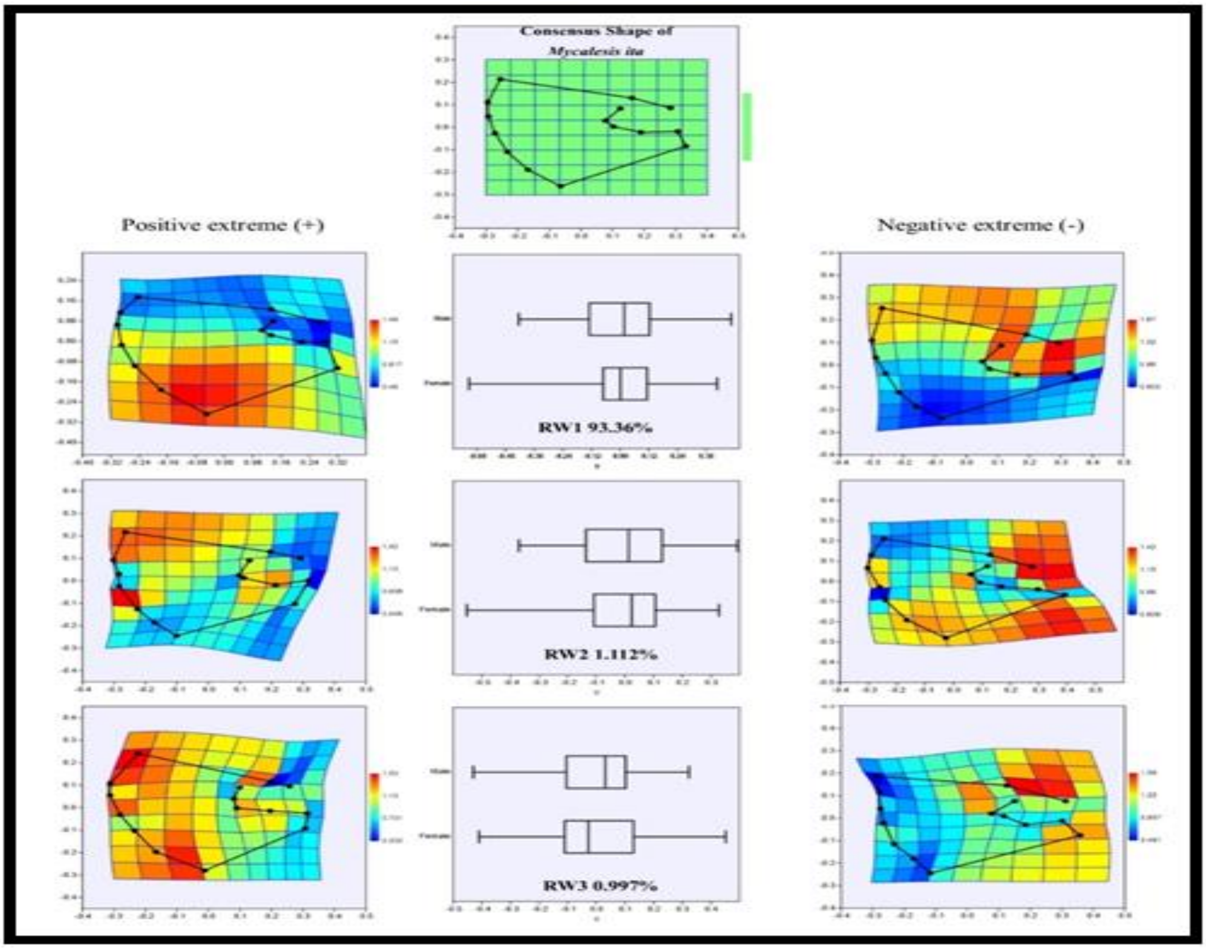

Fig. 3. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the wing shapes between sexes of *Mycalesis ita* of site 1 Barangay Banga (Agricultural landscape) as explained by each of the significant relative warps.

The determination of sexual dimorphism is important to further understand the species behavior, its ecology and life history, knowledge that is critical in making comparison between populations.

The pattern of wing shape variation within the population of *M. ita* from site 1 and 2 is summarized in Figs 3 and 4. Boxplots of the relative warp scores for both sexes are shown together with the deformation grids with expansion factor scale.

The uppermost relative warp is the mean wing shape for each of the two populations.

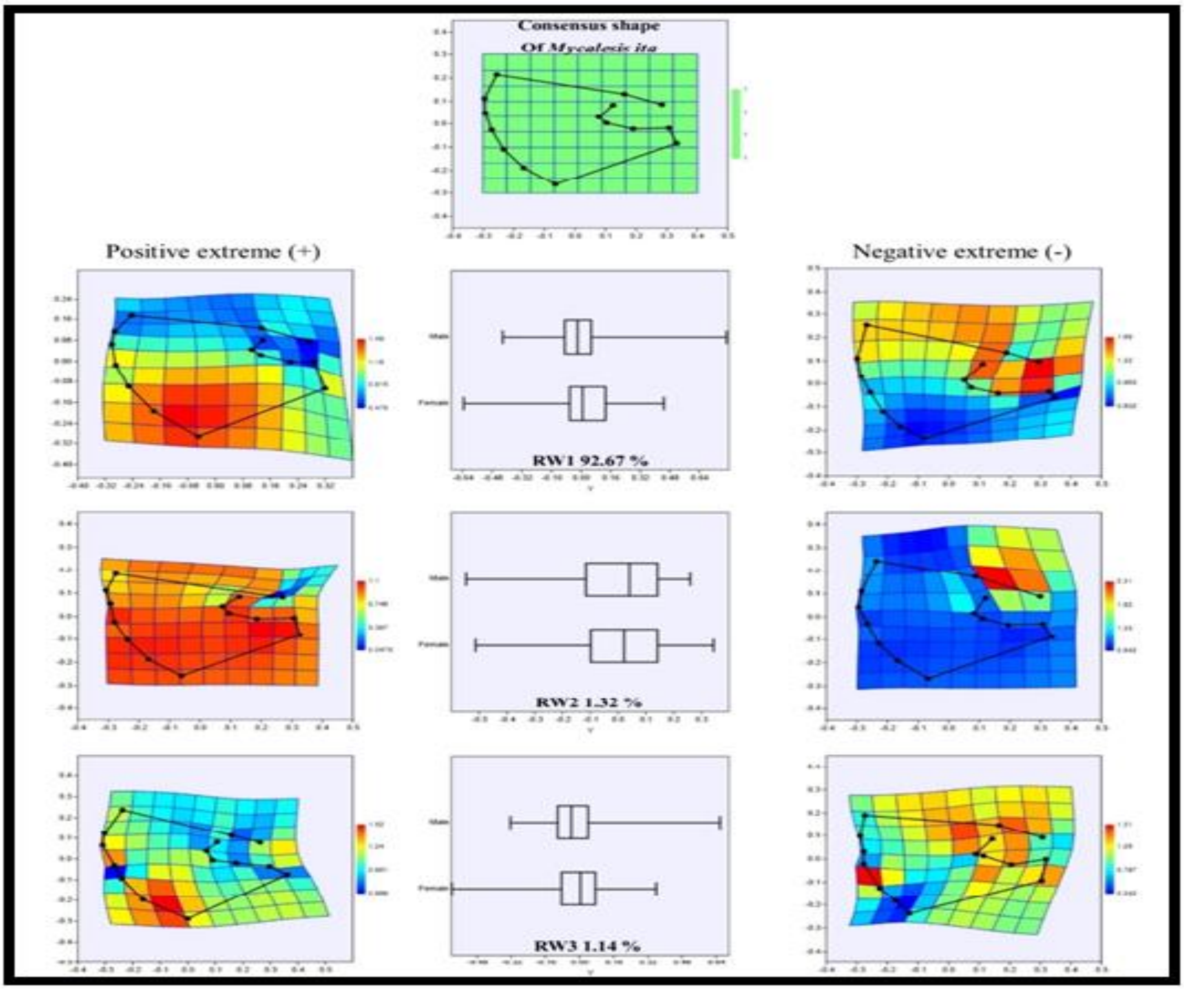

Fig. 4. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the wing shapes between sexes of *Mycalesis ita* of site 2 Barangay Park Area (Forested woodland) as explained by each of the significant relative warps.

In Fig. 3, RW1 account for 93.36% variation among male and female individuals in site 1, and in Fig. 4, RW1 account for 92.67% variation among male and female individuals in site 2.

To further emphasize the comparison between the difference of the wing shapes between both sexes from each population, MANOVA, CVA and DFA were done. Tables 1 and 2 contain the results from MANOVA and DFA for each of the population.

The MANOVA results obtained from comparing the females and males from each population justifies that there is significant differences existing between the two sexes ($p=<0.05$, see Table 1). Thus, sexual dimorphism has been detected and is existing in the two populations of *M. ita*. Wilks' lambda and Pillai trace also strengthen this finding. DFA and CVA are tools that test whether groups or populations can be well separated from each other or blend into each other into a continuum (Hammer, 2002). Looking at the CVA plot (Fig. 5), it can be seen that the males and females have no overlap allowing separation of the two sexes. Thus, it suggests that there are differences between the two sexes which can be attributed to sexual dimorphism.

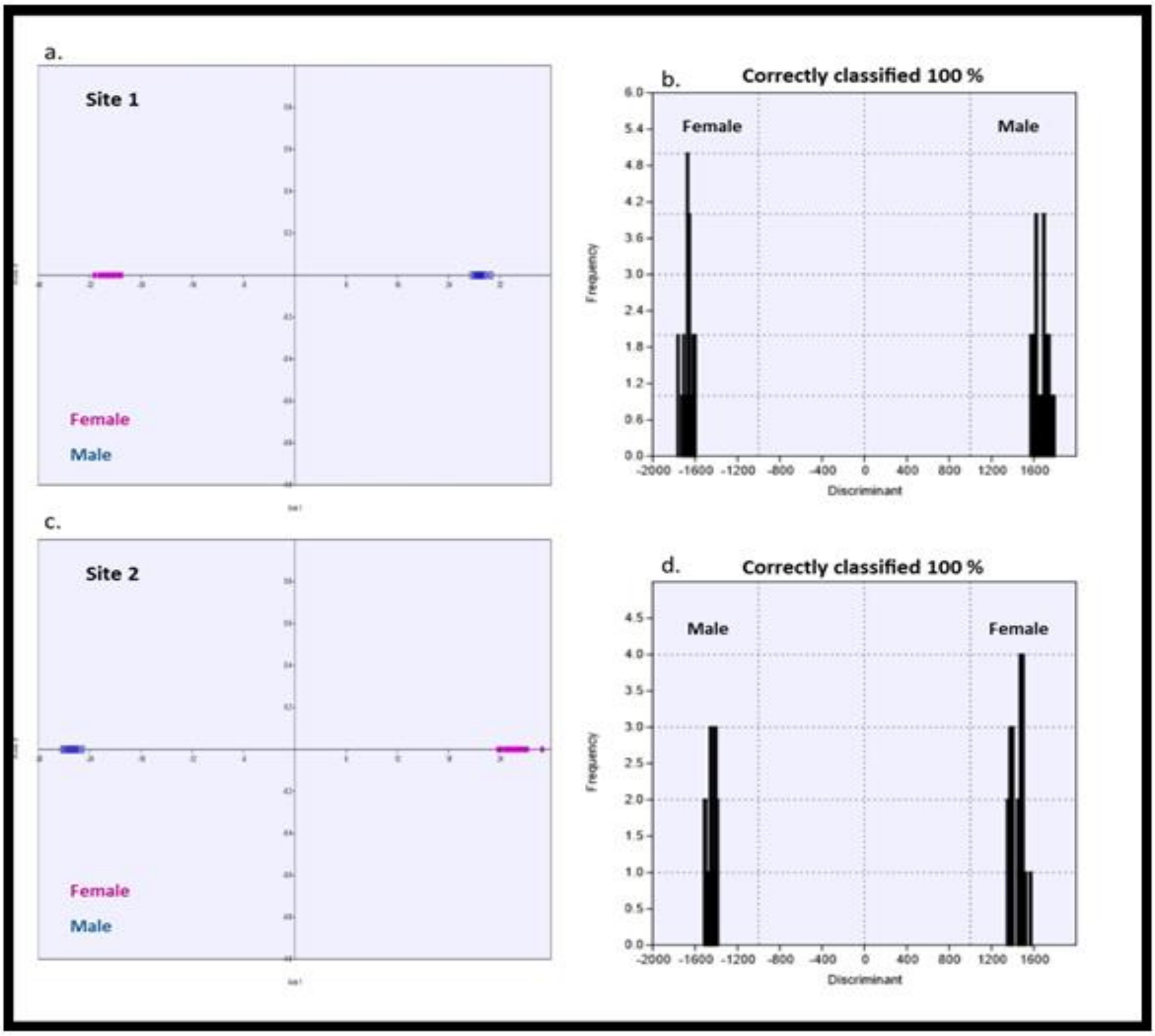

Fig. 5. Canonical Variate Analysis and Discriminant Function Analysis plots of the relative scores of *Mycalesis ita* population.

The DFA further supports the result obtained in CVA since it no overlap (100% correct classification) of the morphological attributes between populations. This result may suggest that geographic separation could be a contributing factor to the population's distinction from each other, since there is a little to no interaction/intermingling and/or migration between these populations (Turan *et al.*, 2004). Isolation also permits populations to be subjected to varying selection pressures, one of the preconditions for allopatric speciation. Such isolated populations may become morphologically and genetically differentiated through adaptive or non-adaptive processes (Grant *et al.*, 2000) eventually leading to formation of distinct gene pools. Hence, the ability of populations to adapt and evolve as separate biological entities is limited by the exchange of genes among populations.

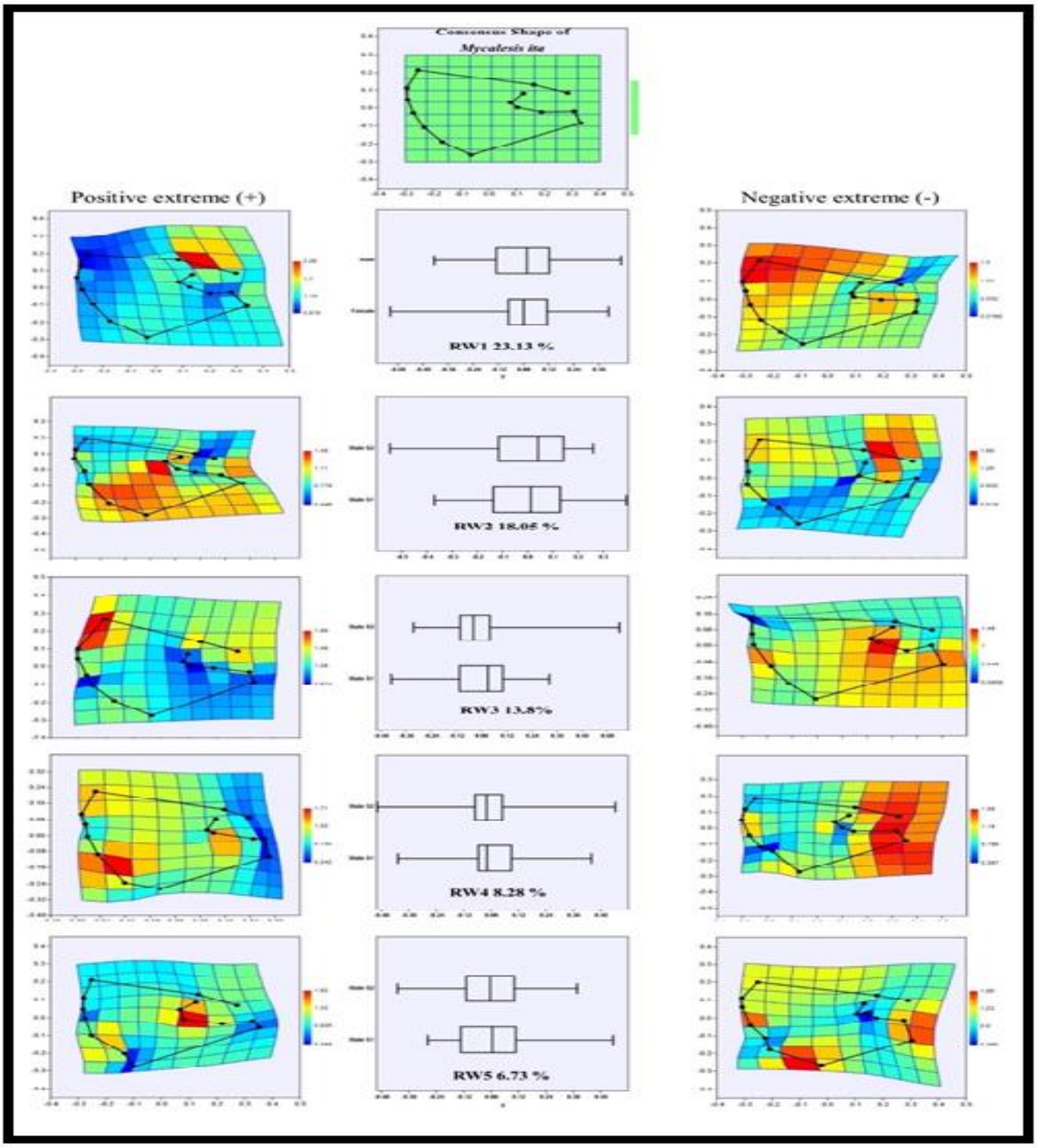

Fig. 6. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the wing shapes within sexes of *Mycalesis ita* male population across the two site as explained by each of the significant relative warps.

The pattern of wing shape variation in population within sexes summarized in Figs. 6 and 7. Boxplots of the relative warp scores for both sexes are shown together with the deformation grids with expansion factor scale.

The uppermost relative warp is the mean wing shape for each of the two populations.

In Fig. 6, RW1, 2, 3, 4 and 5 account for 23.13%, 18.05%, 13.08%, 8.28% and 6.73% respectively (total of 69.27 % variation of the population) the variation among male in both sites and in Fig. 7, RW1, 2, 3, 4 and 5 accounts for 22. 13%, 16.65%, 13.73%, 8.38% and 6.16 % (total of 67.05% variation of the population) 92.67% the variation among female in both populations.

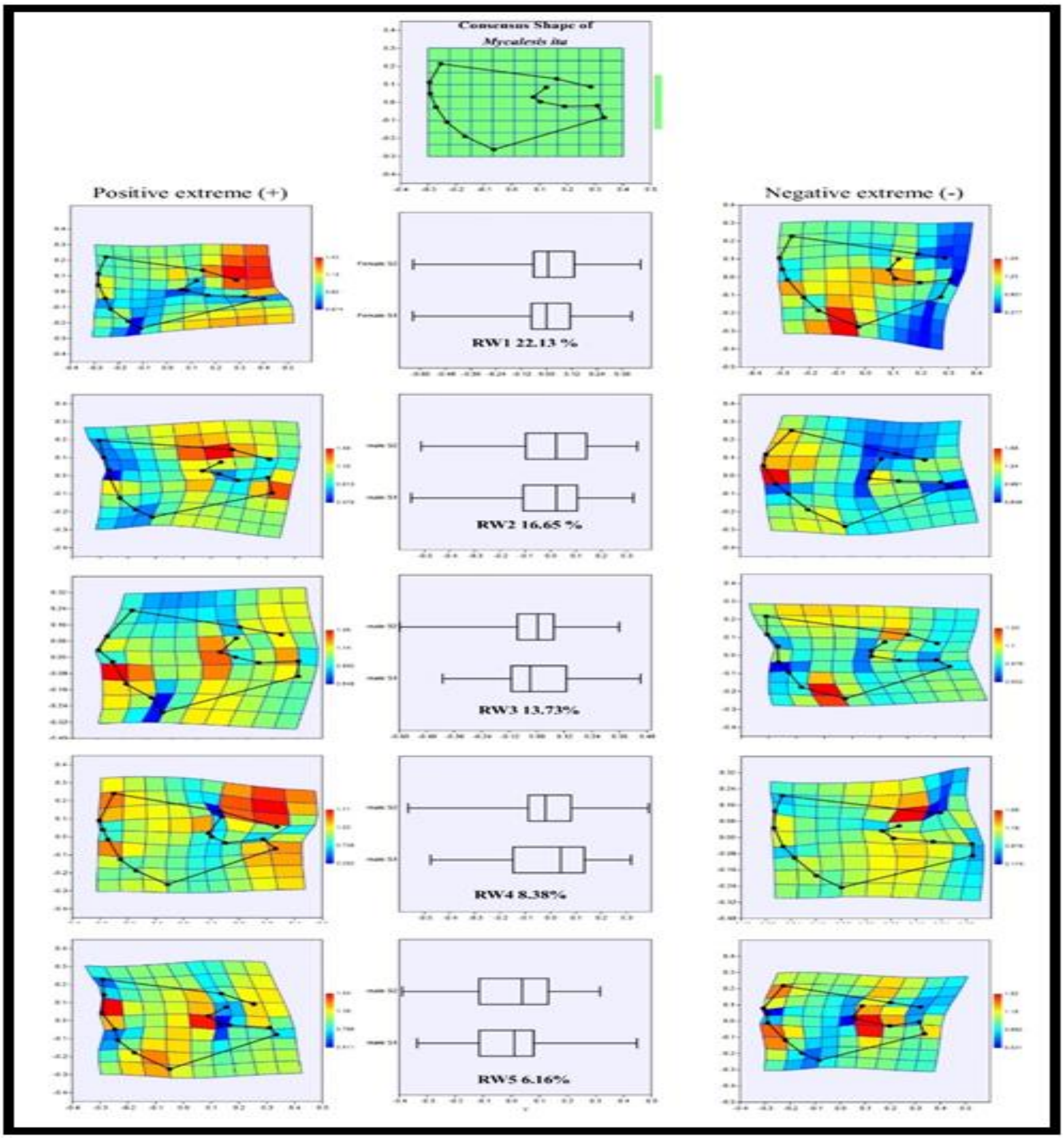

Fig. 7. Summary of landmark based geometric morphometric analysis showing the boxplot and variation of the wing shapes within sexes of *Mycalesis ita* female population across the two site as explained by each of the significant relative warps.

To show whether there are significant differences in the wing shapes of males and females from the two populations, CVA and DFA plots for the pooled male from both sites and pooled female also from both sites were generated (see Fig. 8 a, b, c and d). Fig. 8b show the DFA between the two population of male (pooled male population of site 1 and 2) where 55% is correctly classified, this means that 45% of the population shares the same morphological attributes. Fig. 8d shows the DFA between the two population of Female (pooled female population of site 1 and 2) where 72.5% is correctly classified, only 27.5% portion of the population shares the same morphological attributes. This is further supported by the CVA plots showing overlaps of their morphological characteristics. Tables 3 and 4 contain the results for the MANOVA and DFA between the two populations.

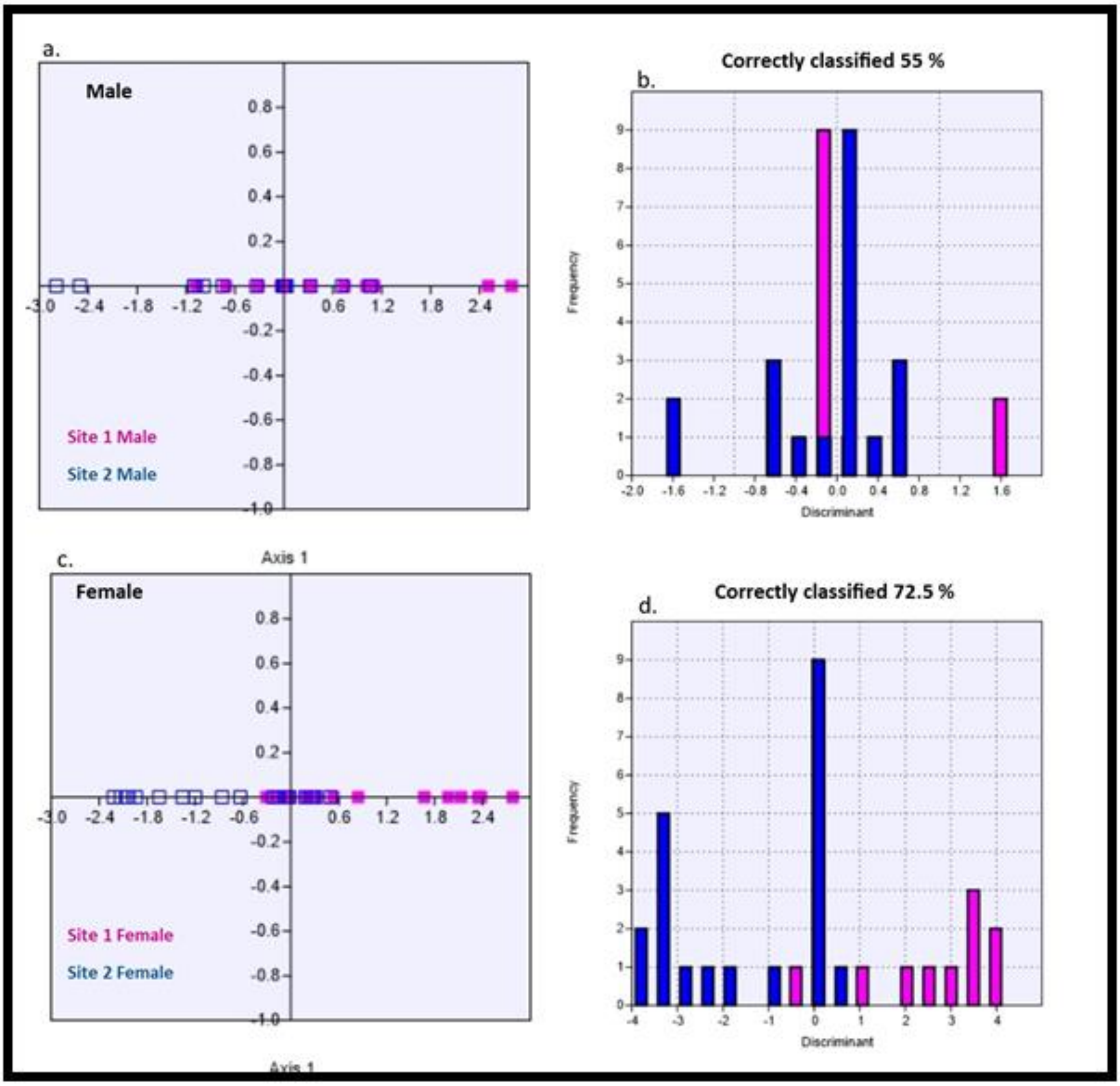

Fig. 8. Canonical Variate Analysis and Discriminant Function Analysis plots of the relative scores of *Mycalesis ita*. Male (a,b) and Female (c,d). CVA (a,c). DFA (b,d).

Although variation has been detected within the population of males and females, it was found to be statistically not significant in p=>0.05 (see Tables3 and 4).

Conclusions

Geometric morphometric analysis was used in the analysis of wing shapes of *M. ita* forewing from two selected Barangays of the Municipality of Wao, Lanao del Sur, in which Barangay Banga and Barangay Park Area as sites 1 and 2, respectively. Data from the MANOVA, CVA, and DFA revealed that there are significant differences between sexes of *M. ita* in both sites' population. Therefore, sexual dimorphism is detected and variation within sexes is present in both sites although it is not statistically significant. It is also notable that males have more stable wing shapes across population having 45% portion of its population sharing the same morphology vs 27.5% portion of female population. These further highlights the distinction in the physiology and life history of male and female *M. ita* and suggest that female morphology may undergone or undergoing more changes across its lifetime. Seasonal environment can apply a strong and varied selection pressure that moves adaptive phenotypic plasticity, where environment cues determine the developing phenotype with better fitness to ever-changing environment. Overall, geometric morphometric proved that's its able to distinguish variations that exist within and between populations. It is highly recommended that a further study on the genetic aspect responsible in morphological variation would strengthen the foundation because the knowledge of both genetic and phenotypic side is both important in the quest to fully understand the diversity of organism.

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