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Describing compartmentalization in the fore- and hind wings of striped slant-faced grasshopper (*Amphitornus sp.*)

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Abstract. The wings of insects are highly compartmentalized by their major and minor veins. Each compartment in insect wings are believed to be potential candidates for separate developmental modules which may correspond to distinct cell lineages and domains of gene expression. Are the entire wings of striped slant-faced grasshopper (*Amphitornus sp.*) integrated? Or, are the compartments, even smaller part of the wings can be considered as autonomous units of "gene regulation"? Hence, this study was conducted to determine the number and pattern of developmental modules in the wings of *Amphitornus sp.* Different hypothesis were formulated and tested to determine the developmental modules in the wings, as well as the veins that bounds each compartments. TPSUtil, TPSdig2 and, Modularity and Integration tool (MINT ver 1.5) were the software's used in this study. A total of 8 and 14 *a priori* models for the fore- and hindwings were constructed and statistically tested. Results show that fore-and hind wings of *Amphitornus sp.* comprised of 3 and 4 developmental modules bounded by their major veins. Fair consistency in the best fit model and pattern of developmental modules was observed. Therefore, compartments in wings correspond to "units of gene regulation."
Keyword: Modules, modularity, fore wing, hind wing, *Amphitornus sp.*

Introduction. Insect wings are divided into compartments by complex network of netted veins wherein it is hypothesized that the subdivisions correspond to a distinct cell lineages and domains of gene expression (von Dassow & Munro 1999; Klingenberg & Zaklan 2000). Each wing compartment is a potential candidate of being separate and distinct developmental module (Cowley & Atchley 1990; Cavicchi et al 1991; Klingenberg & Zaklan 2000) internally coherent through manifold connections. Modules show strong interaction among their components while showing some degree of mutual autonomy and which corresponds to their developmental origins and functions (Cheverud 1996; Wagner 1996; Klingenberg et al 2003; Tabugo et al 2011). However, the question whether the entire wing is a single module or whether the compartments, even the smaller parts of the wings, could be considered as autonomous units of morphological variation that may correspond to domains of gene expression, still remained ambiguous and is attempted to be investigated in the present study on the wings of striped slant faced grasshopper (*Amphitornus sp.*) using the Modularity and Integration Analysis Tool (MINT ver 1.5, Marquez 2008b). The major objective of this study is to determine the number of developmental modules defining the shape of the fore- and hind wings of *Amphitornus sp.* and to delimit the spatial domain of developmental modules in the wings. Understanding relationship between modules in the wings of the grasshoppers can be informative about the underlying biological process of compartmentalization in the wings. Understanding how covariation between modules can have substantial implications for understanding genetic variation and the potential of the species for evolutionary change (Klingenberg 2008).

Material and Method. Insect samples were collected during the month of May to October, 2011 at selected provinces in Mindanao namely: Lanao del Norte, Misamis Occidental, and Zamboanga del Sur, by using sweep nets. The collected samples were placed in a container filled with 70% ethanol. Through visual inspection in the genitalia the sex of the grasshoppers were identified.

The fore- and hind wings of the samples were detached from their body and were mounted in clean and clear slides. Mounted slides were scanned using a Hewlett-Packard Jacket 2400 Scanner with 1200 dots per inch (dpi) resolution. Images was cropped, labeled and saved one by one. TPSDig2 ver. 2.12 (Rohlf 2008) was used to digitized specified landmarks. A total of 150 and 195 points were used for outlining the shape as well as the wing venation pattern of the fore and hind wings of the grasshopper. After outlining, the TPS curve was then converted into landmarks points (XY) using TPSutil ver 1.44 (Rohlf 2009) which served as the raw data for the analysis.

The wings provide an excellent system for studying these issues because wing veins provide many morphological landmarks. Wings of grasshoppers possess five main vein stems: Costa (C), Subcosta (Sc), Media (M), Radius (R) and Cubitus (Cu) (Figure 1). To investigate this, different hypothesis (Figures 2-4 and Tables 1-2) were formulated and tested to determine the possible developmental modules and boundaries defining the fore- and hind wings of *Amphitornus sp.*
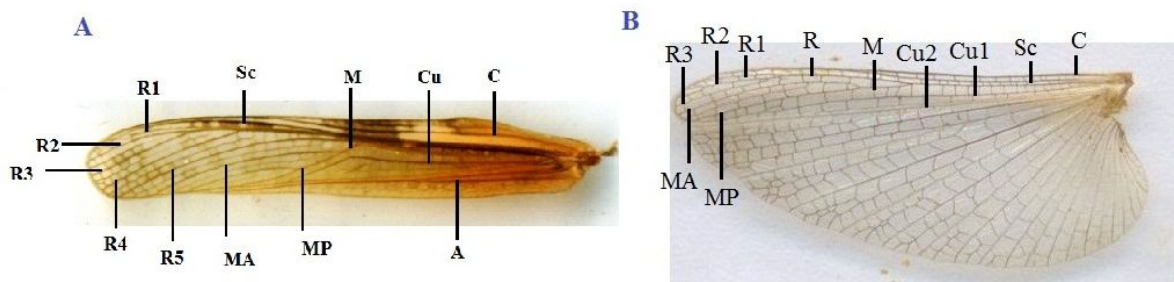

Figure 1. Grasshopper fore- and hind wings showing veins that may serve as boundaries of the hypothesized developmental modules.

Table 1

The veins in the fore wings that possibly defines the boundaries of the developmental modules in the hypothesized models

Models	Modules	Description
H_0	0	Null model, predicting absence of modular structure
H_1	6	First module is bounded by Anterior margin and Costa (C) Second module is bounded by Costa (C) and Subcosta (Sc) Third module is bounded by Subcosta (Sc) and Media (MP) Fourth module is bounded by Media (MP) and Cubitus (Cu) Fifth module is bounded by Cubitus (Cu) and Anal vein (A) Sixth module is bounded by Anal vein (A) and posterior margin
H_2	4	First module is bounded by Anterior margin and Costa (C) Second module is bounded by Costa (C) and Radius (R5) Third module is bounded by Radius (R5) and Media (MP) Fourth module is bounded by Media (MP) and posterior margin

Models	Modules	Description
H_3	3	First module is bounded by Anterior margin and Radius (R5) Second module is bounded by Radius (R5) and Cubitus (Cu) Third module is bounded by Cubitus (Cu) and posterior margin
H_4	4	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded Subcosta (Sc) and Media (MP) Third module is bounded by Media (MP) and Anal vein (A) Fourth module is bounded by Anal vein (A) and posterior margin
H_5	3	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Media (MP) Third module is bounded by Media (MP) and posterior margin
H_6	8	First module is bounded by Anterior margin and Costa (C) Second module is bounded by Costa (C) and Radius (R2) Third module is bounded by Radius (R2) and Radius (R4) Fourth module is bounded by Radius (R4) and Media (MA) Fifth module is bounded by Media (MA) and (MP) Sixth module is bounded by Media (MP) and Cubitus (Cu) Seventh module is bounded by Cubitus (Cu) and Anal vein (A) Eighth module is bounded by Anal vein (A) and posterior margin
H_7	6	First module is bounded by Anterior margin and Radius (R1) Second module is bounded by Radius (R1) and (R3) Third module is bounded by Radius (R3) and (R5) Fourth module is bounded by (R5) and Media (MP) Fifth module is bounded by Media (MP) and Anal Vein (A) Sixth module is bounded by Anal vein (A) and posterior margin
H_8	6	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Radius (R4) Third module is bounded by Radius (R4) and Media (MA) Fourth module is bounded by Media (MA) and Cubitus (Cu) Fifth module is bounded by Cubitus (Cu) and Anal vein (A) Sixth module is bounded by Anal vein (A) and posterior margin

Table 2
The veins in the hind wings that possibly defines the boundaries of the developmental modules

Models	Modules	Description
H_0	0	Wings don't have modular structure
H_1	2	First module is bounded by Costa (C) and Cubitus 2 (Cu2) second module is bounded by Cubitus 2 (Cu2) and posterior margin
H_2	3	First module is bounded by Costa (C) and Media (MP) Second module is bounded by Media (MP) and Cubitus 2 (Cu2) Third module is bounded by Cubitus 2 (Cu2) and Posterior margin
H_3	3	First module is bounded by Costa (C) and Radius (R3) Second module is bounded by Radius (R3) and Cubitus 2 (Cu2) Third module is bounded by Cubitus 2 (Cu2) and Posterior margin

Models	Modules	Description
H_4	4	First module is bounded by Costa (C) and Radius (R3) Second module is bounded by Radius (R3) and Media (MP) Third module is bounded by Media (MP) and Cubitus 2 (Cu2) Fourth module is bounded by Cubitus 2 (Cu2) and posterior margin
H_5	6	First module is bounded by Costa (C) and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Radius (R3) Third module is bounded by Radius (R3) and Media (MP) Fourth module is bounded by Media (MP) and Cubitus 1 (Cu1) Fifth module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2) Sixth module is bounded by Cubitus 2 (Cu2) ans posterior margin
H_6	3	First module is bounded by Costa (C) and Cubitus 1 (Cu1) Second module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2) Third module is bounded by Cubitus 2 (Cu2) and posterior margin
H_7	4	First module is bounded by Costa (C) and Radius (R1) Second module is bounded by Radius 1 (R1) and Media (MA) Third module is bounded by Media (MA) and Cubitus 1 (Cu1) Fourth module is bounded by Cubitus 1 (Cu1) and posterior margin
H_8	4	First module is bounded by Costa (C) and Radius (R2) Second module is bounded by Radius (R2) and Media (MP) Third module is bounded by Media (MP) and Cubitus 2 (Cu2) Fourth module is bounded by Cubitus 2 (Cu2) and posterior margin
H_9	3	First module is bounded by Costa (C) and Media (MA) Second module is bounded by Media (MA) and Cubitus 2 (Cu2) Third module is bounded by Cubitus 2 (Cu2) and posterior margin
H_{10}	5	First module is bounded by Costa (C) and Radius (R3) Second module is bounded by Radius (R3) and Media (MP) Third module is Media (MP) and Cubitus 1 (Cu1) Fourth module is Cubitus (Cu1) and Cubitus 2 (Cu2) Fifth module is bounded by Cubitus 2(Cu2) and posterior margin
H_{11}	4	First module is bounded by Costa (C) and Radius (R2) Second module is bounded by Radius (R2) and Media (MP) Third module is bounded by Media (MP) and Cubitus 2 (Cu2) Fourth module is bounded by Cubitus 2 (Cu2) and posterior margin
H_{12}	3	First module is bounded by Costa and Radius 1 (R1) Second module is bounded by Radius (R1) and Cubitus 2 (Cu2) Third module is bounded by Cubitus 2 (Cu2) and posterior margin

Models	Modules	Description
H_{13}	4	First module is bounded by Costa (C) and Radius (R1) Second module is bounded by Radius (R1) and Media (MP) Third module is bounded by Media (MP) and Cubitus 2 (Cu2) Fourth module is Cubitus (Cu2) and posterior margin
H_{14}	5	First module is bounded by Costa (C) and Radius (R1) Second module is bounded by Radius (R1) and Radius (R3) Third module is bounded by Radius (R3) and Media (MP) Fourth module is bounded Media (MP) and posterior margin

After outlining, the TPS curve was then converted into landmarks points (XY) using TPSutil which served as the raw data for the analysis.

Modularity and Integration Tool (MINT) for Morphometric Data version 1.5 (Marquez 2008b) was used to study modularity and integration in grasshoppers' fore- and hind wings. The software calculates the matrix correlations between expected and observed covariance matrices. The data sets were loaded, and then a set of models were built and loaded. A total of a *priori* models for the fore wings (Figure 2) and a total of 14 a *priori* models for the hind wings (Figure 3) were constructed with the help of the model building tool option of the software. MINT assumes that the data themselves have modular structure, and by partitioning the entire data space into orthogonal subspaces, covariance matrices were then computed based on the modified data structures (Marquez 2008a).

The patterns of variational modularity were tested using γ* (Gamma) test for the Goodness of fit (GoF) on the alternative a *priori* models to evaluate whether a proposed model or hypothesis is good enough to explain variation in the data set. The lower γ* value imply will high degree of similarity between the observed data and the proposed model. Meanwhile, a low P- value (<0.05) corresponds to large values of γ* value, which implies a large difference between data and the model and thus a poor fit model (Marquez 2008a).
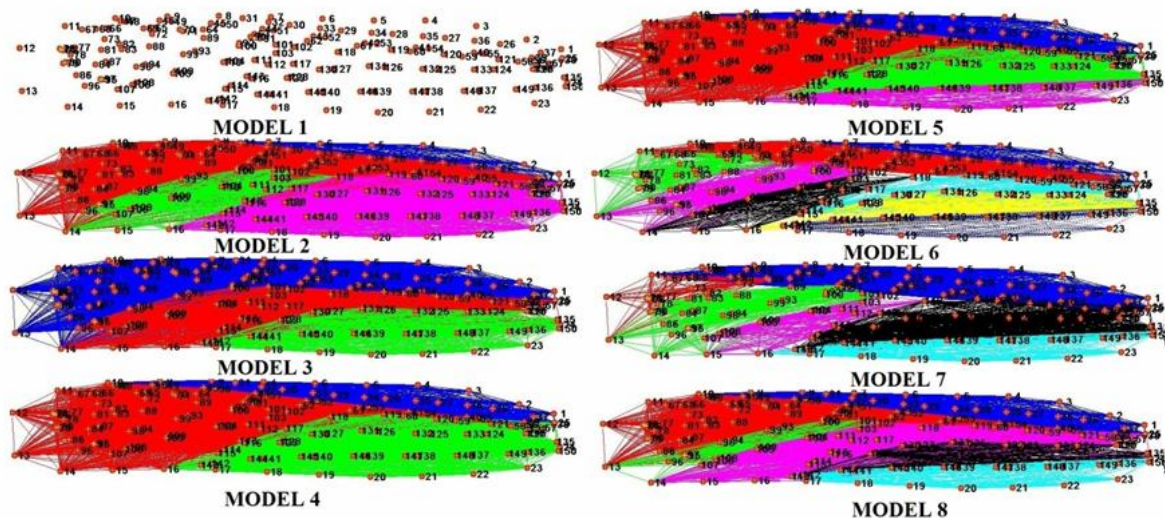

Figure 2. Models used in this study for the fore-wings of striped slant-faced grasshopper (*Amphitornus sp.*).
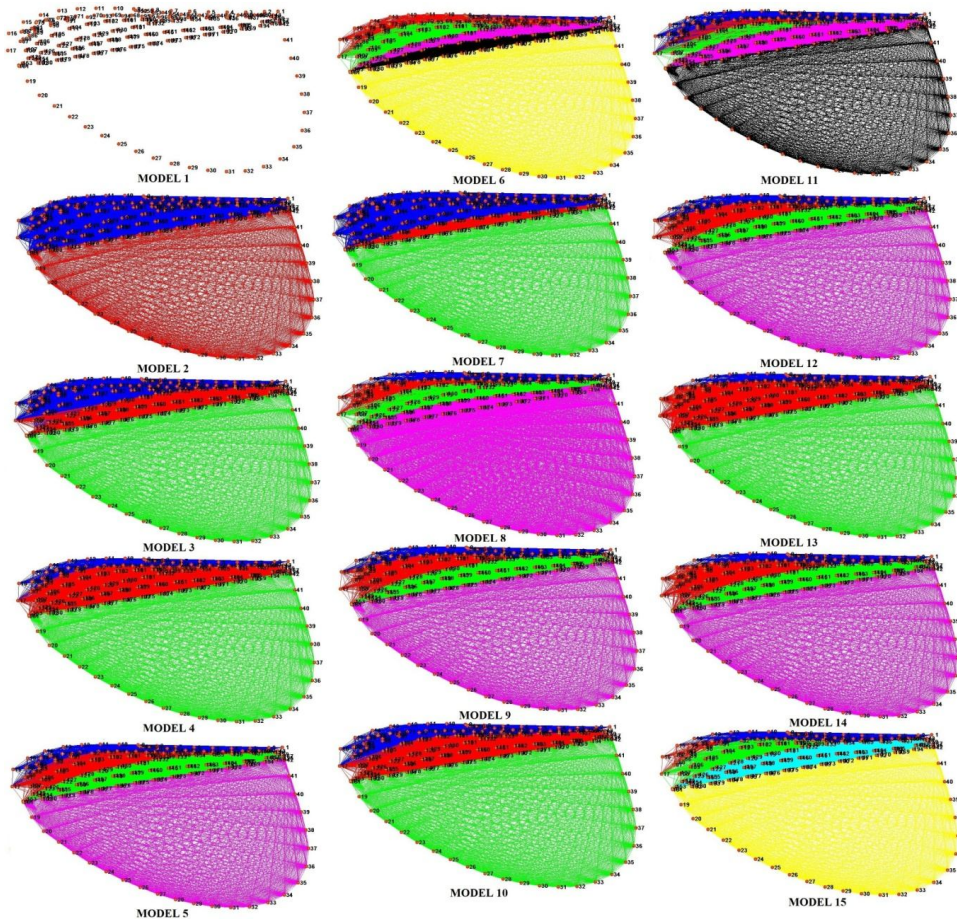
Figure 3. Models used in this study for the hind wings of *Amphitornus sp.*

Results and Discussion. For the male and female fore-wings a common model, model 5, is the most supported best fit model (Figure 4). Results show that the best fit model was consistent for both male and female left and right fore-wings. Model 5 is partitioned into 4 modules wherein, it is bounded by the major veins in the wings: (1) bounded by anterior margin and subcosta, (2) bounded by bounded by subcosta and media posterior, (3) bounded by media posterior and anal vein, (4) bounded by anal vein and posterior margin.
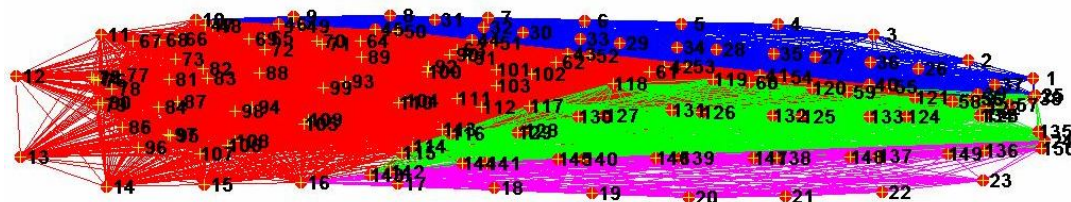

Figure 4. Best fit model for the fore-wing of *Amphitornus* sp. for both sexes.

Results also show that in the hind wings for both male and female a common model, model 7, is the most supported best fit model (Figure 5). Model 7 consisted with three modules: (1) bounded by Costa and Cubitus 1, (2) bounded by Cubitus 1 and Cubitus 2 and (3) bounded by Cubitus 2 and posterior margin.
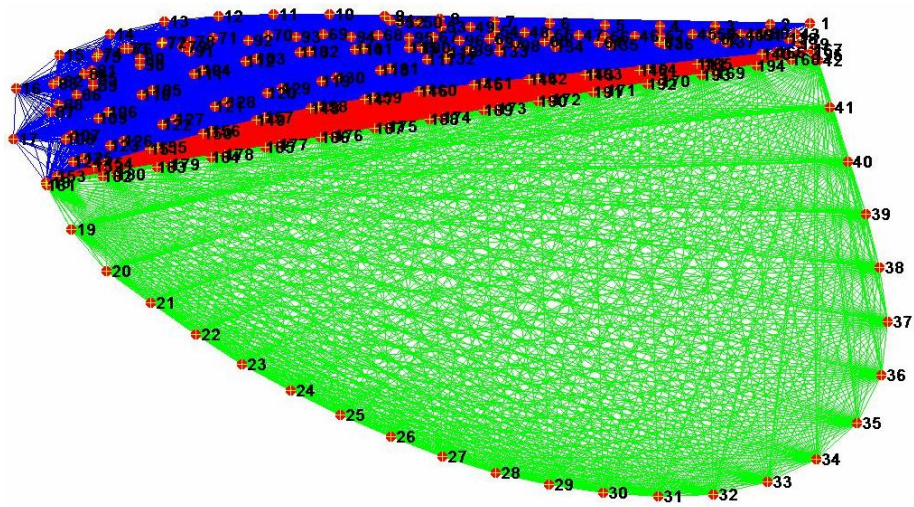
Figure 5. Best fit model for the hind wing of *Amphitornus sp*. for both sexes.

In Monte Carlo test, resulting P-values and gamma (*γ) values depict associations within integrated sets of traits or variation modules. Table 3 shows the computed P-values and gamma (*γ) values of the forewings and hind wings for both male and female *Amphitornus sp*, respectively. Low gamma values were observed model 5 for fore-wings and model 7 for hind wings for both male and female. Also both models yielded P-values greater than α= 0.05, which accepts the hypothesis that proposed models and observed data are not significantly different. Thus, indicating that these models qualify as the best fit models.

Results in this study show that the fore- and hind wings of *Amphitornus sp.* is divided into three developmental modules where it can also be viewed as units of gene regulation. It shows that the main veins serve as boundaries for each developmental module. The compartment boundary (veins) apart from being a delimiter between autonomous unit of developmental domains or modules also serves as an active center of integration, from which crucial patterning signal originate (Lawrence & Struhl 1996; Milan & Cohen 2000; Klingenberg & Zaklan 2000; Tabugo et al 2011). Signals originating from compartments boundary constitute a direct connection between the developmental processes which is responsible for positioning the various vein (Sturtevant & Bier 1995; de Celis 1998). Accordingly, all steps in wing development have different specificities which may vary in their abilities to transmit developmental perturbations, and that the genes involved may have phenotypic effects that are localized to various degrees wherein specificity in this process gives vein its identity. Processes occurring specifically to the smaller regions in the wings appear to contribute only a small portion of the observed morphometric variation (Klingenberg & Zaklan 2000).

Results show a fair consistency in the best fit model and in the number and pattern of developmental modules which may imply that the wings of *Amphitornus sp.* are highly conserved and that both male and female follow the same patterns of development. Highly conserved wings of *Amphitornus sp.* imply genetic conservatism in the morphological spaces in the fore- and hind wings. Consistency also suggests that there is not much variation in the arrangement of developmental module between sexes of *Amphitornus sp*.

The existence of other top alternative modules may indicate evidence of genetic differentiation in the wings of *Amphitornus sp*. wherein, developmental interactions are considered to be the major contributor of such variation (Klingenberg 2009). Other possible factor is the existence of external factors such as environmental plasticity, which may affect the signaling pathways between modules and network interactions, and between set of

genes which may lead to a possible phenotypic variation between populations of *Amphitornus sp.* (West-Eberhard 2003). Accordingly, selection would act on wing shape to optimize flight characteristics, wherein, individual variation in shape may strongly be dependent on environmental condition (Benitez et al 2011). Variation between male and female may due to their different roles, wherein, male grasshoppers favor flight as mode of movement while females frequently hop (Gade 2002). The variation between the left and right hind wings may be a result from small perturbations in the developmental and processes that take place on the left and right side of the body (Klingenberg & Zaklan 2000; Klingenberg et al 2001).

Table 3

Computed γ- VALUE and P-value for the left and right fore- and hind wings of both male and female *Amphitornus sp.* Only the top three (3) best fit models are tabulated.

Gender	Wing	Model	Rank	γ- value	P-value
		Hind wing			
		7	1	0.17265	1
	Left	4	2	0.18794	1
		5	3	0.20267	1
Male		7	1	0.13902	1
	Right	3	2	0.17299	1
		5	3	0.19976	1
		7	1	0.13712	1
	Left	10	2	0.17817	1
		3	3	0.17918	1
Female		7	1	0.16264	1
	Right	4	2	0.19645	1
		10	3	0.20608	1
		Forewing			
		5	1	0.43876	1
	Left	6	2	0.45032	1
		4	3	0.4512	1
Male		5	1	0.32953	1
	Right	2	2	0.34045	1
		6	3	0.35465	1
		5	1	0.34262	1
	Left	2	2	0.34919	1
		6	3	0.35745	1
Female		5	1	0.27721	1
	Right	2	2	0.28112	1
		6	3	0.28639	1

Results also confirmed a number of studies suggesting that compartments of the wings are autonomous units or morphological variation (Cavicchi et al 1991; Zimmerman et al 2000; Torres et al 2010; Tabugo et al 2011). Each may be considered as modules as "morphogenetic field" (Gilbert et al 1996; Klingenberg & Zaklan 2000; Wilkins 2002) that are distinct from other kind of modules in that they are morphological units with clear spatial boundaries. Therefore, each module can evolve independently, at least to some

extent, without disrupting any function at the level of the whole organism (Klingenberg et al 2001; Klingenberg 2008; Torres et al 2010; Tabugo et al 2011).

Conclusion. By using the morphometric data of the fore- and hind wings of *Amphitornus sp.*, the concept of modularity was studied. TPSUtil, TPSdig2 and, Modularity and Integration tool (MINT ver 1.5) softwares used in this study were useful in understanding the phenomenon. Results suggest that compartments in the wings can be considered as autonomous units of morphological variation that may correspond to domains of gene expression. That the forewing is partitioned into 4 developmental modules: (1) first module is bounded by the anterior margin of the wing and subcosta, (2) bounded by subcosta and media posterior, (3) bounded by media posterior and anal vein, (4) bounded by anal vein and posterior margin. The hind wings comprise of 3 developmental modules: (1) bounded by Costa and Cubitus 1, (2) bounded by Cubitus 1 and Cubitus 2 and (3) bounded by Cubitus 2 and posterior margin. The study has shown that each module is bounded by their main veins. Fair consistency in the number and patterns of the hypothesized developmental modules imply that the wings of *Amphitornus sp.*, are highly conserved.

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