Morphometric Analysis of Some Nymphalidae Butterflies (Rhopalocera) from the Bannerghatta Biological Park (BBP), Bengaluru, Karnataka

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Abstract: The current study was carried out to delineate the interrelationship of Nymphalidae (brush-footed butterflies) viz., *Ariadne merione, Danaus genutia, Europa core, Phalanta phalantha* and *Tirumala limiace* in and around Bannerghatta Biological Park (BBP), Bengaluru, Karnataka. To understand morphometrics of Nymphalidae, measurement of the body, proboscis, antenna, wings, and legs of these butterflies was carried out and analyzed. Principal Component Analysis (PCA) was performed for nine variables to examine the principle variable components among the species. The first Principal Component accounted for 84.70% between females and 87.30% between males, followed by the second PCA. Thus, we conclude that morphometric analysis of the five species revealed the importance of interspecies descriptions in characterizing the butterfly species and would also help in conserving the species.

Keywords: Antenna, Body, Brush-footed butterflies, Legs, Morphometry, Proboscis, Wings

A rich heritage of biological diversity is seen in India, consisting of over 89,000 species of organisms and 45,000 plants in very diverse bio-geographical regions (Mudgal and Hajra 1999). Butterflies are arguably the best-loved group of invertebrates and have been a source of inspiration for generations of natural historians and scientists (Wahlberg et al 2005). The butterfly family Nymphalidae approximately contains 6000 species, placed into 542 genera, distributed worldwide. Currently, these genera are put into 12 subfamilies and 40 tribes. These are generally average-sized to large butterflies; some are smaller in size. The butterflies are brightly coloured from above, but their underwings are dull in colour. The majority of the subfamilies species has a reduced pair of forelegs except for the subfamily Libytheinae and holds their iridescent wings flat when at rest. They are also referred to as brush-footed butterflies or four-footed butterflies and all other butterflies have three pairs. The reason is the foreleg pair is reduced as compared to other legs.

The classification of butterflies and skippers is extensively based on the morphology of adults for close to 250 years (Ackery et al 1999). Regardless of the extensive homoplasy in these morphologically variable insects (Kjer 2004). However, character's effectiveness from juvenile stages has long been acknowledged for species analysis (Freitas et al 2001), but they have only recently been automatically incorporated in a phylogenetic context (Penz and Peggie 2003, Freitas and Brown 2004). Generally, butterflies' classification is based on morphometric characters which body, proboscis, antenna, wings, and legs are mainly dependent on body length, proboscis, antenna, wings, and legs. Henceforth, morphometric analysis generally reveals the interrelationship between the various features such as length, leg spinnerets, and other body parts. Therefore, it is a reliable technique for recognizing the degree of reproduction maturity without sacrificing the animal (Ramakrishnaiah et al 2018). Morphological characteristics of various butterflies have certain connections with their evolution environment and natural selection (Azrizal-Wahid et al 2015). In this regard, only beneficial characters for survival are usually retained, while those not beneficial characteristics are lost. The lost features often serve have taxonomic keys in species identification and characterization (Pollard and Yates 1994). As a result, a revision of the current status of butterflies is required to verify the accuracy of taxonomic keys butterfly identification and conservation. Therefore, in the present study, a morphometric approach applying PCA was carried out to give the complete description of the variation in body length, proboscis, antenna, wings, and legs for five species belonging to the Nymphalidae family from Bannerghatta biological park, Bengaluru, Karnataka.

MATERIAL AND METHODS

Butterflies were collected using the standard insect (butterfly) nets or using the ground hand collection methods. The specimens were collected in a live insect box and taken to the laboratory. The work was conducted in the Bannerghatta Biological Park (Butterfly Park), Bengaluru and permission number for the present work is No. A3/BBP/Research Work/2018-19. The identification of the species was accomplished based on the morphology as presented in Kunte (2000) and Butterflies of India, v. 3.17. Indian Foundation for Butterflies (Website reference: http://www.ifoundbutterflies.org/tx/8-Nymphalidae), along with the support from the staff of Bannerghatta Biological Park. The morphometric measurements were carried out using the electronic digital Vernier calipers in mm scale, and the data were analyzed for mean ± standard deviation (n=10). Principal Component Analysis (PCA) was performed using 'ClustVis', a web tool for visualizing clustering multivariate data.

RESULTS AND DISCUSSION

Five species viz**.,** *Ariadne merione, Danaus genutia, Europa core,* and *Phalanta phalantha Tirumala limiace* (Fig. 1 and Table 1), collected from in and around Bannerghatta

Table 1. Morphological characteristics and morphometrics of adult nymphalid butterflies

Scientific names	Character Code (CC)	*Ariadne merione* (Mean ± SD)	*Danaus genutia* (Mean ± SD)	*Euploea core* (Mean ± SD)	*Phalanya phalantha* (Mean ± SD)	*Tirumala limiace* (Mean ± SD)
Antenna	-	Reddish-brown to brownish ochraceous club-shaped antennae small in size	Black Club shaped antennae	Black to Dark brown Club shaped antennae	Brownish black to Reddish-brown club-shaped antennae	Black Club shaped antennae
Legs	-	Three sets of Brownish black legs with non-functioning forelegs	Three sets of black legs with non-functioning forelegs with white dots/stripes.	Three sets of dark brown/black legs with sparsely spotted with white	Three sets of Brownish black to reddish-brown legs with sparsely dotted with white	Three sets of black legs with non-functioning forelegs with white spots/stripes.
Abdomen / body	-	Brownish ochraceous abdomen and body	Black with bluish-white semihyaline spots, Tawny abdomen.	Black with bluish-white semihyaline spots with glossy black and brown underside	Bright yellowish ochreous body and abdomen with a brown underside.	Black with bluish-white semihyaline spots & streaks, the abdomen is brownish-black
Body length (Head+abdomen)	BL	M:26.214 ± 0.02 F:16.584 ± 0.05	M:26.214 ± 0.03 F:26.692 ± 0.03	M:28.922 ± 0.01 F:29.830 ± 0.04	M:17.720 ± 0.05 F:19.001 ± 0.02	M:29.072 ± 0.05 F:29.658 ± 0.04
Abdomen length	AL	M:8.416 ± 0.03 F:8.484 ± 0.04	M:15.680 ± 0.02 F:15.850 ± 0.03	M:17.698 ± 0.04 F:18.750 ± 0.05	M:9.210 ± 0.05 F:9.370 ± 0.03	M:16.836 ± 0.03 F:17.174 ± 0.02
Abdomen width	AW	M:2.310 ± 0.02 F:3.290 ± 0.04	M:3.634 ± 0.03 F:4.108 ± 0.03	M:3.250 ± 0.01 F:3.307 ± 0.04	M:2.790 ± 0.05 F:3.070 ± 0.05	M:3.684 ± 0.01 F:3.990 ± 0.02
Probosis length	PL	M:8.975 ± 0.02 F:8.824 ± 0.05	M:11.101 ± 0.02 F:11.663 ± 0.03	M:11.720 ± 0.02 F:13.32 ± 0.03	M:9.210 ± 0.04 F:9.655 ± 0.03	M:11.542 ± 0.01 F:12.235 ± 0.02
Antenna length	AnL	M:11.110 ± 0.04 F:11.145 ± 0.03	M:13.778 ± 0.05 F:13.900 ± 0.02	M:14.146 ± 0.01 F:15.457 ± 0.02	M:11.890 ± 0.02 F:12.420 ± 0.03	M:13.470 ± 0.02 F:13.832 ± 0.03
Wings	W	M: FW L:26.050 ± 0.04 W:15.360 ± 0.05 HW L:21.966 ± 0.01 W:19.130 ± 0.04	M: FW L:38.960 ± 0.05 W:24.101 ± 0.01 HW L:28.042 ± 0.02 W:24.360 ± 0.03	M: FW L:41.150 ± 0.01 W:23.880 ± 0.03 HW L:28.384 ± 0.02 W:26.476 ± 0.01	M: FW L:24.860 ± 0.01 W:15.500 ± 0.01 HW L:18.460 ± 0.02 W:17.870 ± 0.03	M: FW L:41.598 ± 0.01 W:24.068 ± 0.02 HW L:30.530 ± 0.03 W:25.272 ± 0.01
		F: FW L:26.098 ± 0.04 W:16.418 ± 0.05 HW L:21.778 ± 0.05 W:16.418 ± 0.03	F: FW L:39.654 ± 0.01 W:24.088 ± 0.03 HW L:28.382 ± 0.03 W:24.896 ± 0.04	F: FW L:41.660 ± 0.01 W:24.352 ± 0.02 HW L:29.490 ± 0.03 W:27.452 ± 0.04	F: FW L:27.860 ± 0.01 W:16.030 ± 0.02 HW L:18.590 ± 0.03 W:18.330 ± 0.01	F: FW L:42.686 ± 0.04 W:24.894 ± 0.05 HW L:29.792 ± 0.01 W:25.676 ± 0.02
Leg 1	L1	M:5.880 ± 0.01 F:6.750 ± 0.02	M:5.760 ± 0.01 F:5.985 ± 0.03	M:7.220 ± 0.02 F:7.230 ± 0.03	M:5.590 ± 0.01 F:8.030 ± 0.04	M:6.306 ± 0.02 F:6.773 ± 0.03
Leg 2	L2	M:11.112 ± 0.02 F:11.330 ± 0.04	M:13.620 ± 0.03 F:14.090 ± 0.02	M:15.375 ± 0.01 F:15.116 ± 0.02	M:11.820 ± 0.02 F:12.520 ± 0.03	M:14.245 ± 0.04 F:14.673 ± 0.05
Leg 3	L3	M:11.930 ± 0.03 F:11.980 ± 0.02	M:14.010 ± 0.04 F:14.330 ± 0.03	M:15.120 ± 0.05 F:15.326 ± 0.05	M:11.310 ± 0.02 F:11.990 ± 0.04	M:14.429 ± 0.05 F:14.649 ± 0.04

CC= Character code, M= Male, F= Female, FW= Fore wing, HW= Hind wing, L/W= Length/Width

Biological Park, Bengaluru, Karnataka. The measurements (in mm) and description of the morphometrics of the five species studied are given in Table 1.

***Ariadne marione*:** The female body length represents 16.584±0.05 mm and males presents 16.572±0.05 mm; females are generally larger than males. The main host plant for this butterfly is *Ricinus communis*.

Danaus genutia: The body length of the female represents 26.692±0.03 mm and males presents 26.214±0.03 mm; females are generally larger than males. The main host plants for this butterfly are *Asclepias curassavica* and *Ceropegia intermedia*.

Euploea core: The body length of the female represents 29.830±0.04 mm and males presents 28.922±0.04 mm; females are generally larger than males. The main host plants for this butterfly are *Nerium oleander* and *Icnocarpus fruitescens*.

Phalanta phalantha: The female body length represents 19.001±0.02 mm and males presents 17.720±0.02 mm; females are generally larger than males. The main host plants for this butterfly are *Flacourtia montana* and *Salix tetrasperma*.

Tirumala limiace: The body length of the female represents 29.658±0.04 mm, and males presents 29 females are generally larger than males. The main host plant for this butterfly is *Wattakaka volubilis*. The morphometric lengths of different traits such as wingspan, body length, forewing length, forewing width, hindwing length, hindwing width and basal length of *Eurema* species showed the variations in wing and body size that can be used for taxonomic delineation (Azrizal-Wahid et al 2015). Accordingly, the present study characterizes the phenotypes based on metric properties and typifies differences by measuring the body and leg parts, emphasizing the size assessment. The significant phenotypic variations observed in the length of the total body, abdomen, proboscis, antenna, wings, and legs between the five species studied were brought out. Understanding the mechanics of evolution, its cause, and consequences on the phenotypic variation are significant (Miner et al 2005). Further, the data were tested for normal distribution and the Principal Component Analysis using a correlation matrix and varimax rotation. PCA method adopted herein is used as a dimension reducing technique (Foottit and Mackauer 1990) to investigate morphological variations. PCA is specifically designed to analyze a set of correlated variables without the prior assumption of multiple groups and helps in the minimum selection of the parameters for correct groupings (Humphries et al 1981). In the present study, PCA analysis showed that a clear separation of the two morphs was possible along with PCI, which explains 84.70%

for females and 87.30% for males of the variance. Additionally, clustering is one of the foremost techniques of multivariate analyses (Kettenring 2006). In the current study
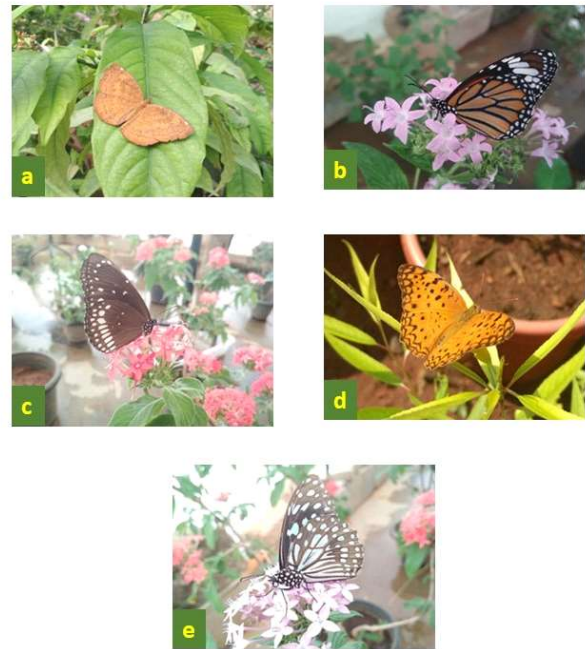

Fig. 1. Photographs of studied Nymphalid butterflies a. *Ariadne merione*, b. *Danaus genutia*, c. *Europa core*, d. *Phalanta phalantha* and e. *Tirumala limiace*
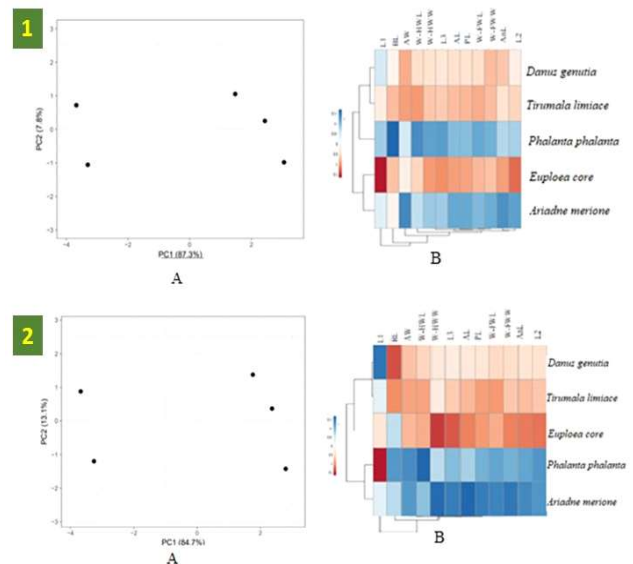

Fig. 2. 1A) Principle component analysis depicting the percentage of principle variables in males. 1B) Heat map morphological characters relationship between the Nimphalidae species (Males). 2A) Principle component analysis depicting the percentage of principle variables in females. 2B) Heat map morphological characters relationship between the Nimphalidae species (Females)

cluster, overall morphological characters were represented in the form of a scatter plot based on PCA (Fig. 2). In conclusion, the identification of Nymphalidae species based on morphological characteristics can be made confidently. Each species possesses unique features that can aid in its identification and conservation efforts.

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