Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2022; 10(5): 30-34

Received: 09-06-2022
Accepted: 12-07-2022

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A study of morphometric traits and length-length relationships of the butterfly, *Melanitis phedima* (Lepidoptera: Nymphalidae)

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DOI: https://doi.org/10.22271/j.ento.2022.v10.i5a.9047

Abstract
The present study deals with the investigation of the morphometric traits including morphometric lengths and length-length relationships (LLRs) of the dark evening brown butterfly, *Melanitis phedima* (Lepidoptera: Nymphalidae). Butterflies were sampled randomly from the Rajshahi University campus, Bangladesh. The pictures of butterflies were taken with a DSLR camera (Canon 750D), and a total of 14 different morphometric lengths from the body, antenna, wings (6 parameters), legs (2 parameters) and wing venation (4 parameters) were measured using ImageJ software (1.52a). The minimum and maximum body length (BL) were recorded at 19.15 mm and 24.85 mm from *M. phedima* respectively, whereas the mean body length was 22.02±1.45 mm. This study found FL (Foreleg) as the short trait at 12.90 mm, while FWBA (Forewing Base-Apex) was the long trait at 45.86 mm. The length of FWBA was 88.75% longer than BL and HWAT (Hindwing Apex-Tornus) was 40.25% longer. On the other hand, lengths of antennae length (AL), total foreleg (TFL) and total hindleg (THL) were shorter compared to BL. According to the coefficient of determination (r^2), all values were ≥ 0.75 which indicates the positive relationship between BL and selected 13 traits and the best-fitted model of LLRs was BL *vs.* HWBA (Hindwing Base-Apex) among these equations (r^2= 0.89). The allometric coefficient '*b*' indicated the isometric growth for BL *vs.* HWBT and BL *vs.* HWAT, and negative allometric growth for BL *vs.* FWBA ($b < 1$), and the rest 10 traits showed positive allometric growth ($b > 1$). The results would be very effective for the study of butterfly taxonomy as a great tool in species and subspecies identification.

Keywords: *Melanitis phedima*, morphometric traits, length-length relationships (LLRs), linear regression, coefficient of determination (r^2), growth patterns

Introduction
One of the largest families of the order Lepidoptera is the Nymphalidae, which includes over 6,000 species of butterflies worldwide. The nymphalids are also known as four-footed or brush-footed butterflies because they can stand on only four legs while the other two are curled up [1]. *Melanitis phedima* belongs to the family Nymphalidae was first recorded in Indonesia by Cramer (1780), and the dark evening brown butterfly (*M. phedima bela*) was recorded in Bangladesh by Alam [2]. Females' (*M. phedima*) forewings have a white spot that is round to ovoid in shape between the veins 3-4 and 4-5, lower 1/2 surrounded with an orange band on the inside, an inner orange and outer dark brown band are bordered the veins 5-7 [2]. In both sexes, the hindwings have a small white spot between veins 2-3 dorsally; the apical area is usually paler than the basal area [2].

Morphometric features are considered to be the most straightforward and reliable way of identifying specimens, a process known as morphological systematics [3], and developing statistical relationships between them is crucial for taxonomic work [4], and taxonomic status [5]. These characteristics aid in the identification and classification of species. Morphometric approaches are used to assess size, form, and the relationship between the two (allometry), and shape was an abstraction before the so-called "revolution" [6], a residue after scaling for size, and it was impossible to perceive the "residue." The substitution of initial variables indicating a distance between two anatomical sites with their coordinates, as well as the subsequent visualization techniques, constituted a huge step forward in the direct study of shapes.

The growth of image processing techniques aided the transition from traditional morphometrics to more complicated geometric functions. Today, landmark methods are utilized alongside "outline methods" [6], and various textures and surface patterning techniques [7].

The most visible aspect of an organism's phenotype is its morphological shape. It establishes a strong link between a species' genotype and its surroundings [8]. Environmental-cause variations differ among individuals of the same species and are dependent on the individual's ability to defend against environmental challenges [9].

Morphological variations in moths and butterflies are mostly associated with environmental effects [10]. Variation in body size is said to be a natural feature of populations and has important implications for gaining a better knowledge of population dynamics and stability [11-12] of an ecological system. Individuals' well-being can be determined using the correlations between length-length and length-weight, as well as probable variances between the same species in various areas [13].

In fisheries management, length-length relationships (LLRs) and length-weight relationships (LWRs) are crucial for growth studies among species [14], but length-length and length-weight relationships are still rare in the morphometric study of insects.

In Bangladesh, very few morphometric studies on insects were reported [15-17]. An attempt was taken to establish a detailed picture of the variations in lengths (body, antenna, wings, and legs), LLRs, and growth patterns of *Melanitis phedima* from Rajshahi, Bangladesh.

Materials and Methods

Study site

To morphometric analysis, the dark evening brown butterflies, *Melanitis phedima* (Cramer, 1780) (Lepidoptera: Nymphalidae) were collected from Rajshahi University Campus, Rajshahi, Bangladesh. Rajshahi University is on a 305-hectare campus in Motihar, 3 kilometers from the Rajshahi city center. The geographical distribution of Rajshahi University is 24.370° latitude and 88.637° longitude.

Sampling method

The random sampling technique was used to acquire the samples. Sweep net and hand-picking methods were utilized to collect specimens. The specimens were narcotized with menthol (naphthalene) crystals and then air dried for preservation at the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi.

Photography

For morphometric measures, photographs of the preserved specimens were taken with a DSLR camera (Canon 750D). Photographs are taken by placing different parts of the butterfly on a scale according to Mahdi *et al.* [17].

Morphometric measurements

For the morphometric study, captured pictures were measured using Image J software (l.52a). Fourteen traits of *M. phedima* (n= 40) were taken into consideration for morphometric analysis, where 4 traits belonged to wing venation (Fig 1). Traits that were taken into consideration for morphometric analysis were shown below belong with abbreviation and description (Table 1).

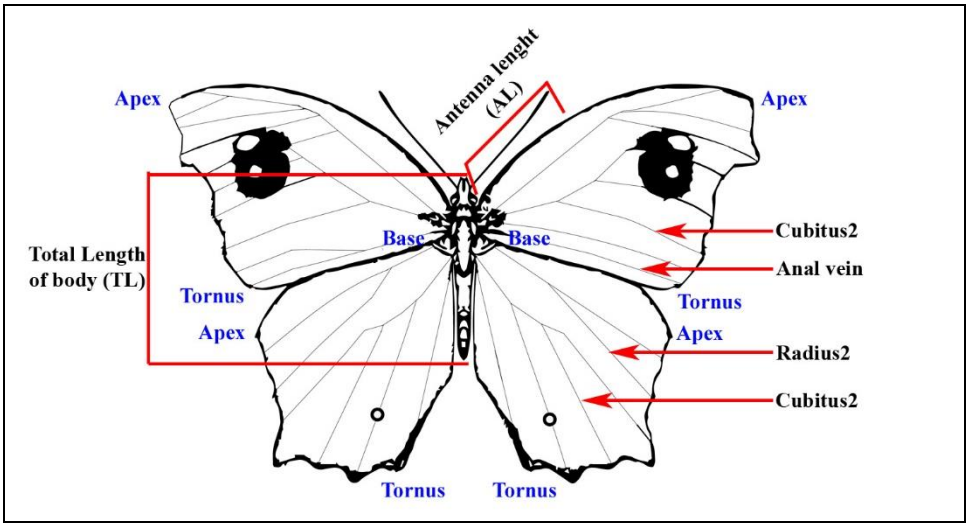

Fig 1: Morphology and measurement sites of different morphometric traits of *Melanitis phedima* (Cramer, 1780).

Data analysis

MS Excel 2019 was used to perform the descriptive statistics. The length-length relationship (LLR) was calculated using the formula $W = a \times L^b$, where W represents the total length (mm), L represents 13 different lengths (mm), 'a' represents regression intercept, and 'b' represents regression coefficient (slope). The LLRs' regression parameters a and b were computed using natural logarithms and linear regression analysis: $\ln(W) = \ln(a) + b \ln(L)$. In addition, the coefficient of determination (r^2) and the 95 percent confidence limit (CL) of a and b were calculated. The r^2 was utilized as a quality indicator for the linear regressions [18]. According to Sokal and Rohlf [19], a t-test was used to determine significant variations from the isometric value (b = 1.0) for the length-length relationship, using the equation ts=(b1)/ Sb, where ts is the t-test value, b is the slope, and Sb is the standard error of the slope (b). The deviation of the b value from the theoretical isometric value was used to determine whether the growth patterns were positive (b > isometric value) or negative (b < isometric value). Based on the maximum value of determination r^2, the best model for LLRs was chosen. The Graph Pad Prism 6.5 software was used for statistical analysis, and the results were declared significant at 5% (p<0.05).

Table 1: Trait abbreviation and description

Trait Abbreviation	Description
TL	Total Length
AL	Antenna Length
FWBA	Forewing Base-Apex
FWBT	Forewing Base-Tornus
FWAT	Forewing Apex-Tornus
HWBA	Hindwing Base-Apex
HWBT	Hindwing Base-Tornus
HWAT	Hindwing Apex-Tornus
TFL	Total Foreleg
THL	Total Hindleg
VFCU2	Vein Forewing Cubitus2
VFA	Vein Forewing Anal
VHR2	Vein Hindwing Radius2
VHCU2	Vein Hindwing Cubitus2

Results

During this study period, 40 butterflies belonging to *Melanitis phedima* were collected from different spots of Rajshahi University Campus, Rajshahi, Bangladesh. Morphometric measurement of different parameters (fourteen lengths), descriptive statistics on length-length relationships, and other regression analyses are shown in Tables 2, 3, and 4.

In this study, 14 traits of *M. phedima* were taken into consideration for morphometric analysis, where FL (Foreleg) was found as the short trait, while FWBA was the long trait, and the recorded length was 12.90 mm and 45.86 mm respectively. The minimum and maximum body length (BL) were observed at 19.15 mm and 24.85 mm respectively from *M. phedima*, while the mean body length was 22.02±1.45 mm. The average length of AL was 14.39±0.64 mm, and 13.03 mm and 15.83 mm were recorded as the minimum and maximum lengths respectively (Fig 2, Table 2).

In the forewing, the mean length of the three traits (FWBA, FWBT, and FWAT) was recorded as follows: 41.56±1.55 mm, 26.64±0.64 mm, and 25.26±1.22 mm successively, and in the hindwing, 30.30±0.79 mm (HWBA), 28.24±1.19 mm (HWBT), and 30.88±1.21 mm (HWAT) lengths were examined consecutively. The length of the foreleg (14.47±0.83 mm) was found as slightly longer than the hindleg (14.05±0.40 mm) (Fig 2, Table 2).

In the present study, cubitus2 (VFCU2) and anal vein (VFA) of the forewing, and radius2 (HVRS) and cubitus2 (VHCU2) vein of the hindwing were taken under consideration to study wing-venation, where VFCU2 (28.24±0.88) was detected longer than the VHCU2 (24.22±0.41) (Fig 2, Table 2). The average lengths of the VFA and HVR2 were 25.50±0.65 and 27.32±0.36 gradually. In percentage, the length of the 13 parameters compared to the body length (BL) was showed as follows: 188.75% (FWBA) > 140.25% (HWAT) > 137.61% (HWBA) > 128.26% (HWBT, VFCU2) > 124.08% (VHR2) > 120.99% (FWBT) > 115.81% (VFA) > 114.72% (FWAT) >109.99% (VHCU2) > 65.72% (TFL) > 65.35% (AL) > 63.81% (THL) (Table 2).

The coefficient of determination (r^2) value was ≥ 0.75 which indicates the positive relationship between body length and selected 13 traits. The best-fitted model among the 13 equations for the *M. phedima* was BL *vs.* HWBA (r^2 = 0.89) (Table 3). The allometric coefficient '*b*' of the LLRs was close to the isometric value in BL *vs.* HWBT and BL *vs.* HWAT, and it showed negative allometric growth in BL *vs.* FWBA (b < 1). The positive allometric growth was found in BL *vs.* AL, BL *vs.* FWBT, BL *vs.* FWAT, BL *vs.* HWBA, BL *vs.* TFL, BL *vs.* THL, BL *vs.* VFCU2, BL *vs.* VFA, BL *vs.* VHR2 and BL *vs.* VHCU2 (b > 1) (Table 4).

Table 2: Morphometric measurement of different parameters (fourteen lengths) of the dark evening brown butterfly, *Melanitis phedima* (n= 40) (Min, minimum; Max, maximum; SD, standard deviation)

Traits	Min (mm)	Max (mm)	Mean (±SD)	BL (%)
BL	19.15	24.85	22.02±1.45	-
AL	13.03	15.83	14.39±0.64	65.35
FWBA	38.14	45.86	41.56±1.55	188.75
FWBT	25.26	28.05	26.64±0.64	120.99
FWAT	22.76	29.01	25.26±1.22	114.72
HWBA	28.84	31.76	30.30±0.79	137.61
HWBT	25.76	31.10	28.24±1.19	128.26
HWAT	28.86	33.29	30.88±1.21	140.25
TFL	12.90	16.71	14.47±0.83	65.72
THL	13.22	14.66	14.05±0.40	63.81
VFCU2	26.57	31.43	28.24±0.88	128.26
VFA	24.11	27.98	25.50±0.65	115.81
VHR2	26.57	28.19	27.32±0.36	124.08
VHCU2	23.54	25.01	24.22±0.41	109.99

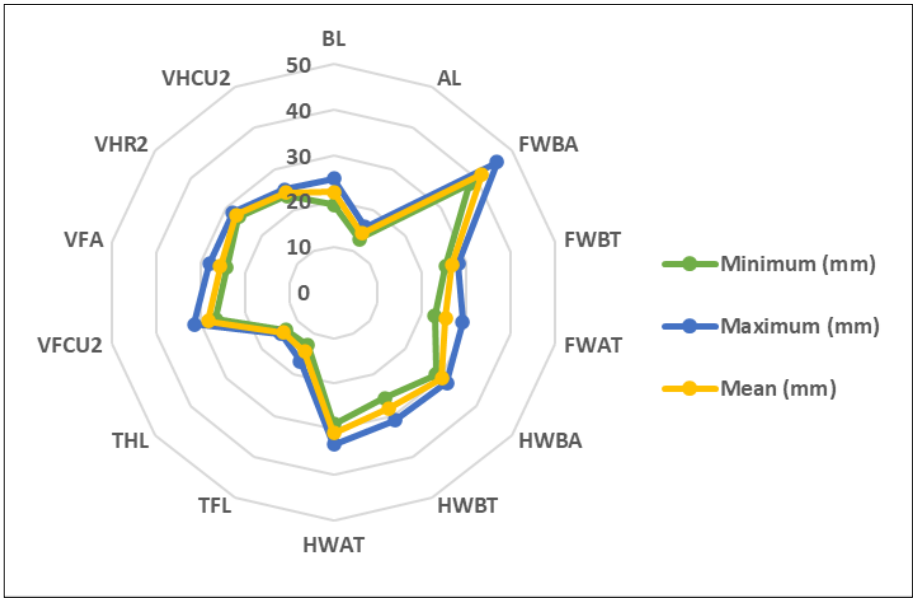

Fig 2: Minimum, Maximum and Average length of the different morphometric traits of *Melanitis phedima* (Cramer, 1780).

Table 3: Descriptive statistics and estimated parameters of of the dark evening brown butterfly, *Melanitis phedima* (n= 40) (See table 1 for abbreviations; *a*, intercept; *b*, slope; CI, confidence intervals; r^2, coefficient of determination)

Equation	Regression Parameters		95% Cl of *a*		95% Cl of *b*		r^2
	a	*b*	Lower	Upper	Lower	Upper	
BL = *a* + *b* × AL	-7.03	2.02	-11.87	-2.18	1.68	2.36	0.80
BL = *a* + *b* × FWBA	-11.81	0.81	-18.20	-5.42	0.66	0.97	0.75
BL = *a* + *b* × FWBT	-32.42	2.04	-41.14	-23.69	1.72	2.37	0.81
BL = *a* + *b* × FWAT	-4.05	1.03	-8.99	0.89	0.84	1.23	0.75
BL = *a* + *b* × HWBA	-30.76	1.74	-36.84	-24.69	1.54	1.94	0.89
BL = *a* + *b* × HWBT	-8.32	1.07	-13.77	-2.87	0.88	1.27	0.77
BL = *a* + *b* × HWAT	-11.62	1.09	-16.84	-6.40	0.92	1.26	0.82
BL = *a* + *b* × TFL	-0.33	1.54	-4.16	3.50	1.28	1.81	0.79
BL = *a* + *b* × THL	-24.08	3.28	-30.98	-17.17	2.79	3.77	0.83
BL = *a* + *b* × VFCU2	-18.17	1.42	-25.76	10.58	1.16	1.69	0.75
BL = *a* + *b* × VFA	-27.54	1.94	-36.57	-18.50	1.59	2.30	0.77
BL = *a* + *b* × VHR2	-76.44	3.60	-92.06	-60.82	3.03	4.18	0.81
BL = *a* + *b* × VHCU2	-54.72	3.17	-67.11	-42.34	2.66	3.68	0.81

Table 4. Growth patterns of *M. phedima* (n= 40)

Equations	t_s- value	Growth pattern
BL = *a* + *b* × AL	6.14	A+
BL = *a* + *b* × FWBA	-2.45	A-
BL = *a* + *b* × FWBT	6.45	A+
BL = *a* + *b* × FWAT	0.33	A+
BL = *a* + *b* × HWBA	7.49	A+
BL = *a* + *b* × HWBT	0.78	I
BL = *a* + *b* × HWAT	1.07	I
BL = *a* + *b* × TFL	4.17	A+
BL = *a* + *b* × THL	9.40	A+
BL = *a* + *b*× VFCU2	3.19	A+
BL = *a* + *b* × VFA	5.39	A+
BL = *a* + *b* × VHR2	9.22	A+
BL = *a* + *b* ×VHCU2	8.59	A+

t_s**:** t- test value; I: isometric; +A positive allometric; -A: Negative allometric

Discussions

Alpha taxonomy, the science of properly distinguishing and characterizing species and higher taxa, relies heavily on morphometrics data. Many butterfly species have striking resemblances, which, when combined with individual variation and morphological plasticity, makes identification challenging [20-21]. To distinguish between taxa, identification keys use objective and plainly evident characteristics as well as minute differences [22-25]. In butterflies, cases of morphologically similar species that are difficult to identify are common, and traditional morphometry or geometric morphometry has been used to reliably aid in their identification [26-28].

The present study was conducted on the morphometric analysis of *Melanitis phedima* (Cramer, 1780) belonging to the family Nymphalidae, and due to a dearth of literature data on this butterfly, present findings were compared with the following findings: Akand *et al.* [15] on Lycaenidae, Mahdi *et al.* [16-17], on Lycaenidae and Pieridae, Dhungel and Otaki [29] on Nymphalidae, Roy *et al.* [30], Goonesekera *et al.* [31], and Wells *et al.* [32] on Nymphalidae. In this study, the average body length of *M. phedima* was recorded as 22.02±1.45 mm, while the mean body length was 16.10 2.55 mm in *Eurema hecabe* (L.), 9.942±0.98 mm in *Chilades pandava*, 9.116±0.42 mm in *Chilades lajus* was recorded by Mahdi *et al.* [16-17]. The antenna length was recorded as 4.39±0.64 mm in this study, but the antenna length was recorded as 7.19±0.70 mm in *Eurema hecabe* (L.) and 10.55 mm in *Arhopala pseudocentaurus* by previous researchers [15, 17].

The forewing was examined longer than the hindwing in terms of the average length of FWBA (41.56±1.55 mm) and HWBA (30.30±0.79 mm) in this study. This finding was supported by Mahdi *et al.* [16-17], who showed that the average length of FWBA was larger than the HWBA for 3 butterflies namely, *Eurema hecabe* (L.), *Chilades pandava*, and *Chilades lajus*. Akand *et al.* [15] reported that the mean length of the forewing was larger than the hindwing when they worked on 44 species belonging to the Lycaenidae. Wells *et al.* [32] worked on f *Speyeria diana* (Nymphalidae) and found a significant difference between wing shape in male and female specimens and concluded that male forewings were narrower and more elongated which possibly made them suitable for patrolling behavior. Several authors revealed in their works on migratory populations of the monarch butterfly, *Danaus plexippus* L. (Nymphalidae) has narrower, more elongated forewings for stronger flight than non-migratory individuals, which have smaller, rounder forewings [33-34]. Based on average length, the foreleg (14.47±0.83 mm) was recorded as slightly longer than the hindleg (14.05±0.40 mm), and a similar result was recorded in *Eurema hecabe* (L.) by Mahdi *et al.* [17].

In the present study, All LLRs revealed a positive relationship between body length and selected 13 traits ($r^2 \geq 0.75$), and the best-fitted model among the 13 equations for the *M. phedima* was BL *vs.* HWBA ($r^2 = 0.89$). Mahdi *et al.* [16-17] worked on *Eurema hecabe* (L.), *Chilades pandava*, and *Chilades lajus*, where they showed a positive relationship between body length with other selected traits ($r^2 \geq .88$, $r^2 \geq 0.66$, and $r^2 \geq 0.71$ respectively). They also established best-fitted model among the selected traits as follows: BL *vs.* HWBT ($r^2 = 0.91$ for *Chilades pandava*), BL *vs.* THL ($r^2 = 0.94$ for *Chilades lajus*), and BL *vs.* FL ($r^2 = 0.92$ for *Eurema hecabe*) [16-17]. The allometric coefficient *b* of the LLRs was close to the isometric value in BL vs. HWBT and BL vs. HWAT, which is similar to the findings (BL *vs.* HWAT in *Chilades pandava*, and BL vs. HWBT and BL vs. FWAT in *Chilades lajus*) by Mahdi *et al.* [16]. We also observed negative allometric growth in BL vs. FWBA ($b < 1$), and positive allometric growth in remaining traits compared to body length ($b > 1$) during the study.

Conclusion

Currently, there is no information on the morphometrics and length-length relationships of the dark evening brown butterfly, *M. phedima*. This study was undertaken to establish primary data on morphometrics and LLRs of this species, and future researchers may be able to use this data as a starting point.

Acknowledgements

Authors express gratefulness to Md. Yeamin Hossain, Professor, Department of Fisheries, University of Rajshahi for helping in data analysis and discussion.

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