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Shah HA Mahdi
Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh

Sujan Malo
Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh

Meherun Nesa
Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh

Md. Abdur Rahim
Institute of Environmental Science, University of Rajshahi, Rajshahi, Bangladesh

Corresponding Author:
Shah HA Mahdi
Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh

First report of morphometrics and length-length relationships of the common grass yellow butterfly, *Eurema hecabe* (L.) (Lepidoptera: Pieridae)

Shah HA Mahdi, Sujan Malo, Meherun Nesa and Md. Abdur Rahim

Abstract
The present investigation was conducted to study the morphometrics and length-length relationships (LLRs) of the Common Grass Yellow Butterfly, *Eurema hecabe* (L.) (Lepidoptera: Pieridiae). Samples were collected randomly from the Rajshahi University Campus (RUC), Bangladesh, and the pictures were taken with a DSLR camera (Canon 750D) and measured using ImageJ software (l.48v). Here, a total of 14 different morphometric lengths *viz*. total body, antenna, wings (fore- and hindwing) and legs (fore-, mid- and hindleg) were evaluated. Total length (TL) was recorded as a minimum of 11.51 mm and a maximum of 22.01 mm, whereas antenna length (AL) has differed from 5.78-8.68 mm. The highest morphometric mean was found in forewing base-apex (FWBA) as 21.45±2.21 mm, and the lowest mean of length was recorded in the hindleg (HL) as 6.21±0.67 mm. The FWBA was larger than the TL about 33% followed by the forewing base-tornus (FWBT), hindwing base-apex (HWBA), hindwing base tornus (HWBT) and forewing cubitus2 vein (C2V). According to co-efficient of determination (r^2) values, the best-fitted model of LLRs was TL *vs.* foreleg (FL) among 13 equations. The allometric coefficient '*b*' was suggested positive allometric growth. These works will be very useful for species recognition and taxonomic study of butterflies in the RUC as well as in Bangladesh.

Keywords: *Eurema hecabe* (L.), morphometric analysis, length-length relations (LLRs), linear regression, growth patterns

Introduction
The butterfly *Eurema hecabe* (L.) (Lepidoptera: Pieridiae) is commonly known as the common grass yellow butterfly and widely distributed in the tropics and subtropics such as Asia, Africa and Australia [1]. *E. hecabe* (L.) shows sexual dimorphism in the ground color of wings; females have paler yellow wings than males, and this species also shows seasonal polyphenism [2]. The butterfly *E. hecabe* (L.) consists of brown and yellow types, which are linked with expression of seasonal wing morphs and with ability for host-plant use [3]. This butterfly plays an essential role as pollinators and thus serves as a vital factor in fruit and crop production [4].

Morphometric analysis is one of the important tools for the identification of many groups of insects [5, 6]. Wing morphometrics also use as a tool in species identification of many flies [7]. Morphometric characteristics can play a vital role in determining animal systematic and growth variability [8]. Other important functions of morphometric characters in the research were compared the life cycle and morphological trends of populations across regions [9]. The significance of wing and body morphometry in selective six species of *Eurema* butterflies were studied in Peninsular Malaysia [6].

At the present time, the descriptive statistical analyses are characteristically applied for morphometric study [10]. The regression analysis of the parts of the body has exposed a significant element for determining the complex taxonomy [11]. The coefficient of determination (r^2) was also utilized as a sign of the quality of the linear regressions [12]. However, no study has yet done on the morphometric lengths, length-length relationships (LLRs) and growth patterns of *E. hecabe* (L.). In the present study, the morphometric dimension of the different parameters of the body, LLRs and growth patterns of the common grass yellow butterfly, *E. hecabe* (L.) were examined, and this report was the first time in Rajshahi District as well as Bangladesh.

2. Materials and Methods

2.1. Animals

The Common Grass Yellow Butterfly, *Eurema hecabe* (L.) was used in this study that belongs to the order Lepidoptera and family Pieridae. The butterfly was collected from bushland, open field, botanical garden, grassland, paddy field and mulberry garden of Rajshahi University Campus (RUC), Bangladesh. The RUC area is about 305 ha (753 acres) and located between 24.370°N 88.637°E.

2.2. Sampling and preservation

Eurema hecabe (L.) was collected randomly from RUC using a sweeping net and handpicking method. Collected butterflies were placed in a plastic container, and a little amount of chloroform soaked cotton bud was put inside. Samples were then transferred to Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi for preservation and study.

2.3. Photography

The photographs were taken using a DSLR camera (Canon 750D) for morphometric analysis of different parts of the butterfly's body (Fig. 1).

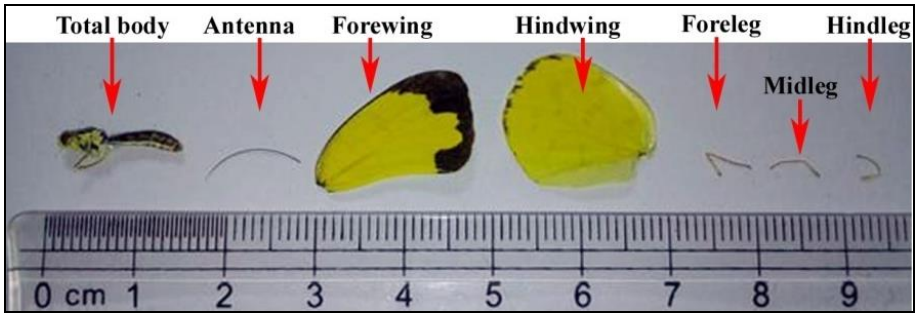

Fig 1: Morphology of different parts of the common grass yellow butterfly, *Eurema hecabe* (L.)

2.4. Morphometric analysis

For the morphometric study, captured pictures were measured using ImageJ software (l.48v). The fourteen measurement sites were calculated *viz*. total length (TL), antenna length (AL), forewing base-apex (FWBA), forewing base-tornus (FWBT), forewing apex-tornus (FWAT), hindwing base-apex (HWBA), hindwing base-tornus (HWBT), hindwing apex-tornus (HWAT), forewing radius2 vein (R2V), forewing cubitus2 vein (C2V), forewing anal vein (AV), foreleg (FL), midleg (ML) and hindleg (HL) (Fig. 1 and 2).

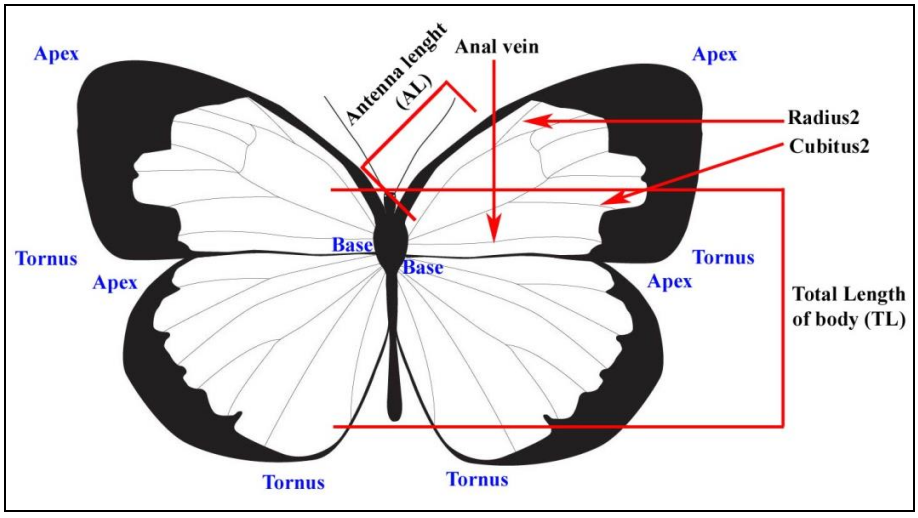

Fig 2: Measurement sites of the common grass yellow butterfly, *E. hecabe* (L.)

2.5. Data analysis

The descriptive statistics were performed by MS Excel 2013. The length-length relationship (LLR) was determined using the formula: $W = a \times L^b$, where the *W* is the total length (mm), *L* for 13 different lengths (mm), "*a*" the intercept of the regression and "*b*" is the regression co-efficient (slope). Regression parameters *a* and *b* of the LLRs were calculated through the linear regression analysis depending on natural logarithms: $\ln(W) = \ln(a) + b \ln(L)$.

Furthermore, 95% confidence limit (CL) of *a* and *b* and the co-efficient of determination (r^2) were estimated. The r^2 was used as an indicator of the quality of the linear regressions [12]. According to Sokal and Rohlf [13], a t-test was applied to determine significant differences from the isometric value (b = 1.0) for length-length relationship by the equation: $ts = (b-1)/Sb$, where *ts* is the *t*-test value, *b* the slope and *Sb* the standard error of the slope (*b*). To evaluate the growth patterns, either positive ($b >$ isometric value) or negative ($b <$ isometric value) allometric growth was indicated by the deviation of the *b* value from the theoretical isometric value. The best model for LLRs was chosen based on the maximum value of determination r^2.

Statistical analyses were done through the Graph Pad Prism 6.5 software, and these analyses were considered significant at 5% ($p < 0.05$).

3. Results and Discussion

Morphometric analysis of 14 parameters *Eurema hecabe* (L.) was evaluated **(**Table 1). We examined the total length (TL); antenna length (AL); three lengths from forewing: base-apex (FWBA), base-tornus (FWBT), apex-tornus (FWAT); three lengths from hindwing: base-apex (HWBA), base-tornus (HWBT), apex-tornus (HWAT); three lengths from forewing's vein: radius2 (R2V), cubitus2 (C2V), anal (AV); and three lengths from leg: foreleg (FL), midleg (ML) and hindleg (HL) (Table 1).

Table 1: Morphometric measurement of different parameters (fourteen lengths) of the common grass yellow butterfly, *Eurema hecabe* (n= 40)

Parameters (length)	Min (mm)	Max (mm)	Mean (± SD)	Median	% TL
Total Length (TL)	11.51	22.01	16.10 ± 2.55	15.89	
Antenna Length (AL)	5.78	8.68	7.19 ± 0.70	7.12	44.69
Forewing Base-Apex (FWBA)	17.53	26.10	21.45 ± 2.21	21.18	133.21
Forewing Base-Tornus (FWBT)	16.16	21.12	18.50 ± 1.27	18.8	114.92
Forewing Apex-Tornus (FWAT)	11.87	20.31	15.60 ± 1.90	15.29	96.88
Hindwing Base-Apex (HWBA)	15.78	19.92	17.81 ± 1.15	17.91	110.65
Hindwing Base-Tornus (HWBT)	12.81	19.98	16.30 ± 2.00	16.42	101.24
Hindwing Apex-Tornus (HWAT)	12.67	17.12	14.75 ± 1.17	14.57	91.61
Forewing Radius2 Vein (R2V)	13.17	17.03	15.37 ± 0.94	15.36	95.45
Forewing Cubitus2 Vein (C2V)	14.69	18.21	16.30 ± 0.91	16.12	101.24
Forewing Anal Vein (AV)	12.45	16.78	14.50 ± 1.08	14.19	90.08
Foreleg (FL)	5.76	8.37	6.91 ± 0.63	6.88	42.89
Midleg (ML)	4.77	7.71	6.28 ± 0.64	6.21	39.03
Hindleg (HL)	5.01	7.54	6.21 ± 0.67	6.09	38.60

Min, minimum; Max, maximum; SD, standard deviation;

In this study, the average TL was 16.10 ± 2.55 mm, and the minimum and maximum TLs were 11.51 and 22.01 mm respectively. The mean of AL was 7.19 ± 0.70 mm and the minimum to maximum length was 5.78 to 8.68 mm (Table 1). The length of the FWBA was the maximum length of all the parameters as 26.10 mm in this study. Among the three characters of the forewing, the lowest length was recorded in FWAT at 11.87 mm Furthermore, the morphometric length (mean) of hindwing was 17.81 ± 1.15, 16.30 ± 2.00 and 14.75 ± 1.17 mm for HWBA, HWBT and HWAT respectively (Table 1).

In addition, the average length of R2V, C2V and AV was recorded as 15.37 ± 0.94, 16.30 ± 0.91 and 14.50 ± 1.08 mm respectively. In the present study, the length of fore-, mid- and hindleg was calculated as 6.91 ± 0.63, 6.28 ± 0.64 and 6.21 ± 0.67 mm correspondingly (Table 1). The FWBA was larger than the TL about 33% followed by the forewing base-tornus (FWBT), hindwing base-apex (HWBA), hindwing base tornus (HWBT) and forewing cubitus2 vein (C2V). The length of the HL was only 38.60%, which was the lowest percentage compared to the TL (Table 1).

The regression parameters *a* and *b* of LLRs, their 95% confidence intervals of (*a* and *b*) and coefficients of determination (r^2) of the *E. hecabe* (L.) were determined (Table 2). The calculated allometric coefficient *b* ranged from a minimum of 1.1036 for TL *vs.* FWBA, to a maximum of 3.9016 for TL *vs.* FL. The mean value of *b* was 2.4521. All LLRs were significant ($p< 0.05$) with r^2 values ≥ 0.8777. Based on r^2 value, LLR by TL *vs.* FL was the best fitted model among 13 equations for the *E. hecabe* (L.). In term of growth type, the LLRs between 13 equations of length indicated positive allometric (A+) growth for the common grass yellow butterfly, *E. hecabe* (L.) (Table 3).

Table 2: Descriptive statistics and estimated parameters of the length-length relationship of the common grass yellow butterfly, *Eurema hecabe* (n= 40)

Equation	Regression Parameters		95% CI of a		95% CI of b		r^2
	a	*b*	Lower	Upper	Lower	Upper	
TL = a + b × AL	-8.9179	3.4768	-11.4349	-6.4010	3.1286	3.825	0.9149
TL = a + b × FWBA	-7.6264	1.1036	-9.9031	-5.3497	1.0006	1.2119	0.9220
TL = a + b × FWBT	-19.3297	1.9149	-22.994	-15.6655	1.7173	2.1125	0.9101
TL = a + b × FWAT	-3.9219	1.2836	-5.9925	-1.8512	1.1518	1.4152	0.9109
TL = a + b ×HWBA	-21.2634	2.0972	-25.2406	-17.2862	1.8749	2.3199	0.9052
TL = a + b ×HWBT	-3.3644	1.1940	-5.7676	-0.9612	1.0477	1.3404	0.8777
TL = a + b ×HWAT	-14.3754	2.0662	-17.6993	-11.0515	1.8415	2.2909	0.9012
TL = a + b × R2V	-22.9738	2.5424	-27.5893	-18.3582	2.2426	2.8421	0.8858
TL = a + b × C2V	-27.0199	2.6452	-31.7149	-22.3249	2.3576	2.9328	0.9012
TL = a + b ×AV	-16.434	2.2431	-19.8872	-12.9808	2.0057	2.4806	0.9058
TL = a + b × FL	-14.848	3.9016	-13.3678	-8.3281	3.5382	4.2649	0.9255
TL = a + b × ML	-7.8469	3.8103	-10.3913	-5.3025	3.4056	4.2132	0.9060
TL = a + b × HL	-6.2658	3.5986	-8.9782	-3.5534	3.1646	4.0326	0.8811

See table 1 for abbreviations; *a*, intercept; *b*, slope; CI, confidence intervals; r^2, co-efficient of determination

Table 3: Growth patterns of the common grass yellow butterfly, *Eurema hecabe* (n= 40)

Equation	t_s-value	Growth pattern
TL = a + b × AL	14.408	A+
TL = a + b × FWBA	2.040	A+
TL = a + b × FWBT	9.373	A+
TL = a + b × FWAT	4.356	A+
TL = a + b × HWBA	9.974	A+
TL = a + b ×HWBT	2.686	A+
TL = a + b × HWAT	9.614	A+
TL = a + b × R2V	10.415	A+
TL = a + b × C2V	11.581	A+
TL = a + b × AV	10.606	A+
TL = a + b × FL	16.173	A+
TL = a + b × ML	14.129	A+
TL = a + b × HL	12.125	A+

t_s: t-test value; A+: positive allometric

Due to lack of literature data on family Pieridae butterflies, it restrains to compare our results with other findings except the study by Mahdi *et al.* [14] and Akand *et al.* [15] on family Lycaenidae. Earlier, Akand *et al.* [15] reported the lengths of the forewing, hindwing, body and antenna of 44 species of butterflies under the two subfamilies of the family Lycaenidae. In this study, the mean of total length of the body was recorded as 16.10 ± 2.55 mm for *E. hecabe*, while the mean of the body length was recorded as average 9.942± 0.98 and 9.116 ± 0.42 mm for *Chilades pandava* and *C. lajus* respectively [14]. The maximum mean length was found in FWBA as 21.45 ± 2.21 mm and the minimum mean length was recorded as 6.21 ± 0.67 in HL in our study. However, the maximum average length was found in FWBA as 14.473± 1.86 and 12.791 ± 1.16 mm and the minimum average length was recorded in foreleg as 4.185 ± 0.59 and 3.727 ± 0.50 mm in *C. pandava* and *C. lajus* respectively [14].

All LLRs were highly correlated with TL, and they were compared with the available literature based on fish. It has earlier been reported the maximum r^2 value was 0.936 for TL *vs.* FL (fork length) of *A. mola* and 0.998 for TL *vs.* SL (standard length) of Cyprinid fishes [16, 17]. The allometric co-efficient (*b*) values ranging from 2.5 to 3.5 are more common [18]. In the present study, most of the *b* values were in the limit of 1.1–3.9; however, *b* values may vary in the same species due to one or more factors. In this study, the growth patterns of *Eurema hecabe* (L.) were positive allometric growth, which is similar to Hossain *et al.* [16] who recorded the positive allometric growth in *A. mola* in the Mathabhanga River, southwestern Bangladesh.

To the best of our knowledge, no information currently exists on the morphometrics and length-length relationships of the common grass yellow butterfly, *E. hecabe* (L.), this study may provide primary information for future researchers. Further study may be required to investigate the details of biology and physiology.

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