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Assessment of Morphometric Characters of the *Chilades pandava* and *Chilades lajus* (Lepidoptera: Lycaenidae) Butterflies

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Abstract: The present study was carried out to describe the morphometric characters including morphometric lengths and length-length relationships (LLRs) of the butterflies *Chilades pandava* and *Chilades lajus* (Lepidoptera: Lycaenidae). Butterflies were sampled randomly from the Rajshahi University campus, Bangladesh. The pictures of butterflies were taken with DSLR camera (Canon 750D), and total 14 different morphometric lengths from the body, antenna, wings and legs were assessed using ImageJ software (1.48v). Body length (BL) was recorded as average 9.942± 0.98 and 9.116 ± 0.42 mm for *C. pandava* and *C. lajus* respectively. The maximum length was found in forewing base-apex (FWBA) as 14.473± 1.86 and 12.791 ± 1.16 mm and the minimum length was recorded in total foreleg (TFL) as 4.185 ± 0.59 and 3.727 ± 0.50 mm in *C. pandava* and *C. lajus* respectively. While comparing two species, all morphometric lengths were significant ($p < 0.05$), expect antenna length (AL), hindwing base-apex (HWBA) and anal vein (VA). All LLRs also had significance with r^2 values ≥ 0.6974 for *C. pandava* and ≥ 0.7080 *C. lajus*. According to r^2 values, the best-fitted model of LLR was BL *vs.* VC2 (Vein Cubitus2) for *C. pandava* and BL *vs.* THL (Total Hindleg) for *C. lajus* among 13 equations. The allometric coefficient '*b*' of the LLRs between BL vs. AL, BL vs. FWBT, BL vs. FWAT, BL vs. HWAT, BL vs. VR2, BL vs. TFL, BL vs. TML and BL vs. THL for *C. pandava* and BL vs. FWAT, BL vs. HWBT, BL vs. TFL and BL vs. TML for *C. lajus* indicated isometric growth while the remaining parameters of both species indicated negative allometric growth. Among the variations of morphometric characters within *C. pandava* and *C. lajus* show the way of dynamic speciation.

Keywords: Morphometric analysis, *C. pandava*, C. *lajus*, linear regression, growth patterns.

INTRODUCTION

Morphometric analysis is one of the useful tools for the separation and identification of many groups of insects [1-3]. Morphometric techniques have been used to assist quantitative measurement and analysis of morphological variation in size and shape of the organisms [1, 4]. Morphometric character also represent one of the major keys for determining their systematic, growth variability [5]. In addition, morphometric characters play a very important role in research, as it is used for comparing life history and morphological trends of populations across regions [6, 7].

Chilades butterfly is a genus of butterflies in the family Lycaenidae of the order Lepidoptera. Lycaenidae is one of the largest families in the butterflies with over 6,000 species worldwide [8]. *Chilades pandava* (Horsfield) (Cycad Blue) and *Chilades lajus* (Stoll) (Lime Blue) are very common species and widespread, found from India, Bangladesh and Sri Lanka to the Philippines [9]. They show population dynamics and seasonal polyphenism [10].

The morphological shape and size of the body and wing of insects is comprehensively studied to clarify the relationship between closely related taxa and to help in identifying population within and between species of insects [2, 11-14]. It is reported that the variation in body size is an element of natural populations and has vital implications for the understanding of the population dynamics and stability of ecological systems [15, 16].

The uses of statistical techniques are nowadays routinely applied for morphometric study [17]. The linear regression analysis of the body parts has shown a significant element for resolving the complex taxonomy [18]. The determination coefficient (r^2) was also used as an indicator of the quality of the linear regressions [19]. To the best of the knowledge, there is no previous information on morphometric lengths and length-length relationships (LLRs) of *C. pandava* and *C. lajus* from

Rajshahi in Bangladesh. In the present study, a morphometric approach was applied to carry out the first complete and comprehensive description of the variation in lengths (body, antenna, wings and legs), LLRs and growth patterns of these two butterfly species from Rajshahi, Bangladesh.

MATERIALS AND METHODS

Butterflies

The *Chilades pandava* (Horsfield) (Cycad Blue) and *Chilades lajus* (Stoll) (Lime blue) (Lepidoptera: Lycaenidae) were collected for morphometric analysis from Rajshahi University campus, Rajshahi, Bangladesh. Rajshahi University area of 753 acres located in between 24.370° north latitudes and has an 88.637° east longitudes.

Sampling Method

Sample collections were done using random sampling technique. For collection of specimens, sweep net and hand picking methods were used. Collected specimens were narcotized with menthol (naphthalene) crystals and brought into the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi and air dried for preservation.

Photography

The pictures of the preserved specimens were taken using a DSLR camera (Canon 750D) for morphometric measurements.

Morphometric measurements

For the comparison of morphometric characters, fourteen parameters were measured using ImageJ software (1.48v). Measurement sites in butterflies are shown in Figure-1.

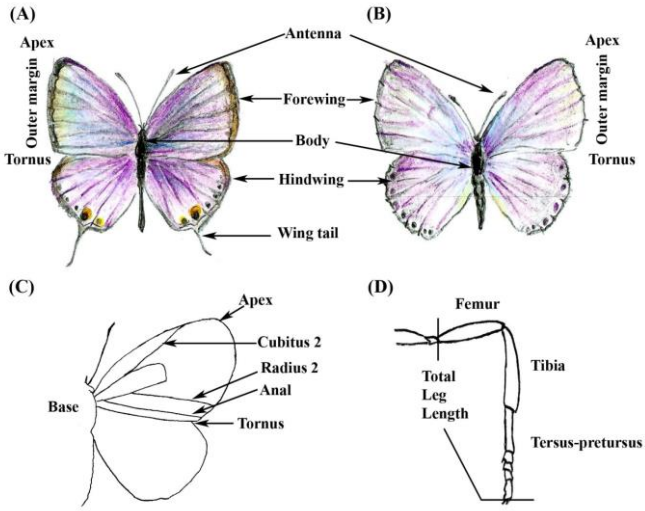

Fig-1: Morphology and measurement sites of the two species of *Chilades* butterflies. Dorsal side of *C. pandava* (A) and *C.lajus* (B). Wing venation (C) and leg position (D) for morphometric analysis.

Data Analysis

The comparison between lengths of different measurement sites of *C. pandava* and *C.lajus* was analyzed by *t*-test. The length-length relationship (LLR) was calculated with the equation: $W = a \times L^{b}$, where W stand for Body Length (BL) and L for 13 different lengths in mm. The parameters a and b were calculated through linear regression analyses depending on natural logarithms: $\ln(W) = \ln(a) + b \ln(L)$. Moreover, 95% confidence limit (CL) of a and b and the co-efficient of determination (r^2) were estimated. Extreme outliers were excluded from the regression analyses. In order to confirm whether b values obtained in the linear regressions were significantly different from the isometric value ($b = 1$), a *t*-test was applied, expressed by the equation according to Sokal and Rohlf [20]: $t_s = (b\text{-}1) / S_b$, where t_s is the *t*-test value, b the slope and S_b the standard error of the slope (b). Deviation of the b value from the theoretical isometric value indicates either positive ($b >$ isometric value) or negative ($b <$ isometric value) allometric growth. The best model for LLRs was selected depending on the highest value of determination r^2. Statistical analyses were carried out with Graph Pad Prism 6.5 software. All statistical analyses were significant at 5% ($p < 0.05$).

RESULTS

Morphometric analyses of fourteen parameters *viz.* length of body and antenna; length of base-apex, base-tornus and apex-tornus of forewing and hindwing; length of radius2, cubitus2 and anal vein of forewing; length of foreleg, midleg and hindleg were measured in the two species of *Chilades* butterflies (Table 1). The length of body of the two species was 9.942± 0.98, 9.116 ± 0.42 mm respectively and considered significant ($p<0.05$) although length of antenna (6.568 ± 0.63, 6.087 ± 0.77 mm) was not significant. The mean

lengths of base-apex, base-tornus and apex-tornus of forewing for *C. pandava* were 14.473± 1.86, 10.944 ± 0.91 and 9.963 ± 1.16 mm and for *C. lajus* were 12.791 ± 1.16, 9.934 ± 0.98 and 8.935 ± 0.50 mm. The variation of the wing parts of forewing was statistically significant (p<0.05). For hindwing, base-apex, base-tornus and apex-tornus were 11.232 ± 1.47, 9.152 ± 1.49 and 9.711 ± 1.10 mm for *C. pandava* and 10.937 ± 0.89, 7.205 ± 0.52, 8.743 ± 0.64 mm for *C. lajus*. All wing parts of hindwing were found significance differences (p<0.01 and p<0.05) except the length of base-apex.

Table-1: Morphometric measurements of different characters of the two species of *Chilades* butterflies (*n* =10)

Measurement sites		*C. pandava*			*C. lajus*			*P*- value
		Min (mm)	Max (mm)	Mean ±SD	Min (mm)	Max (mm)	Mean ±SD	
Body length (BL)		8.51	11.10	9.942± 0.98	8.20	9.74	9.116 ± 0.42	0.01288*
Antenna length (AL)		5.52	7.53	6.568 ± 0.63	4.77	7.28	6.087 ± 0.77	0.05599 ns
Forewing (FW)	Base-Apex (BA)	11.66	16.41	14.473± 1.86	10.25	14.14	12.791 ± 1.16	0.02513*
	Base-Tornus (BT)	9.58	11.86	10.944 ± 0.91	8.36	11.76	9.934 ± 0.98	0.01575*
	Apex-Tornus (AT)	8.38	11.80	9.963 ± 1.16	7.93	9.62	8.935 ± 0.50	0.01011*
Hindwing (HW)	Base-Apex (BA)	9.10	12.98	11.232 ± 1.47	9.01	12.27	10.937 ± 0.89	0.30290 ns
	Base-Tornus (BT)	6.96	10.99	9.152 ± 1.49	6.27	8.12	7.205 ± 0.52	0.00121**
	Apex-Tornus (AT)	8.13	11.51	9.711 ± 1.10	7.60	9.70	8.743 ± 0.64	0.01921*
Vein Forewing (V)	Radius2 (R2)	8.69	11.59	10.107 ± 0.93	7.18	9.86	8.725 ± 0.80	0.00477**
	Cubitus2 (C2)	9.62	13.92	11.936 ± 1.37	9.08	12.20	11.102 ± 0.84	0.04882*
	Anal (A)	9.05	12.80	10.969 ± 1.34	8.52	10.86	10.162 ± 0.74	0.07243ns
Foreleg	Total (TFL)	3.21	5.07	4.185 ± 0.59	2.79	4.35	3.727 ± 0.50	0.0132*
Midleg	Total (TML)	4.73	6.28	5.507 ± 0.58	4.17	5.17	4.639 ± 0.30	0.00016***
Hindleg	Total (THL)	4.33	5.98	5.401 ± 0.64	3.08	5.61	4.569 ± 0.71	0.01260*

n, sample size; Min, minimum; Max, maximum; SD, standard deviation; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant

For wing venation of forewing, the length of radius2, cubitus2 and anal vein were 10.107 ± 0.93, 11.936 ± 1.37 and 10.969 ± 1.34 mm for *C. pandava* and 8.725 ± 0.80, 11.102 ± 0.84 and 10.162 ± 0.74 mm for *C. lajus* respectively. The length of radius2 and cubitus2 was considered significant (p<0.01 and p<0.05) whereas the length of anal vein did not show any significant differences for these two species. The mean lengths of foreleg, midleg and hindleg were 4.185 ± 0.59, 5.507 ± 0.58 and 5.401 ± 0.64 mm for *C. pandava* and 3.727 ± 0.50, 4.639 ± 0.30, 4.569 ± 0.71 mm for *C. lajus* respectively. The difference between midleg was highly significant (p<0.001) and fore-hindleg was significant at p<0.05 level for *C. pandava* and *C. lajus*.

Table-2: Descriptive statistics and estimated characters of the length-length relationships of *C. pandava* (*n* = 10)

Equation	Regression parameters			95% CI of *a*			95% CI of b		r^2
	a	*b*		Lower	Upper		Lower	Upper	
BL = a + b × AL	0.8620	1.3824		-3.0807	4.8047		0.7846	1.9803	0.7804
BL = a + b × FWBA	2.6966	0.5006		0.7820	4.6112		0.3692	0.6319	0.9061
BL = a + b × FWBT	-1.0122	1.0009		-4.4743	2.4499		0.6855	1.3163	0.8700
BL = a + b × FWAT	2.8109	0.7157		-0.7880	6.4100		0.3567	1.0747	0.7253
BL = a + b × HWBA	3.7058	0.5552		0.3315	7.0801		0.2570	0.8533	0.6974
BL = a + b × HWBT	4.1934	0.6281		2.7202	5.6665		0.4690	0.7872	0.9119
BL = a + b × HWAT	1.9640	0.8215		-0.7306	4.6588		0.5456	1.0974	0.8549
BL = a + b × VR2	1.4073	0.8444		-3.7209	6.5355		0.3389	1.3498	0.6497
BL = a + b × VC2	1.7298	0.6880		-0.2048	3.6645		0.5268	0.8491	0.9237
BL = a + b × VA	2.4144	0.6862		0.1395	4.6893		0.4802	0.8922	0.8806
BL = a + b × TFL	3.3756	1.5690		0.6787	6.0726		0.9296	2.2083	0.8001
BL = a + b × TML	1.6957	1.4974		-1.9163	5.3079		0.8446	2.1501	0.7776
BL = a + b × THL	2.8052	1.3213		-0.5399	6.1504		0.7059	1.9368	0.7539

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

The regression parameters (*a* and *b*), their 95% confidence intervals, coefficients of determination (r^2) for LLRs, of *C. chilades* are given in Table 2. All LLRs were highly significant ($p< 0.05$) with r^2values ≥ 0.6497. Based on r^2 value, LLR by BL *vs.* VC2 was the best fitted model among 13 equations for *C. pandava*.

All length-length relations of *C. lajus*, which are shown in Table 3, were also highly correlated with r^2 values being greater than 0.7080. Based on maximum values of r^2, LLR by BL *vs.* THL was the best-fitted model among 13 equations for *C. lajus*.

Table-3: Descriptive statistics and estimated characters of the length-length relationships of *C. lajus* (*n* = 10)

Equation	Regression parameters			95% CI of *a*			95% CI of b		r^2
	a	*b*		Lower	Upper		Lower	Upper	
BL = a + b × AL	6.0635	0.5014		5.0044	7.1226		0.3287	0.6742	0.8485
BL = a + b × FWBA	5.0353	0.3190		3.2478	6.8228		0.1797	0.4582	0.7772
BL = a + b × FWBT	5.0860	0.4056		3.5410	6.6310		0.2507	0.5605	0.8200
BL = a + b × FWAT	2.7459	0.7129		-0.5937	6.0856		0.3396	1.0861	0.7080
BL = a + b × HWBA	4.2532	0.4446		2.8132	5.6932		0.3133	0.5758	0.8840
BL = a + b × HWBT	3.9811	0.7126		1.7100	6.2521		0.3982	1.0271	0.7734
BL = a + b × HWAT	3.9107	0.5886		1.8508	5.9706		0.3562	0.8210	0.8100
BL = a + b × VR2	4.9261	0.4802		3.4428	6.4093		0.3108	0.6495	0.8423
BL = a + b × VC2	3.9837	0.4622		2.2672	5.7001		0.3080	0.6164	0.8565
BL = a + b × VA	4.0150	0.5019		1.8322	6.1978		0.2876	0.7162	0.7848
BL = a + b × TFL	6.3584	0.7398		5.1518	7.5650		0.4187	1.0610	0.7791
BL = a + b × TML	3.4544	1.2204		1.0065	5.9024		0.6937	1.7470	0.7811
BL = a + b × THL	6.5092	0.5705		5.9695	7.0489		0.4536	0.6873	0.9406

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

The calculated allometric coefficient *b* of LLRs between BL vs. AL, BL vs. FWBT, BL vs. FWAT, BL vs. HWAT, BL vs. VR2, BL vs. TFL, BL vs. TML and BL vs. THL indicated isometric growth while BL vs. FWBA, BL vs. HWBA, BL vs. HWBT, BL vs. VC2 and BL vs. VA indicated negative allometric growth in *C. pandava* (Table 4). In *C. lajus*, the calculated allometric coefficient *b* of LLRs between BL vs. FWAT, BL vs. HWBT, BL vs. TFL and BL vs. TML showed isometric growth while BL vs. AL, BL vs. FWBA, BL vs. FWBT, BL vs. HWBA, BL vs. HWAT, BL vs. VR2, BL vs. VC2, BL vs. VA and BL vs. THL showed negative allometric growth (Table 4).

Table-4: Growth patterns of *C. pandava* and *C. lajus* butterflies

	C. pandava	***Growth pattern***	***C. lajus***	***Growth pattern***
BL = a + b × AL	1.46	I	-6.65	-A
BL = a + b × FWBA	-8.76	-A	-11.28	-A
BL = a + b × FWBT	0.0068	I	-8.85	-A
BL = a + b × FWAT	-1.82	I	-1.72	I
BL = a + b × HWBA	-3.42	-A	-9.75	-A
BL = a + b × HWBT	-5.39	-A	-2.11	I
BL = a + b × HWAT	-1.49	I	-4.08	-A
BL = a + b × VR2	-0.71	I	-7.08	-A
BL = a + b × VC2	-4.46	-A	-8.04	-A
BL = a + b × VA	-3.49	-A	-5.36	-A
BL = a + b × TFL	2.03	I	-1.87	I
BL = a + b × TML	1.76	I	0.97	I
BL = a + b × THL	1.20	I	-8.48	-A

I: isometric; -A: negative allometric

DISCUSSION

Information regarding any biological and morphological aspects of *Chilades* spp. from the Rajshahi is quite insufficient and the data quality of previous studies on all morphometric characters and LLRs from these areas are not well defined. A recent study has only shown that the lengths of forewing, hindwing, body and antenna were 13.33, 11.33, 9.33 and 6.66 mm for *C. pandava* and 13.75, 10.5, 8.33 and 6 mm for *C. lajus* respectively [21]. From this study, the lengths of body and antenna were found as 9.942± 0.98 and 6.568 ± 0.63 mm for *C. pandava* and 9.116 ± 0.42 and 6.087 ± 0.77 mm for *C. lajus* respectively. Moreover, additional twelve characters were evaluated in the present study.

It is also reported that the morphometric lengths of seven characters *viz*. wingspan, body length, forewing length, forewing width, hindwing length, hindwing width and basal length for six *Eurema* species showed the variations in wing and body size that can be used for taxonomic discrimination [3]. Due to the lack

of references dealing with LLRs for *Chilades* spp., it was not possible to make comparisons with the previous literature. In addition, the morphometric characters, LLRs and growth patterns were used frequently as a parameter in fish research [6, 22]. The estimated length-length relationships and growth patterns of these species could provide valuable information for future research.

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