SELINUS UNIVERSITY
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The Use of Morphometrics and Growth Models to differentiation between two species of Butterfly of the genus *Colotis*

By

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Abstract

Morphometric analysis and two growth models were utilized to examine the efficacy of using these methods to differentiate between two species of butterflies. Body length, body width, forewing length, forewing width, head diameter, body length/head diameter ratio and body width/head diameter ratio were determined of a total of 342 butterflies, *Colotis phisadia* (174) and *Colotis chrysonome* (168). The results of the morphometric analyses showed large differences in six measured parameters the Body width : Wing Length; body length : head diameter; body length : body width/head diameter; body width : body width/head diameter; wing width : body width/head diameter; and wing length : body width/head diameter. These results showed that morphometric analysis is capable of differentiating between the two species. The results of the two growth models showed slight variations in the growth coefficients not enough to indicate capability to differentiate between the two species. In conclusion, morphometric analysis can be used to differentiate between species of the same genus. Other studies are needed to find out if the differences between species revealed by the morphometric analysis is due to natural selection or to environmental pressure during ontogenetic development.

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Chapter 1 Introduction

The aim of this study is to use morphometrics of two butterfly species commonly available in Jordan and two types of growth models to examine if these two methods can be used to differentiate between two species of the same genus.

1.1 Definition of Morphometry

Morphometry of an animal or a plant is defined as quantitative relationship between growth and allocation of the organism (Weiner, 2004). Morphometric measurements are used to study the relative sizes of the organism parts in which the relationship between two morphology parameters is calculated. This relationship will be either allometric or isometric and any factor that affects size will also affect the percent allocation to different structures and functions (Weiner, 2004).

Allometry means "of other or different measures" (allo=other or different, metry=measure) and it is used to describe the differences in magnitude in form or function that are correlated with changes in form or function of another factor. (Trombulk, 1991).

Allometry is used sometimes in place of Morphometry as a powerful tool for shape analysis since it allows for the characterization of growth trajectories and the visualization of growth patterns (Loy *et al*., 2000). It is a powerful tool for the understanding of comparative studies of individuals, populations or species (Harvey and Pagel, 1991).

Julian Huxley and George Teissier came up with the term allometry in 1936 to avoid confusion with relative growth studies (Gayon, 2000). Both agreed to use the allometric growth formula " $Y = a X^b$, although several authors have used similar formula in various contexts and under various titles (Gayon, 2000).

Allometry refers to three alternative cases; 1) Ontogenetic allometry (growth trajectory of an organ relative to body size during the growth of the individual) 2) Static allometry (scaling relationship among individuals between two organisms after the growth has stopped) 3) Evolutionary or Phylogenetic allometry (relationship between organs across species) (Stern and Emlen, 1999).

Huxley's equation $Y = A X^B$, where X is some measure of size, Y is the variable of interest, A is a constant describing a relationship between X and Y and B is the allometric coefficient (German and Meyers, 1989).

Morphometry, can describe an allometric relationship or isometric relationship, has been applied in many fields in biology. It used in animals as a predictor of various ecological relationships (Peters, 1983). Furthermore, it is used in plants to find out relations in a many plant species and for natural selection and adaptive evolutionary changes (Reddy *et al.*, 1998).

Morphometrics are used since the shape and size of an animal are important characteristics originating from their genetic bases in complex association and interactions with the environment both internal and external (Marroig, 2007).

1.2 Growth models

Growth models and rates are important in associating variables to morphological diversification, life history strategies and population dynamics (Moran, 2000). Gould (1966) stated that different proportions of an organism can be correlated with changes in the absolute magnitude of the organism or of a specific part of the organism. Changes in body size and shape of an organism occur during the course of evolution and can be seen in the microscopic to the macroscopic scale during the growth and maturation of an organism and during the evolution of populations (Tschinkel *et al*., 2003).

Two growth models that use length to determine growth will be used in this study; 1) Von Bertalanffy growth model where the growth in terms of length is determined by $\{L(t) = L_{max} (1 – A * e^{(-kt)})\}$ where Lmax is the maximum growth in length of the organism, k is the growth rate of the organism and A is a constant determined by the relation between the minimum length and maximum length of an organism. 2) Gombertz growth model where growth is determined in terms of length in the equation {$L(t) = L_{max}e^{(-A * e(-kt))}$} in which two exponentials appear in the equation and k, L_{max} and A are defined as in the Von Bertalanffy model (Elkarmi and Ismail, 2006).

1.3 Butterfly Species

1.3.1 *Colotis phisadia phisadia* GODART 1819

This species belongs to family **Pieridae** sub-family **Pieridae** (Katbeh-Bader *et al.,* 2004), intermediate in size, with wing ground color is usually white, yellow or orange, with black or greenish markings. The head is rounded and all the legs are equally developed (Korshunov and Gorbunov, 1995). Green color larvae, mostly with markings and stripes, and larvae feed predominantly on Brassicaceae and Fabaceae (Katbeh-Bader *et al.*, 2004) (Figure 1). It is known as the Blue Spotted Arab and is common in addition to Jordan in tropical Africa and Arabia. It is limited to the Dead Sea area in Jordan. The food plant is *Scdvadora persic* (Katbeh-Bader *et al.*, 2004).

Figure 1: Picture of *Colotis phisadia*

1.3.2 *Colotis chrysonome* KLUG 1829

This species belongs to family **Pieridae** sub-family **Pieridae** (Katbeh-Bader *et al.*, 2004), it is an afrotropical species and known as the Golden Arab. Larsen and Nakamura (1983) indicated that tropical oasis in southern part of the Dead Sea are typical localities for this butterfly. It is associated with *Maerua crassifouaa*s as a food source (Katbeh-Bader *et al.*, 2004) (Figure 2). It is known that the population fluctuation of this species is dependent on the survival of the food source (Walker and Bittaway, 1987).

Figure 2: Picture of *Colotis chrysonome*

Chapter 2 Literature Review

2.1 Allometry of Butterflies

After extensive literature review there are no morphometric, allometric or growth model studies on the two butterfly species of this study namely *Colotis phisadia* and *Colotis chrysonome*. On the other hand, there are few allometric studies on other butterfly species. Dudley (1990) studied the morphometrics and kinematics of neotropical butterflies. He examined the Wing and body kinematics of free cruising flight of Panamanian butterflies and concluded that there is no consistent correlation exists between wing kinematics and absolute flight speed. In another research, Dudley and Srygley (1994) conducted a research on the allometry of air speeds during natural free flight of a total of 270 neotropical butterflies. They concluded that butterfly air speeds under natural conditions can reasonably be predicted from morphological measurements. Kunte (2007) examined the allometry and functional constraints on proboscis lengths using Costa Rican butterflies. He concluded that a strong positive relationship exists between proboscis length in relation to body size and handling time per flower on nectar plants. In another study the fresh weight, dry weight, and C and N content of the eggs, egg shells and neonate larvae of twelve satyrine butterflies were determined (Garcia-Barros, 2006). The results show that the evidence for intra-specific allometry between the traits investigated and egg weight varied among the species, indicating that the slope of such relationship may be a specific feature. Palmer *et al.* (2019) carried out an interesting study of the scaling and allometry of butterfly wing patterns. Their results indicated that the color patterns showed that the positions and size of the pattern elements scaled perfectly isometrically with wing size. Mirth *et al.* (2016) conducted a research on the allometry and size control in order to answer the question what can studies of body size regulation teach us about the evolution of morphological scaling relationships. They stated that allometric studies and population genetics provide a power tool for the understanding of evolution and allometry. Wolfe *et al.* (2010) carried out a study to evolutionary reduction of the first thoracic limb of members of butterflies of families Nymphalidae and Riodinidae. They stated that limb evolution in butterfly members of the families Nymphalidae and Riodinidaeare likely evolved reduced forelimbs in parallel. Steppan (2000) compared the flexural stiffness of dried forewings of ten butterfly species to the butterflies' gross morphological parameters in order to calculate the allometric relationships. He stated that the distal regions of the wings are stiffer against forces applied to the ventral side; on the other hand, the basal region is much stiffer against forces applied dorsally. Akand *et al.* (2017) studied the morphometric variations in the species of two sub-families of butterflies of the family Lycaenid. They reported that there were significant differences between the two subfamilies and these differences are good indicators to identify the species more correctly. Chazot *et al*. (2015) used morphometrics to find out what drives the evolution of wing size and shape in the *Morpho* butterflies. Their results showed that microhabitat has driven wing shape evolution while it has little effect on forewing and hindwing integration. Bai *et al*. (2015) conducted a geometric morphometric study of the wing shapes of the butterfly *Pierisrapae*. They reported that there are significant differences in the forewings and hindwings of the butterfly. Furthermore, they stated that their finding indicate that the wing shapes of the butterfly are sensitive to environmental heterogeneity.

2.1 Allometry of Other Animals

Belaev *et al*. (2018) studied the allometry of wing shape and venation in Hymenoptera. They reported that in hindwings most families that have increase in body size showed elongation of the cells in proximal zone while showed shortening of the cells in distal zone. On the other hand, the cells of central region of the forewings increase in longitudinal direction. Moreover, Palestrini et al. (2019) studied the geometric morphometrics of two species of the small dung beetle to examine if these species have polymorphic males that might show different reproductive tactics. Their results showed that these species lack male polymorphism possibly due to functional constraints. Polilov and Macarova (2017) examined the scaling and allometry of organ size associated with miniaturization in insects using Coleoptera and Hymenoptera as a case study. They indicated that the relative volume of the nervous and reproductive systems increases as body size decrease. Therefore, this relation can determine the limit to which an insect can get. Ramirez-Ponce *et al*. (2017) studied the nature of allometry in an exaggerated trait choosing the postocular flange of the dobsonfly genus *Platyneuromus* in their study as an example. Their results showed a positive allometry of the postocular flange in males of two species and a negative allometry in males one species. Dillon and Frazier (2013) studied the allometry of development time in insects. They reported that warm-adapted insects develop more quickly regardless of body size which is in support of the hypothesis that ectotherms have limited ability to evolutionary compensate for the effects of low temperatures on the biological processes rate. Benitez *et al*. (2013) studied the allometric and non-allometric patterns in sexual dimorphism discrimination of wing shape in *Ophion intricatus*. They stated that there are significant differences between sexes and sites and point of intersection of radial and cubital-anal veins could be used as keys characters to differentiate between the sexes. Paciencia*et al*. (2012) evaluated the allometric growth of two species of Ephemeroptera. Their results showed that the head measurements indicated a negative allometry, the hind leg length in both species showed a positive allometry and the abdominal length in both species showed also a positive allometry. They concluded that many of the structures that showed positive allometry are due to the transition from the aquatic stage to the adult. Tschinkle*et al*. (2003) studied the relationship between worker body size and shape of the body parts in the ant *Solenopsis invicta*. They reported that the head length retained a constant proportion to body length, on the other hand, the antennae became relatively smaller. Elkarmi and Ismail (2006a) examined the allometry of the gastropod *Melanopsis praemorsa*. They reported that there is a linear relationship between shell length and the parameters shell width, aperture width and aperture length. On the other hand, the relationship between shell length to shell weight and dry body weight were nonlinear. The male horn allometry in the beetle *Onthophagus acuminatus* was studied by Emlen (1997). He reported that there is a relationship between horn length and the abundance of diet. Yamaguchi and Ikeda (2000) examined the diet and seasonal vertical distribution, life cycle and body allometry if two oceanic copepods. They reported that there are relationships between prosome length and wet weight, dry weight and ash-free fry weight. Kemp and Bertness (1984) showed that the periwinkle Littorina littorea that lives in densely populated areas exhibited elongated shells in comparison to those that live in sparsely populated areas. Ismail and Elkarmi (1999) studied the age, growth and shell morphometrics of the Limpet *Cellana radiate*. They indicated that there are negative allometries between shell length to each of shell width, height and width/height ratio. Loy *et al*. (2000) examined the allometry of fish and reported that allometry is the most easily perceivable means of assessing the evolutionary adaptation of a species to its environment. Kovac*et al*. (1999) studied the Morphometry of the stone loach fish. They reported that morphological changes coincide with changes in microhabitat.

2.2 Growth models

Oberhauser *et al*. (2016) presented a spatially explicit demographic model to simulate the annual cycle of the eastern monarch population. They suggested that conservation investment in projects across the full monarch range will be more effective than focusing on one region. Heuvel *et al*. (2013) modeled the life history evolution of the butterfly *Bicyclusa nynana* in seasonal environments. They concluded that for this butterfly, early stage cues can direct development to a better adapted phenotype. Duenez-Guzman *et al*. (2009) built a spatial individual based multilocus model of homoploid hybrid speciation. The model is tailored for a case of hybrid origin of butterfly *Heliconius heurippa* from to other species of the same genus. They reported that the model supported the possibility of hybrid origin of this butterfly under certain conditions. Yakubu et al. (2004) studied population cycles of the monarch butterfly using spatially discrete advection model. They reported the success of their approach to examine both migration and local dynamics. Schultz and Crone (2002) applied an empirically based mathematical model for the management of rare species habitat using the Fender's blue butterfly. They reported that better knowledge of rates of habitat change can result in a better ability to make management decisions. Palmer (1983) studied the growth rate as a measure of food value in Thaidid gastropods. He reported that there is a relationship between body growth and predator size, prey size and prey species. Chaitanawisuti and Kritsanapuntu (1999) examined the effects of different feeding regimes on the growth and survival of the gastropod *Babylonia areolata*. They reported that shell length growth rates did not differ with the different feeding regimes. Jara*et al*. (2004) studied the variation in density, shell size and shell growth with shore height and wave exposure of *Calyptrae aspirata*. They reported that there was no significant difference in the size of the shell between the snails found in the upper and lower intertidal zones. Carmichael *et al*. (2004) evaluated the changes in shell, soft tissue growth and survival of *Mercenaria mercenaria* with changes in food supply and habitat. They reported that shell growth increases with increase in food supply and the growth of the soft tissue followed the growth of the shell. Elkarmi and Ismail (2006a) studied age, growth and morphometrics of *Melanopsis praemorsa*. They reported that the snail may survive for five years and by applying Bertalanffy's and Richards' growth models the maximum length may reach 54mm. Stringer et al. (2002) studied the growth and development of the land snail *Paryphanta busbyi*. Their results estimated the growth to the shell stage of the snail to be between 3 to 4.3 years and fast developing snails tended to become larger adults.

Chapter 3 Materials and Methods

3.1 Study Species and Location

The butterflies of the study belong to order **Lepidoptera**, suborder **Rhopalocera**, superfamily **Papilionoidea**, family **Pieridae** and subfamily **Pierinae**. Species number depends on the reference chosen although all references put it at more than one thousand (Heppner, 2008). Adult wings span varies from 23 to 100 mm, antennae often with weak clubs, wings mostly triangular or round and body usually slender but sometimes robust (Heppner, 2008). In Jordan they are found in the area of the Dead Sea, Wadi Arabah and sometimes Aqaba ((Katbeh-Bader *et al.*, 2004) (see Figure 3). Upper side of male forewing has a pale salmon-pink ground color, this color paler outwardly; base heavily sprinkled with bluish-grey scales that extend outwards and are merged with a black patch that occupies the apex of the cell and spreads along the discocellulars (Borror*et al.*, 1981). Hindwing: white, base heavily sprinkled with bluish-grey scales that are extended downwards in a diffuse band parallel to the dorsum; terminal half of wing jet black. Female is very variable, but resembles the male in markings. On the upper side however, the terminal areas on both forewings and hindwings that are black in the male are silky brown on the forewing, the inner sinuate margin of the same posteriorly black; on the hindwing the terminal brown area encloses

Figure 3: A map of Jordan showing the localities of both butterflies

an irregular sinuate black band that does not extend either to the costa or the dorsum (Borror*et al.*, 1981).

3.2 Measurements of the Butterflies

Seven parameters were determined of one hundred and seventy four (174) *Colotis phisadia* and one hundred and sixty eight (168) of *Colotis chrysonome* namely body (thorax and abdomen) length (BL), body (abdomen) width (BW), forewing length (WL), forewing width (WW), head diameter (HD), ratio of body length to head diameter (BL/HD) and ratio of body width with head diameter (BW/HD) (Figure 4).

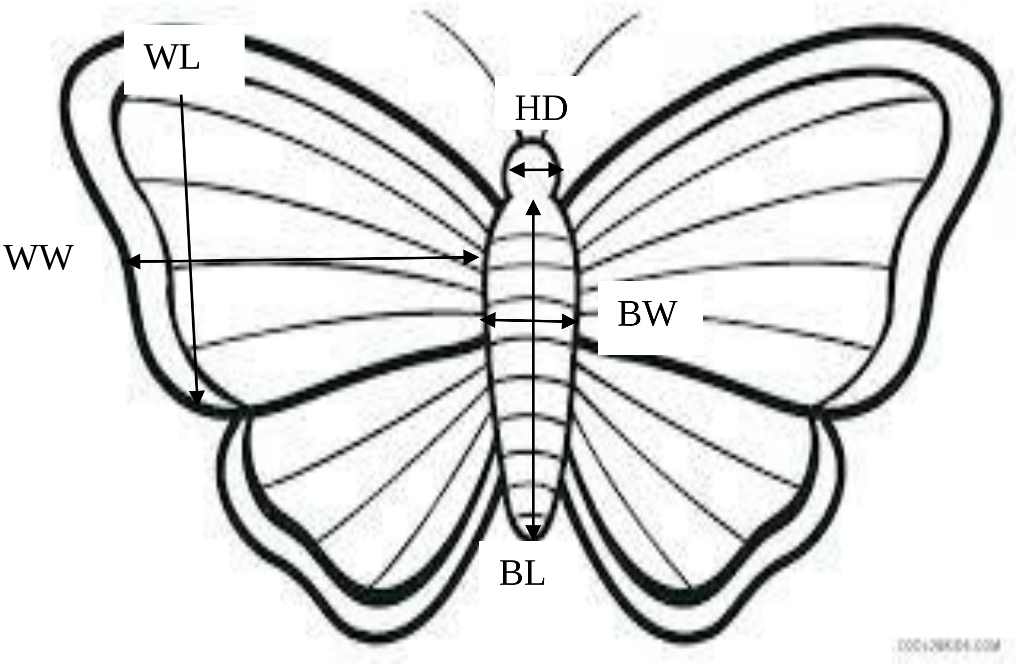

Figure 4: Schematic diagram of a butterfly showing the location of the measurements

The measurements were carried out using a digital caliper accurate to 10 µm (E-Base, MC 02050282-I, China) (Figure 5).

Species identification and confirmation was carried out by the Department of Biology and Biotechnology, Faculty of Science, Hashemite University, Jordan.

3.3 Allometric Analysis

Allometric analysis was conducted using nonlinear regression analysis. All measured parameters and variables (WL, WW, BL, BW, HD, BL/HD and BW/HD) were analyzed using the equation:

$$\mathbf{Y = A*X^{b}}$$

Where b is the allometric coefficient and A reflects the ratio of Y/X (mean value of the ratio Y/X).

Therefore, the nonlinear relationship is allometric if the allometric coefficient $b \neq 1$. The calculations were carried out using STATISTICA software for windows (StatSoft, USA).

The allometric relationships between BL and BW; WL and WW; BL and HD; BW and HD; BL and WL; BW and WW; BL and BL/HD; BW and BL/HD; BL and BW/HD; BW and BW/HD; WL and BL/HD; WW and BW/HD were calculated.

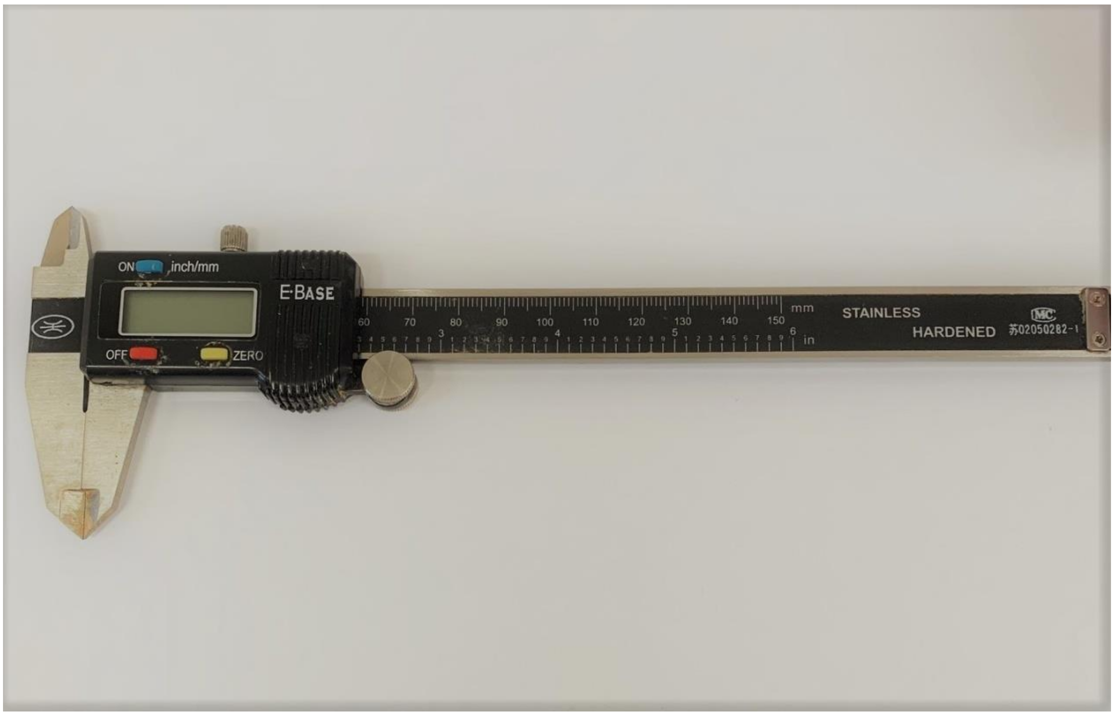

Figure 5: A picture of the digital caliper used in the measurements

The above allometric relationships and allometric coefficients were calculated for both species of butterflies and the obtained values were compared to examine if the differences in these values between the two species can be used as a means of differentiating between these two species.

3.4 Growth Model Analysis

Two growth models were utilized using body length as a measure of growth namely the Von Bertalanffy's growth model:

$$BL(t) = L_{max}(1 - A * e^{(-kt)})$$

And the Gombertz growth model:

$$L(t) = L_{max}e^{(-A * e(-kt))}$$

Where BL is the body length, L_{max} is the theoretical maximum body length, the constant A is a ratio between the maximum body length and minimum body length and k is the growth coefficient. The variables L_{max} , A and K were calculated using the Quasi-Newton method for nonlinear estimates (Ostle and Mensing, 1975) and using STATISTICA software for windows (StatSoft, USA).

The above variables were calculated for both species of butterflies and the obtained values were compared to examine if the differences between the two species can be used as a means of differentiating between these two species.

Chapter 4 Results

The results will be divided into three sections showing the values of the measurements, the allometric analyses results and the growth model results.

4.1 Values of measurements

The measurements of body length, body width, wing length, wing width and head diameter for both butterfly species are shown in figures (6 -11). These figures represent a plot between body length and body width, wing length and wing width, body length and head diameter for both species of butterflies. All values are in millimeter. Furthermore, plots between body length of *Colotis phisadia* and body length of *Colotis chrysonome*, and similarly between body width, wing length, wing width and head diameter of the two species are shown in figures (11 – 15). The results of the morphometric analysis will be represented in the form of tables detailing these results.

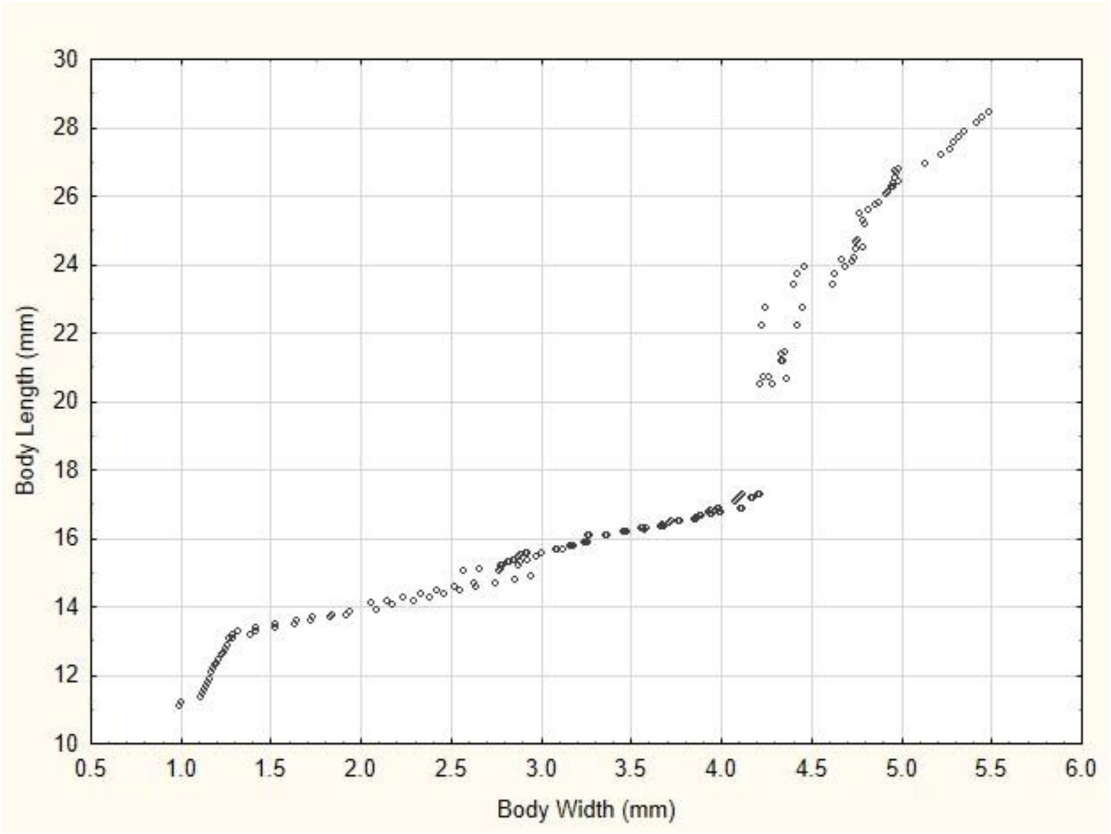

Figure 6: A scatter plot of the body length and body width of *Colotis phisadia*

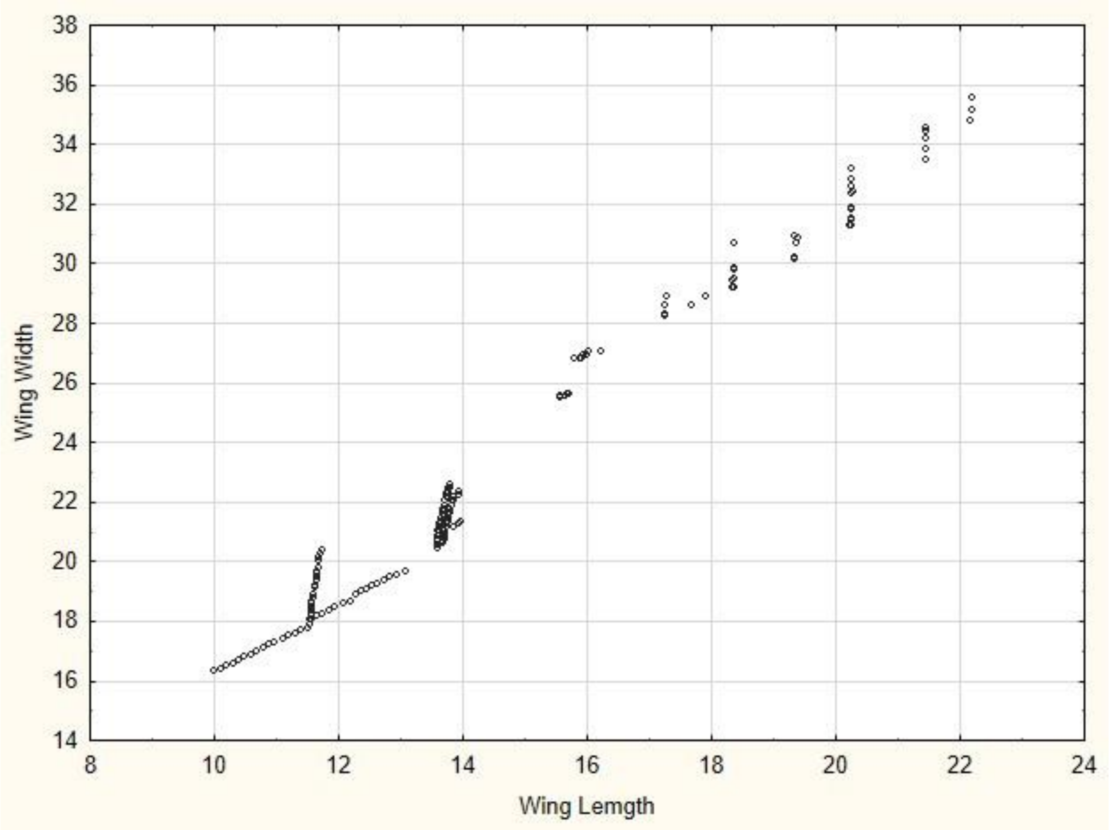

Figure 7: A scatter plot of wing length and wing width of *Colotis phisadia*

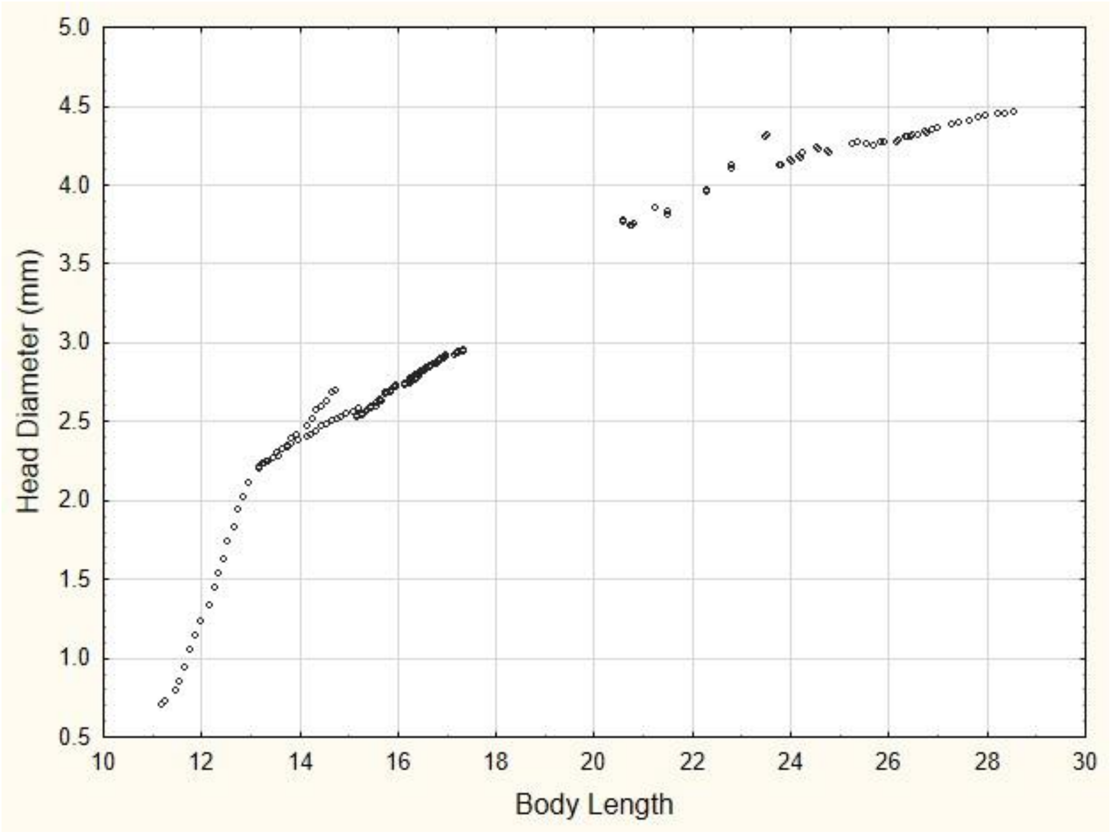

Figure 8: A scatter plot of body length and head diameter of *Colotis phisadia*

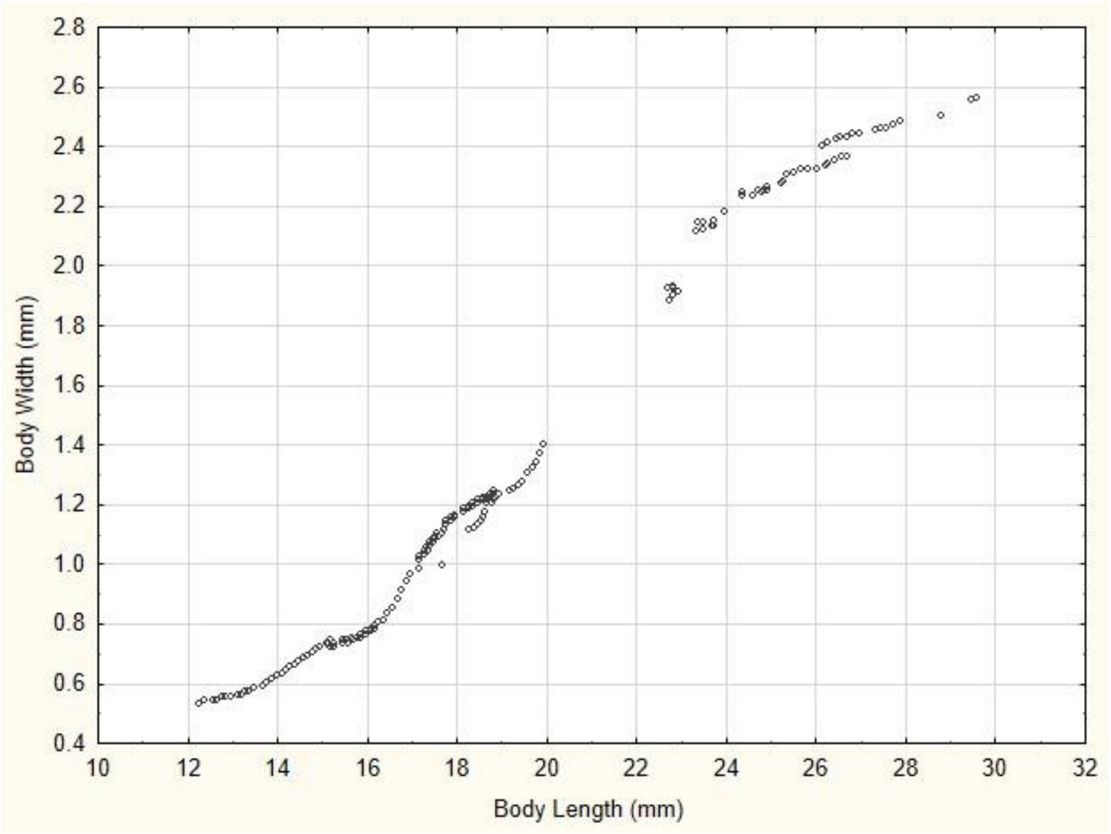

Figure 9: A scatter plot of body length and body width of *Colotis chrysonome*

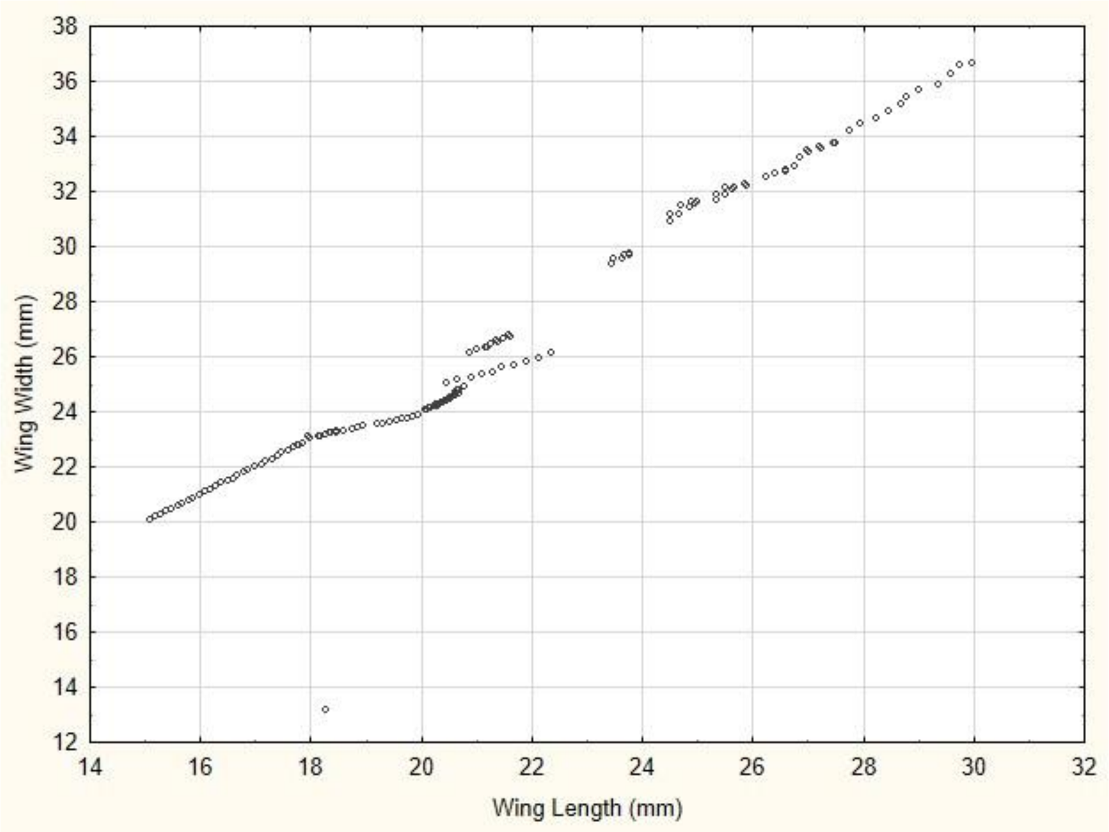

Figure 10: A scatter plot of wing length and wing width of *Colotis chrysonome*

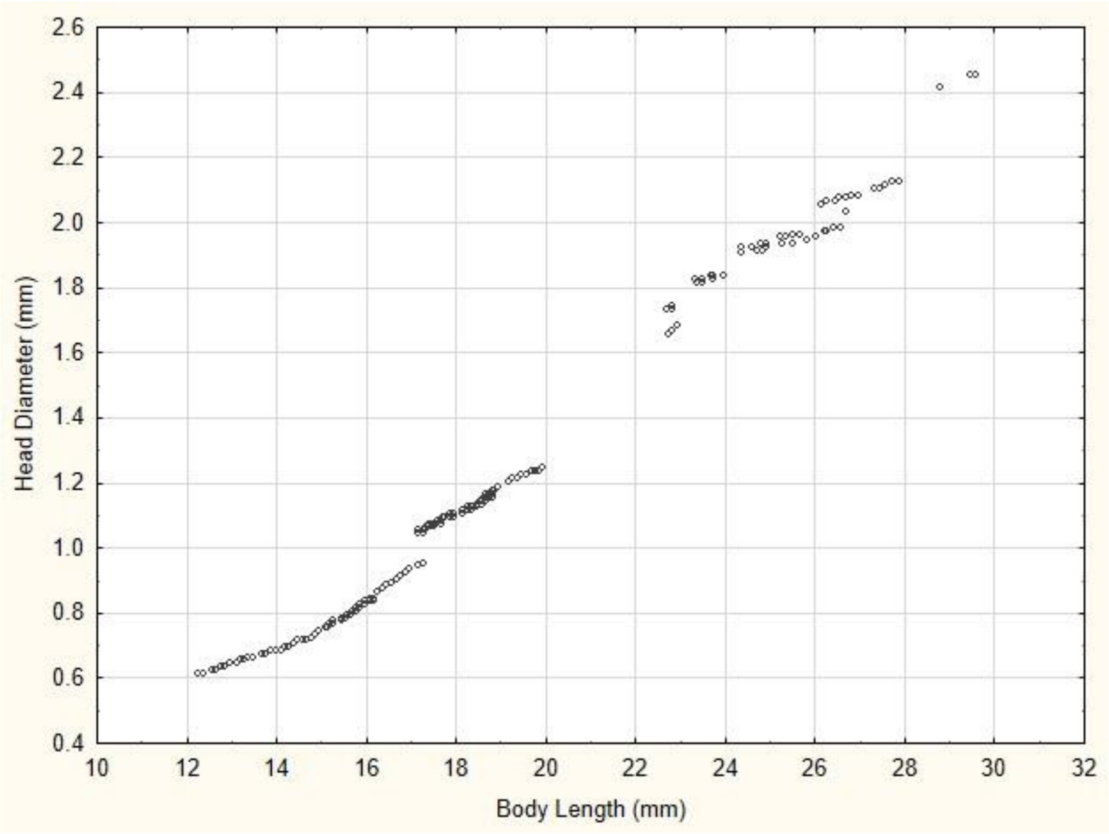

Figure 11: A scatter plot of body length and head diameter of *Colotis chrysonome*

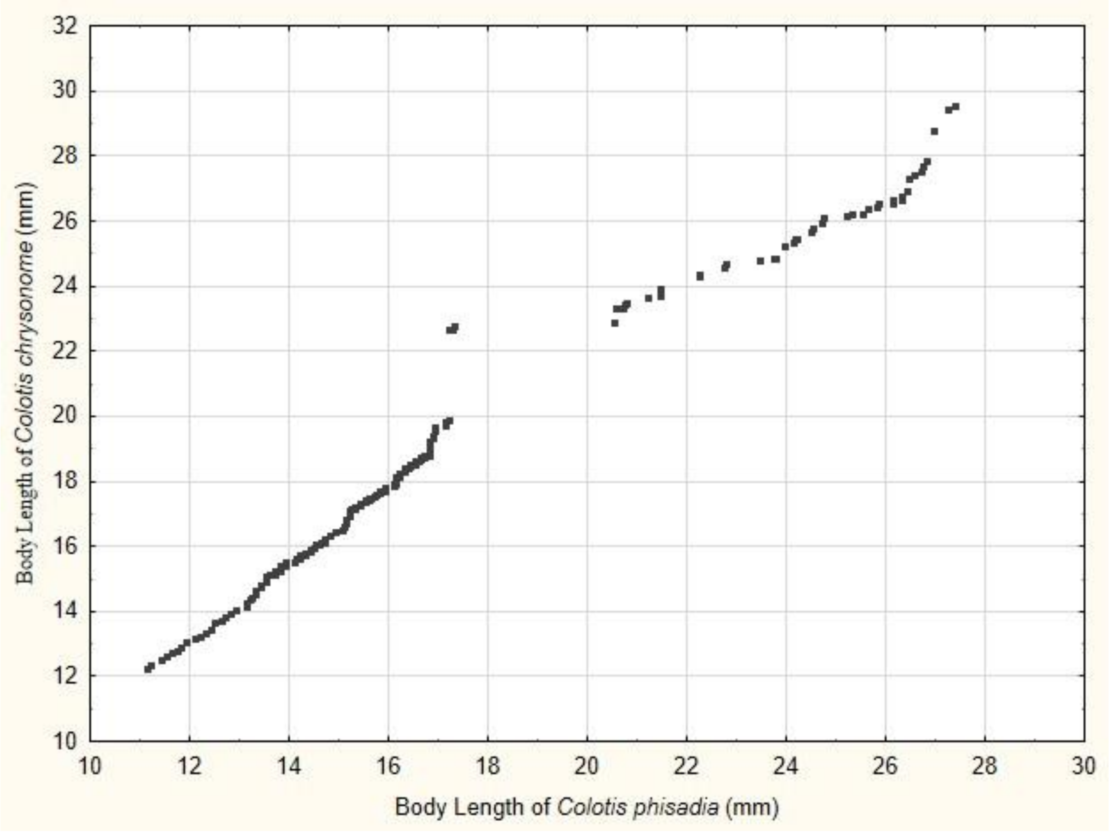

Figure 12: A scatter plot of body lengths of the two species of butterflies

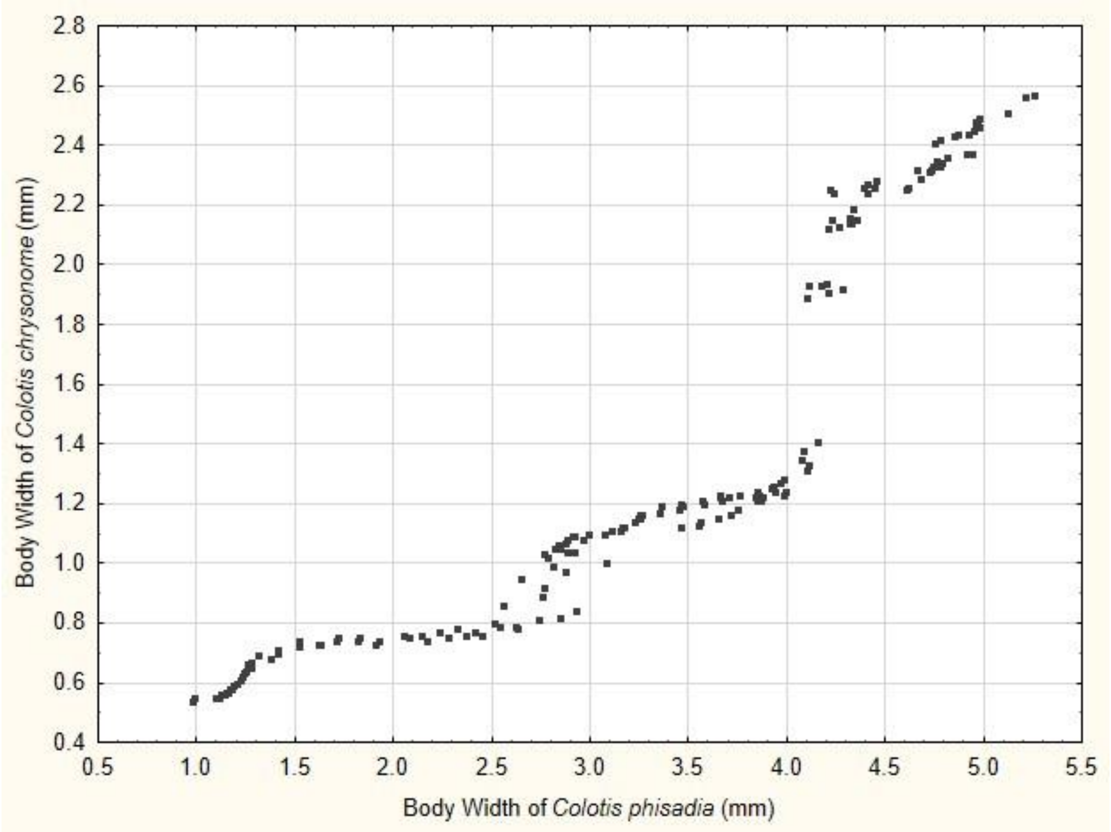

Figure 13: A scatter plot of body width of the two species of butterflies

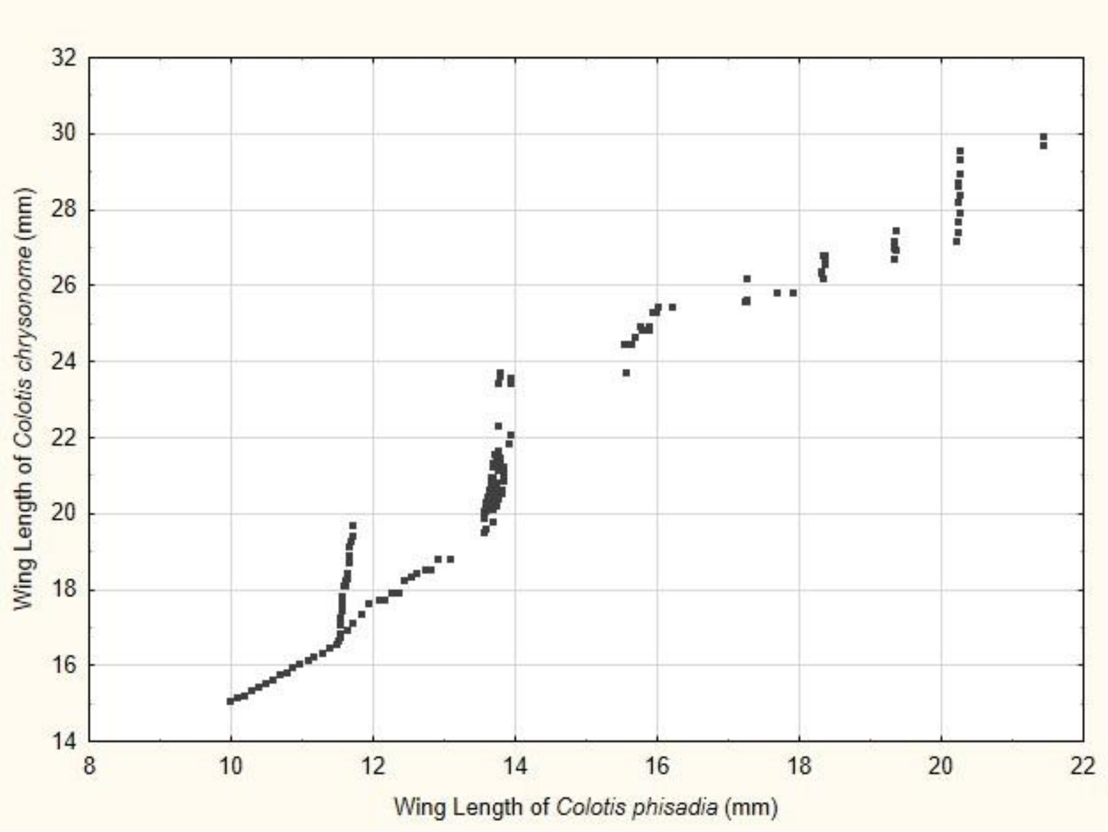

Figure 14: A scatter plot of the wing length of the two species of butterflies

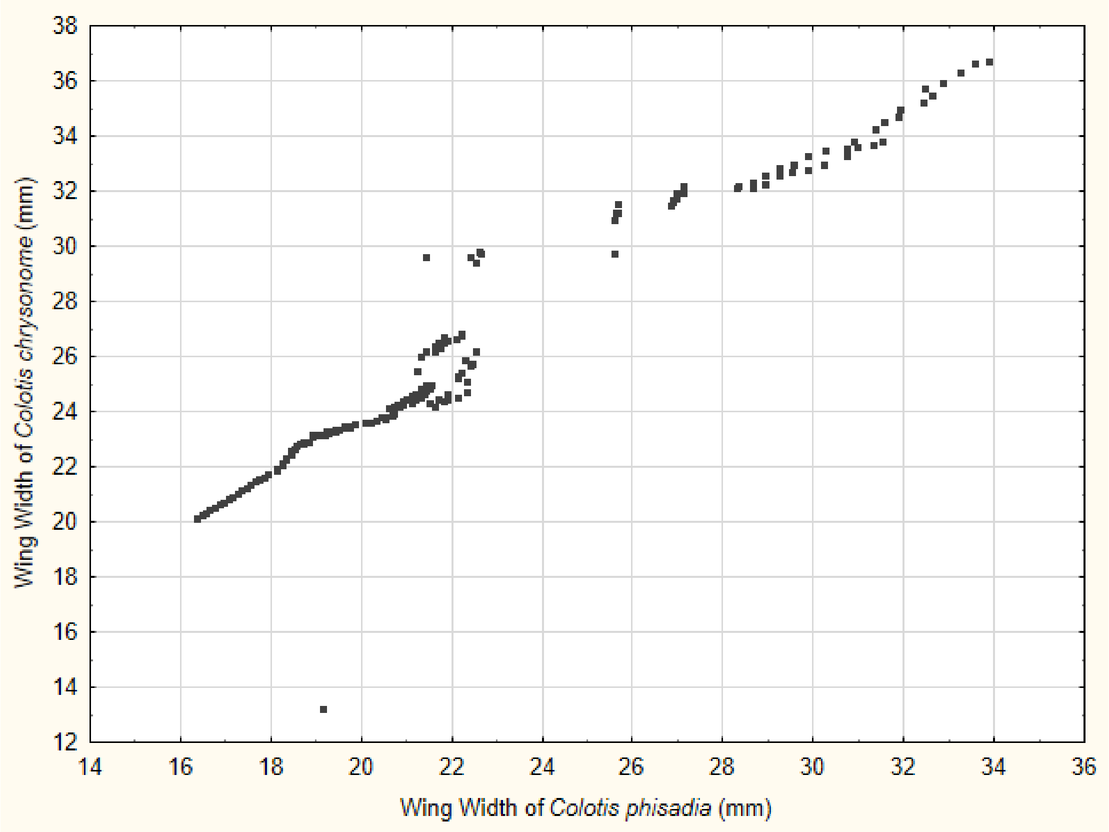

Figure 15: A scatter plot of the wing width of the two species of butterflies

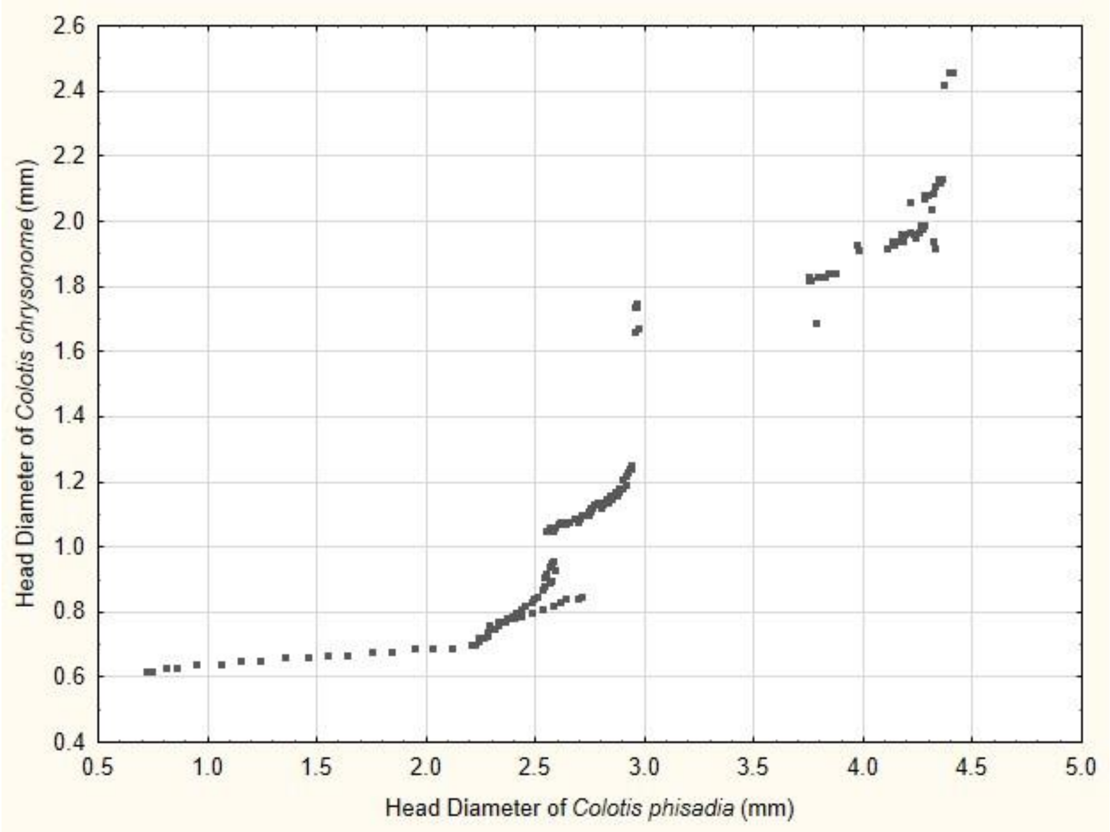

Figure 16: A scatter plot of the head diameter of the two species of butterflies

4.2 Allometric Analysis

The parameters of the nonlinear analysis for the relationships between body length and body width, wing length and wing width, body length and head diameter, body width and head diameter, wing length and head diameter, wing width and head diameter, body length and wing length, body width and wing width, and wing length and wing width with the two ratios body length/head diameter (BL/HD) and body width to head diameter (BL/HD) are listed in tables (1-14). The tables show the estimates of parameters A and B of the allometric equation, the lower and upper 95% confidence interval and the p-value of testing the validity of the A and B parameters.

These relationships are shown in the following equations of body length and body width, wing length and wing width, body length and wing length and body width and wing width for both butterfly species as an example to show the morphometric relationships between body parts.

Allometric values for *Colotis phisadia*

$BL = 8.758 * BW^{0.606}$

$WL = 0.682 * WW^{0.973}$

$BL = 0.668 * WL^{1.227}$

$BW = 0.037 * WW^{1.427}$

Allometric values for *Colotis chrysonome*

$BL = 17.159 * BW^{0.476}$

$WL = 0.806 * WW^{0.999}$

$BL = 0.350 * WL^{1.307}$

$BW = 0.0003 * WW^{2.585}$

Where, BL is body length, BW is body width, WL is wing length and WW is wing length.

Table 1: Parameter values for the relationship between body length and body width ($\alpha = 0.05$).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	8.758	17.159
Low conf. limit	8.075	17.052
Up conf. limit	9.442	17.266
p-value	0.0	0.0
Parameter B		
Estimate	0.606	0.476
Low conf. limit	0.549	0.466
Up conf. limit	0.664	0.486
p-value	0.0	0.0

Table 2: Parameter values for the relationship between body width and wing length (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.072	0.0005
Low conf. limit	0.047	0.0004
Up conf. limit	0.098	0.0006
p-value	0.0	0.0
Parameter B		
Estimate	1.428	2.551
Low conf. limit	1.303	2.461
Up conf. limit	1.554	2.642
p-value	0.0	0.0

Table 3: Parameter values for the relationship between body length and wing width ($\alpha = 0.05$).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.386	0.350
Low conf. limit	0.366	0.329
Up conf. limit	0.405	0.371
p-value	0.0	0.0
Parameter B		
Estimate	1.219	1.307
Low conf. limit	1.204	1.288
Up conf. limit	1.235	1.327
p-value	0.0	0.0

Table 4: Parameter values for the relationship between body width and wing width (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.0374	0.257
Low conf. limit	0.023	0.222
Up conf. limit	0.0518	0.292
p-value	0.0	0.0
Parameter B		
Estimate	1.426	1.315
Low conf. limit	1.308	1.275
Up conf. limit	1.545	1.356
p-value	0.0	0.0

Table 5: Parameter values for the relationship between wing width and head diameter α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	11.053	24.447
Low conf. limit	10.464	24.257
Up conf. limit	11.643	24.637
p-value	0.0	0.0
Parameter B		
Estimate	0.683	0.426
Low conf. limit	0.638	0.410
Up conf. limit	0.727	0.442
p-value	0.0	0.0

Table 6: Parameter values for the relationship between wing length and wing width ($\alpha = 0.05$).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.682	0.806
Low conf. limit	0.63772	0.714
Up conf. limit	0.727	0.898
p-value	0.0	0.0
Parameter B		
Estimate	0.973	0.999
Low conf. limit	0.953	0.965
Up conf. limit	0.993	1.034
p-value	0.0	0.0

Table 7: Parameter values for the relationship between body width and head diameter (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.947	1.013
Low conf. limit	0.845	1.001
Up conf. limit	1.049	1.025
p-value	0.0	0.0
Parameter B		
Estimate	1.146	1.188
Low conf. limit	1.060	1.168
Up conf. limit	1.231	1.208
p-value	0.0	0.0

Table 8: Parameter values for the relationship between body length and head diameter (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	6.668	17.159
Low conf. limit	6.284	17.077
Up conf. limit	7.053	17.242
p-value	0.0	0.0
Parameter B		
Estimate	0.902	0.582
Low conf. limit	0.855	0.572
Up conf. limit	0.948	0.591
p-value	0.0	0.0

Table 9: Parameter values for the relationship between wing length and head diameter (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	7.167	19.657
Low conf. limit	6.766	19.557
Up conf. limit	7.568	19.757
p-value	0.0	0.0
Parameter B		
Estimate	0.653	0.438
Low conf. limit	0.605	0.427
Up conf. limit	0.698	0.440
p-value	0.0	0.0

Table 10: Parameter values for the relationship between body length and body width/head diameter ($\alpha = 0.05$).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	17.293	17.450
Low conf. limit	16.549	17.129
Up conf. limit	18.037	17.771
p-value	0.0	0.0
Parameter B		
Estimate	0.403	2.253
Low conf. limit	0.215	2.093
Up conf. limit	0.592	2.414
p-value	0.0	0.0

Table 11: Parameter values for the relationship between body width and body width/ head diameter (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	2.995	1.027
Low conf. limit	2.813	0.985
Up conf. limit	3.176	1.069
p-value	0.0	
Parameter B		
Estimate	1.125	4.951
Low conf. limit	0.855	4.639
Up conf. limit	1.394	5.263
p-value	0.0	0.0

Table 12: Parameter values for the relationship between wing width and body width/head diameter (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	22.512	24.748
Low conf. limit	21.750	24.393
Up conf. limit	23.273	25.102
p-value	0.0	0.0
Parameter B		
Estimate	0.337	1.641
Low conf. limit	0.189	1.511
Up conf. limit	0.484	1.770
p-value	0.0	0.0

Table 13: Parameter values for the relationship between wing length and body width/head diameter ($\alpha = 0.05$).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	14.128	19.903
Low conf. limit	13.659	19.636
Up conf. limit	14.596	20.171
p-value	0.0	0.0
Parameter B		
Estimate	0.316	1.691
Low conf. limit	0.171	1.570
Up conf. limit	0.460	1.812
p-value	0.0	0.0

Table 14: Parameter values for the relationship between body length and wing length (α = 0.05).

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Parameter A		
Estimate	0.668	0.350
Low conf. limit	0.609	0.329
Up conf. limit	0.727	0.372
p-value	0.0	0.0
Parameter B		
Estimate	1.227	1.307
Low conf. limit	1.195	1.288
Up conf. limit	1.259	1.327
p-value	0.0	0.0

The results show that six of the allometric relationships presented large differences in the allometric coefficient (b) between the two species namely, Body width (1.428) : Wing Length (2.551); body length (0.902) : head diameter (0.438); body length (0.403) : body width/head diameter (2.253); body width (1.125) : body width/head diameter; wing width (0.337) : body width/head diameter; and wing length (0.316) : body width/head diameter (1.691). The differences are most striking when the allometric coefficient is less than one for one species and more than one for the other. This indicates that, for example, the relationship of wing length with the ratio of body width/head diameter for *Colotis phisadia* (b) = 0.316 meaning that wing length increase with time much less than the ratio of body width/head diameter, while for *Colotis chrysonome* (b) = 1.691 meaning that body length increase with time is much more that the increase in the ratio of body width/head diameter.

In the other nine allometric relationships the allometric coefficient (b) is close for the two species indicating that both species show similar allometric growth between their body parts.

An interesting result is that the relationship between wing length and wing width in both species *Colotis phisadia* and *Colotis chrysonome* is isometric not allometric both showing (b) values of (0.973) and (0.999) respectively.

4.3 Growth Models

The results of the two growth models for both species *Colotis phisadia* and *Colotis chrysonome* are shown in tables (15) and (16). Each table shows the theoretically calculated maximum length, the value of A constant, the values of the growth coefficient (k) and the correlation coefficient (r) of the nonlinear estimation.

Von Bertalanffy model showed that for *Colotis phisadia* the growth equation is:

Body Length = $29 * (1 - 0.68 * e^{-0.0141*t})$

and for *Colotis chrysonome*

Body Length = $30 * (1 – 0.643 * e^{-0.015*t})$

While the Gombertz growth model showed that for *Colotis phisadia* the growth equation is:

Body Length = $29 * e^{(-1.097 * (e(-0.0194 * t)}$

And for *Colotis chrysonome*

Body Length = $30 * e^{(-0.991 * (e(-0.02 * t)}$

Table 15: Results of the Von Bertalanffy growth model

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Maximum Length	29	30
A constant	0.68	0.643
Growth Coefficient k	0.0141	0.015
Correlation Coefficient R	0.879	0.923

Table 16: Results of the Gombertz growth model

Parameter Values	*Colotis phisadia*	*Colotis chrysonome*
Maximum Length	29	30
A constant	1.097	0.991
Growth Coefficient k	0.019	0.02
Correlation Coefficient R	0.896	0.936

Chapter 5 Discussion

The results show that the use of morphometric analyses can be used to differentiate between species of the same genus. Six allometric relationships showed large differences in the allometric coefficient (b) as listed in the results section. These differences can be explained by the presence of enough differences in the genetic material between *Colotis phisadia* and *Colotis chrysonome* to warrant large differences in the allometric coefficient or due to environmental pressure during ontogenetic development. Azrizal-Wahid *et al*. (2016) reported the success use of morphometry to discriminate between six species of *Eurema* butterflies. Benitez *et al.* (2013) stated that the differences in wing shape found out after an allometric and non-allometric study raise the question of whether sexual dimorphism of wing shape may be modulated by natural selection which indicate the presence of two clearly different genetic materials. Ramirez-Ponce *et al.* (2017) stated that different models of selective forces have been proposed and tested to explain allometry within a framework of natural or sexual selection, with a link between positive allometry and sexual selection generally accepted when exaggerated traits are present. The results of Akand *et al*. (2017) are in clear support of the findings of this research. They stated that morphometric analysis of forewing length, hind wing length, body length and antenna length of the species under the two subfamilies Polyommatinae and Theclinae showed significant differences and these differences stand among the species of both the subfamilies and produced good results to identify the species more correctly. The results of Chazot *et al*. (2015) demonstrated that microhabitat has driven wing shape evolution, although it has not strongly affected forewing and hindwing integration. Furthermore, sexual dimorphism of forewing shape and color pattern are coupled, suggesting a common selective force. Owen (2012) stated that morphometric analysis has proved to be particularly useful for species identification and classification. The more traditional approaches appear to be as sensitive as geometric morphometrics for many problems. He concluded that a powerful approach is to combine morphometric genetic methods, particularly to help answer questions of systematic and taxonomy.

A number of researchers successfully used allometric analyses for various purposes and few will be mentioned to stress the point that morphometric analyses is a valuable tool for various purposes. Kunte (2007) used allometry to study proboscis length in butterflies. He reported that there is a strong positive relationship between relative proboscis length and handling time per flower. Breuker *et al*. (2010) used geometric morphometrics to understand the effects of the environment on possibly adaptive butterfly wing size and shape variation in ecologically relevant contexts. Patel *et al*. (2017) used allometry to study the allometric variations in the life stages of citrus butterflies. Elkarmi and Ismail (2007) indicated that morphometric studies were useful to differentiate between two populations of the same species of *Melanoides tuberculata* living in hot and in cold waters.

The results of this study as mentioned before showed that six allometric relationships revealed large differences in the allometric coefficient between the two species. The other nine relationships studied in this research did not show significant differences in the allometric coefficients. This can be expected since both butterflies belong to the same genus and thus must exhibit similarities in most allometric relationships.

The results of the two growth models showed slight variations in the growth coefficients for both species. The Von Bertalanffy growth model gave a growth coefficient (k) for *Colotis phisadia* of (0.0141) and for *Colotis chrysonome* (0.015). Furthermore, the Gombertz growth model results are (0.019) for *Colotis phisadia* and (0.02) for *Colotis chrysonome*. This indicates that the growth models are not greatly useful to differentiate between species.

A number of researchers used growth models for a number of purposes. Gould (1966) used growth models to show that different proportions of an organism can be correlated with changes in the absolute magnitude of the organism or of a specific part of the organism. Tschinkel *et al*., (2003) used the growth models to study the changes in body size and shape of an organism occur during the course of evolution. Palmer (1983) studied the growth rate as a measure of food value in Thaidid gastropods. He reported that there is a relationship between body growth and predator size, prey size and prey species. Elkarmi and Ismail (2006b) reported that the snail *Theodoxus macri* may survive for four years and by applying Bertalanffy's and Richards' growth models the maximum length may reach 21.4 mm. These studies indicate that the growth models are useful for a number of purposes. Unfortunately, due to the lack of studies on using growth models to differentiate between species, meaningful comparison with the results of this research cannot be carried out.

Chapter 6 Conclusion

1) Morphometric analysis can be used to differentiate between two species of the same genus.

2) The research determined fourteen allometric relationships between body parts of the two butterfly species and one isometric relationship.

3) The two growth models determined the maximum forewing length and the growth coefficient of both butterflies.

4) The growth models have limited use as a tool to differentiate between two species of the same genus.

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