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RELATIONSHIPS BETWEEN PUPAL SIZE AND SEX IN GIANT SILKWORM MOTHS (SATURNIIDAE)[1]

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ABSTRACT. Weights and dimensions are given for pupae of *Callosamia promethea* (Drury), *Eupackardia calleta* (Westwood) and *Hyalophora cecropia* (Linnaeus). Significant sex-related differences were observed in sample means for all characteristics studied, except antenna length in *C. promethea.* Discriminant function equations were derived for predicting sex in individual pupae of *E. calleta* on the basis of weight and antenna width data; and individual pupae of *H. cecropia* on the basis of circumference and antenna width data. Reliable discriminant function equations could not be derived for determining sex in individual *C. promethea* pupae. Within species, significant differences were observed for male and female antenna surface areas. Between species, antenna length to width ratios did not differ significantly for individuals of the same sex.

The ability to determine sex in lepidopterous pupae precludes the need to await adult emergence to identify individuals for breeding or experimentation, or to determine sex ratios or individual sexes. For lepidopterous pupae the dimensions of the antennae and the morphology of the genital openings have been the most reliable and widely used characteristics for determining sex. Other characteristics such as coloration, body size, and even behavior have been used for certain species. The fact that female pupae are generally larger than males has been noted by many lepidopterists, but such differences have not been quantified. (Mosher, 1914, 1916a, 1916b; Butt & Cantu, 1962; Solomon, 1962; Ehrlick et al., 1969; Villiard, 1969; Kean & Platt, 1973; Jennings, 1974; Muggli, 1974).

Mosher (1914, 1916a, 1916b) is the best available reference on sex-related characteristics of giant silkworm moth pupae; providing detailed descriptions of external morphology, length to width relationships for male and female antennae, and dimensional and weight data. The dimensional and weight data are of limited value, however, because she mentions neither the number of pupae examined nor the sex. Mosher (1916a, Plates V & VI) also illustrates genital openings for a few species but does not discuss these structures.

In some giant silkworm moths, such as *Eupackardia calleta* (Westwood) and *Hyalophora cecropia* (Linnaeus), pupae can be sexed cor-

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rectly in almost every instance because of consistently distinct differences in both the size of the antennae (Figs. 1–2) and the morphology of the genital openings (Figs. 5–6). In pupae of *Callosamia promethea* (Drury) the dimensions of male and female antennae are not sufficiently different to permit reliable sex determinations (Figs. 3–4). Mosher (1916b) reported that the antennae of male *C. promethea* pupae are slightly longer and wider than those of the female, but that the antenna length is never more than three times the width in either sex. Also, the morphology of the genital openings can be highly variable in *C. promethea*. For certain groups of wild and reared pupae many individuals cannot be correctly sexed by examining these structures. The usual genital opening morphology for *C. promethea* pupae is shown in Figs. 7–8.

Although there are observable differences in the size of male and female pupae of *E. calleta*, *H. cecropia*, and *C. promethea*, no information has been found in the literature to indicate that such differences have been quantified or studied to determine their value in discriminating sex. Therefore, certain size characteristics of the pupae of these three species were examined to: (1) quantitatively define sex-related differences in weights and dimensions and; (2) determine statistically whether such differences can be used singly, or in combination, to discriminate sex.

MATERIALS AND METHODS

Measurements made during the study were: body weight (WT); body length (BL); body width (BW); circumference (CE); antenna length (AL); and antenna width (AW). Weights were determined to the nearest 0.01 gram using a Mettler H542 Analytical Balance. Measurements of BL, BW, and AW were made to the nearest millimeter using a vernier caliper. Body length was the distance from the vertex of the head to the posterior end of the abdomen; BW was the width at the 4th abdominal segment; and AW was the maximum width measured perpendicular to the flagellum. Measurement of CE was made by placing a fine thread around the 4th abdominal segment and then measuring the thread on a metric ruler. Measurement of AW was made by placing a piece of monofilament nylon along the length of the flagellum and then measuring the piece of nylon on a metric ruler. Descriptive statistics (mean and 95% confidence interval) were cal-

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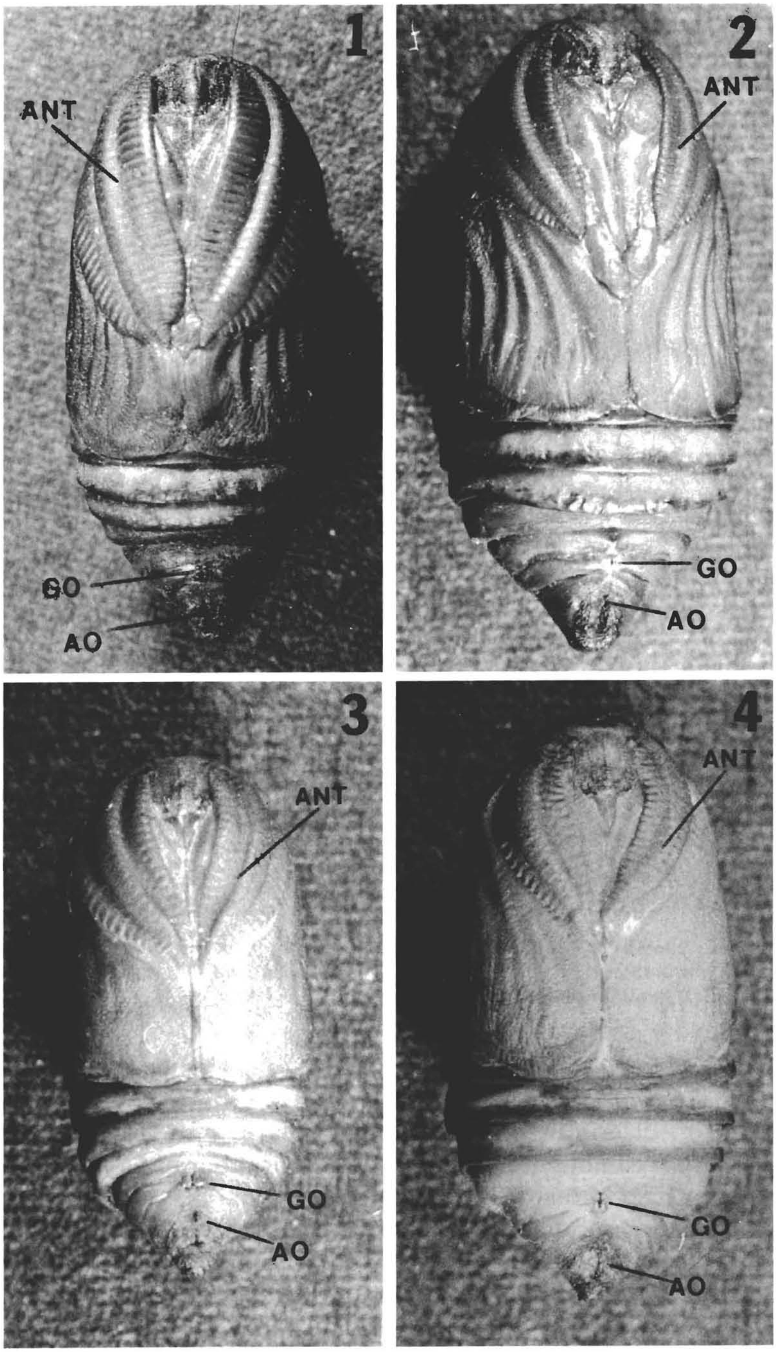
FIGS. 1–4. Ventral views of pupae. **1**, male *H. cecropia*; **2**, female *H. cecropia*; **3**, male *C. promethea*; **4**, female *C. promethea*. ANT, antenna; GO, genital opening; AO, anal opening.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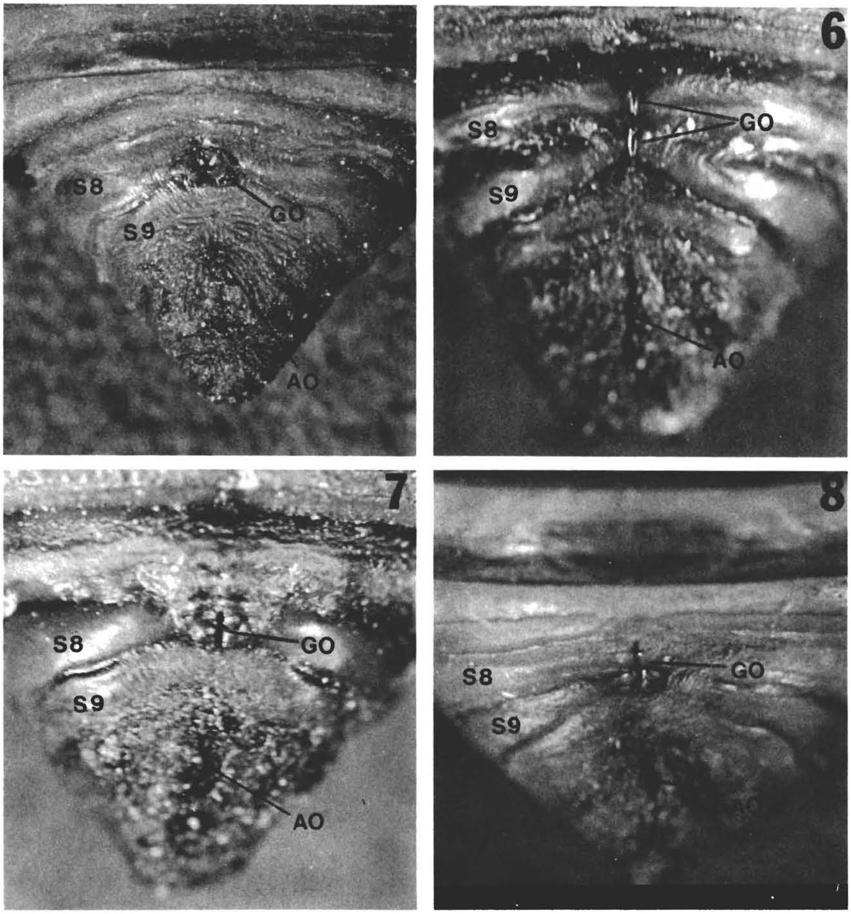


FIGS. 5–8. Details of genital openings. **5,** male *H. cecropia*; **6,** female *H. cecropia*; **7,** male *C. promethea*; **8,** female *C. promethea*. S8 and S9, 8th and 9th abdominal segments; GO, genital opening; AO, anal opening.

culated to quantitatively define sex-related differences. Data were subjected to discriminant function analysis (Freese, 1964) to determine whether combination measurements, or ratios of measurements, were better discriminators of sex than individual measurements. Discriminant function analysis was used to estimate coefficients for a series of pupal measurements. Discrimination of sex is based on the magnitude of the discriminant function (Y) calculated from the formula $Y = a_1X_1 + a_2X_2 + a_3X_3 \ldots + a_nX_n$, where X_i is a pupal measurement and a_i is a coefficient. The method assumes for each type of measurement that the variance is approximately the same for males and females. Generally, only those measurements that improve discrimination are used in the model. By using statistical tests (e.g., F) it is possible to determine, through sequential testing of pupal measurements, which coefficients differ from zero and should be included in the model. The sequential testing of coefficients assumes the normal distribution. In addition to the single pupal measurements discussed earlier, the following ratios were examined by discriminant function analysis: WT/AL, BL/AL, CE/AL, WT/AW, BL/AW, CE/AW, and AL/AW. Sources of specimens from which data were collected were various reared and wild specimens as follows: *C. promethea* were diapausing pupae collected in Harford County, Maryland, during the winter of 1973–74 ($n = 34$) and diapausing pupae collected in Portage County, Ohio, during the winter of 1974–75 ($n = 43$); *E. calleta* were diapausing pupae collected in various south Texas counties during the fall of 1974 ($n = 48$); *H. cecropia* were diapausing pupae reared in Harford County, Maryland, during the summer of 1973 ($n = 65$). Pupae that were later used to test the various discriminant function equations were either specimens reared in Frederick County, Maryland, or wild specimens collected in Frederick and Harford counties, Maryland, or various counties in south Texas.

RESULTS AND DISCUSSION

Pupal weights and dimensions for *C. promethea*, *E. calleta*, and *H. cecropia* are summarized in Table 1. Within species the sample means for males and females are significantly different ($P < 0.05$) for all measurements, except antenna length in *C. promethea*. The statistics shown in Table 1 are sample means, ±95 percent confidence intervals: they define an interval for population means but not individuals in the population. Thus, the interval statistics characterize the values of male and female pupae of these three species but will not validly discriminate sexes in individual pupae.

Discriminant function equations derived for determining sex in individual pupae of the three species are shown in Table 2. The discriminant function analysis program derived numerous equations; those shown in Table 2 are judged the most predictive calculation for each species. The validity of these equations was tested by using them to predict sexes in groups of wild and reared pupae. For *E. calleta*, we collected the appropriate data from individuals in two groups of reared pupae and one group of wild pupae from south Texas. The discriminant function equation ($DF_{ec} = -10.19WT + 39.22AW$) correctly calculated that there were 16 males and 16 females, and 7 males and 12 females, respectively, in the two groups of reared pupae,

TABLE 1. Weights and dimensions of giant silkworm moth pupae.[1]

Measurement	*Callosamia promethea*		*Eupackardia calleta*		*Hyalophora cecropia*	
	Male	Female	Male	Female	Male	Female
Number (n)	44	33	27	21	37	28
Weight (WT)	1.23 ± 0.08	1.83 ± 0.16	2.80 ± 0.12	4.03 ± 0.20	3.87 ± 0.13	5.16 ± 0.29
Length (BL)	21.77 ± 0.74	25.18 ± 0.74	29.18 ± 0.45	35.52 ± 0.55	34.30 ± 0.45	37.92 ± 0.91
Width (BW)	10.06 ± 0.31	11.90 ± 0.37	13.77 ± 0.32	15.57 ± 0.34	15.61 ± 0.20	17.25 ± 0.36
Circumference (CE)	32.63 ± 0.93	36.96 ± 1.09	42.44 ± 0.74	47.85 ± 1.06	45.19 ± 0.69	52.07 ± 1.11
Antenna length (AL)	10.65 ± 0.32	10.51 ± 0.39	14.18 ± 0.38	12.66 ± 0.54	18.38 ± 0.31	16.35 ± 0.34
Antenna width (AW)	3.68 ± 0.10	3.06 ± 0.08	4.94 ± 0.07	3.75 ± 0.24	7.08 ± 0.12	5.00 ± 0.00

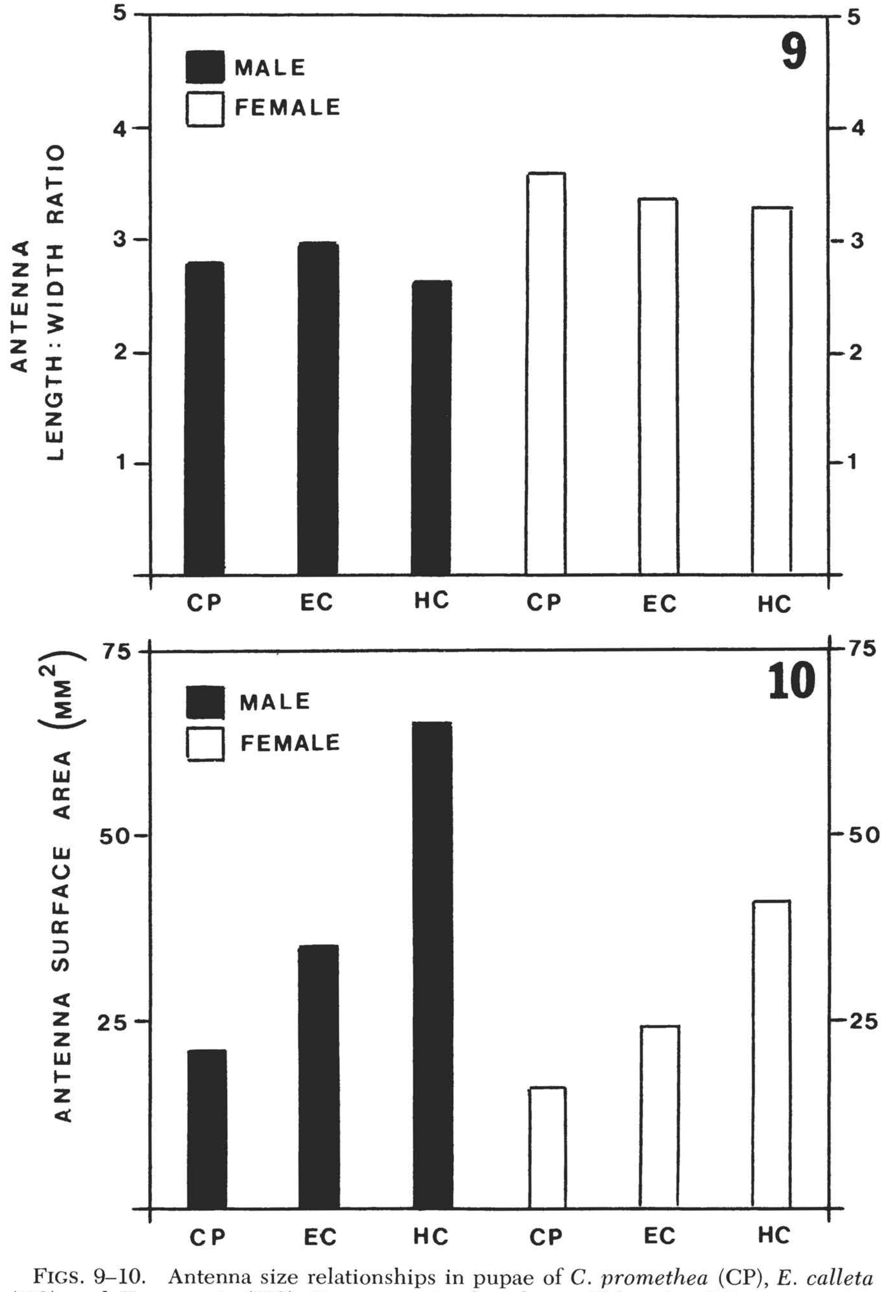
[1] Values are sample means ± 95% confidence intervals; weights in grams; dimensions in millimeters.

TABLE 2. Discriminant functions for determining the sex of giant silkworm moth pupae.

Discriminant function equation	Decision value
Callosamia promethea:	
$DF_{cp} = -2.69BW + 11.42AW$	female < 9.97 > male
$DF_{cp} = 3.66(CE/AL) + 5.35(BL/AW)$	male < 49.33 > female
Eupackardia calleta:	
$DF_{ec} = -10.19WT + 39.22AW$	female < 135.60 > male
Hyalophora cecropia:	
$DF_{hc} = -2.25CE + 33.08AW$	female < 90.28 > male

as confirmed by adult sexes at emergence. Similarly, 11 wild pupae of *E. calleta* were correctly sexed as 5 males and 6 females. For *H. cecropia* we examined an additional 11 reared pupae. This group contained 7 males and 4 females, as calculated by the discriminant function equation ($DF_{hc} = -2.25CE + 33.08AW$) and confirmed at the time of adult emergence. For *C. promethea* we examined two additional groups of pupae containing 16 and 10 reared individuals, respectively; and a third group containing 33 wild individuals. The discriminant function equation ($DF_{cp} = -2.69BW + 11.42AW$) misclassified 2 females in the first reared group, 7 females in the second reared group, and 5 females in the wild group, as determined by adult sexes at emergence. These misclassifications appeared to be due to a shift in the mean of some measurement used in the equation. Therefore, we used a similarly established predictive equation containing ratio information ($DF_{cp} = 3.66(CE/AW) + 5.35(BL/AW)$) to determine whether ratios might remain relatively constant when mean values were shifting. Using this second equation to calculate individual sexes of these three additional groups of *C. promethea* pupae resulted in similar misclassifications.

These studies have quantified differences in the weights and dimensions of male and female pupae of *C. promethea, E. calleta,* and *H. cecropia.* Antenna length in *C. promethea* was the only characteristic that was not significantly sex-related. This may account for the fact that antenna size in male and female individuals of this species is not a good discriminator of sex. Mosher (1916b) discusses antenna dimensions in terms of length to width ratios, but does not present data on absolute sizes. There is no known published information on the origin of describing lepidopterous antennae using ratios. The characteristic actually being perceived by an observer is the antenna surface area, and an approximation of that characteristic would seem



FIGS. 9–10. Antenna size relationships in pupae of *C. promethea* (CP), *E. calleta* (EC), and *H. cecropia* (HC). **9,** comparative length to width ratios; **10,** comparative surface areas.

to be more valuable than the length to width ratio. The antenna surface area (SA) can be approximated (SA = 0.5(AL × AW)) from the length to width information. Antenna length to width ratios and antenna surface areas are compared for male and female pupae in Figs. 9–10. The comparison quantifies the fact that there is less visually-perceptible difference in male and female antennae in *C. promethea* than the other two species and thus, more difficulty in discriminating sex on the basis of antenna size in *C. promethea*. Another point apparent from the length to width information is that for each sex the ratios do not differ significantly among the three species. Whether these very similar ratios for each sex have a relationship to the sensory function of the antennae in the adult moths is not known.

CONCLUSIONS

In *C. promethea* neither the combination measurements nor the ratios of dimensions used in the discriminant function analysis resulted in reliable equations for determination of individual sexes. Examination of the genital openings appears to be the best available way of determining sex in individual *C. promethea* pupae.

For *E. calleta* pupae, weight and antenna width were reliable indicators of individual sex when used in the discriminant function equation derived for this species.

For *H. cecropia* pupae, discriminant function analysis demonstrated that circumference and antenna width data are the most reliable dimensions for discriminating sex in individuals.

ACKNOWLEDGMENT

We acknowledge the assistance of R. S. Peigler in providing wild *Eupackardia calleta* (Westwood) pupae from south Texas; with these we were able to verify the discriminant function equation for that species.

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BOOK REVIEW

MICROLEPIDOPTERA, by Elwood C. Zimmerman. 1978. Insects of Hawaii, volume 9, xxiv + 1903 pages, 1355 cuts. Price: U.S. $60. University of Hawaii Press, Honolulu.

The first 200 pages are an overview of the Lepidoptera that includes a) classification, b) identification keys to the immature stages of the species found in Hawaii and separate keys to the larvae and pupae found in specific habitats, c) morphology of the immatures and adults, and d) techniques for preparing and handling adults and immatures for collections and for wing and genital studies. A checklist of the described (only previously described or misidentified species are treated) genera and species and list of nomenclatural changes are useful. Fourteen of the 80 genera and 605 of the 681 species are endemic. A synopsis of the distribution of genera and species by island illustrates the high degree of species' endemicity; however, lack of adequate collecting makes the tables preliminary.

The systematic treatment is a good survey of the microlepidoptera of Hawaii. Illustrations are abundant and cover the immature as well as the adult stages. Zimmerman has developed identification keys to most of the taxa. A notable exception is to the species of *Hyposmocoma* Butler. New generic names are proposed and defined when necessary. He has brought together published drawings of structural parts of adults and larvae of as many species as possible and reproduced them in this volume; so, the user has before him much of the extant illustrative material.

Zimmerman proposes a relatively conservative higher classification at the family level within the so-called microlepidoptera that is particularly noteworthy in the Gelechioidea. He places the Oecophorinae, Ethmiinae, Xyloryctinae, Blastobasinae, Chrysopeleiinae, Momphinae, Cosmopteriginae, and Gelechiinae as subfamilies of the Gelechiidae. Based on strict priority of family-group names, the superfamily and family should be Oecophoroidea and Oecophoridae (Bruand, 1850), not Gelechioidea and Gelechiidae (Stainton, 1854). I agree with Zimmerman's philosophy on the inflation of the classification of the microlepidoptera but not with all of his conclusions. However, the final word definitely is not written on classification, particularly that of the Gelechioidea. Some major differences are the following: *Thyrocopa* Meyrick is in the Autostichinae of the Oecophoridae rather than in the Xyloryctinae of the Gelechiidae. *Chedra* Hodges and *Batrachedroides* Zimmerman are in the Batrachedrinae of the Coleophoridae rather than in the Momphinae, Gelechiidae. Momphidae, *sensu stricto*, do not occur in Hawaii. Cosmopterigidae have two subfamilies in Hawaii, Cosmopteriginae with four genera, and Chrysopeleiinae with one introduced species and genus. Symmocinae are a subfamily of Blastobasidae rather than a tribe of the Gelechiinae. The correct spelling for Dichomerini is Dichomeridini. *Sitotroga* Heinemann and *Pectinophora* Busck are in the Chelariinae rather than the Gelechiinae. *Merimnetria* Walsingham is in the Anomologinae rather than the Aristoteliini.