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# Alternative ejaculate allocation tactics in relation to male mating history of the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae)

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**Abstract.** In polyandrous species, a male's fertilization success is strongly related to the number of sperm he carries and the mass of the ejaculate substance transferred to the female. However, because ejaculate production is costly and limited, males are expected to allocate their ejaculates adaptively among matings. In order to clarify the ejaculate allocation pattern in the polyandrous swallowtail butterfly, *Papilio xuthus* (Linnaeus, 1767), the spermatophore size and the number of sperm were counted just after the termination of the first and second copulations. Virgin males slowly increased the size of their spermatophores with age after eclosion, while there was a negative correlation between the ratio of sperm transferred to the female and the number of sperm produced. Males seemed to keep some sperm for further matings. On the other hand, the spermatophore size rapidly increased in males that had mated once, and these males transferred most of the sperm in their sperm storage organs at their second mating, irrespective of the number of sperm stored. Therefore, males might use their own mating history to tailor their ejaculates, probably assessing the probability of additional matings.

**Keywords**: apyrene sperm, eupyrene sperm, sperm competition, sperm transfer, spermatophore.

## Introduction

When a female insect receives and stores sperm from several males throughout her life, sperm competition commonly occurs in her reproductive organs (Parker, 1970). It has been shown that a male's fertilization success is strongly related to the number of sperm he carries as well as the mass of the ejaculate transferred to the female (Simmons, 2001). In the Lepidoptera, males transfer a single spermatophore that includes two types of sperm, viz. eupyrene and apyrene sperm, in each mating. Various nutrients contained in a spermatophore have been shown to contribute to somatic maintenance in the female (Boggs & Gilbert, 1979) and to increase her fecundity (Watanabe, 1988), and the spermatophore size may affect the length of the refractory period of the female (i.e., the amount of time the female is unreceptive to male courtship following copulation: Sugawara, 1979). On the other hand, Parker (1982) pointed out that a male's fertilization success is often proportional to its contribution to the total number of fertile eupyrene sperm in the female sperm storage organ. In contrast, apyrene sperm is non-fertile, and its role is not yet well known, though apyrene sperm in the female sperm storage organ, the spermatheca, was found to affect the length of the female refractory period relative to a subsequent mating in pierid butterflies (Cook & Wedell, 1999). Therefore, lepidopteran males who can transfer a larger spermatophore with more sperm generally have higher fertilization success, especially in polyandrous species (e.g. Wedell & Cook, 1998).

Sperm production must incur a cost for males (e.g. Dewsbury, 1982). Moreover, in some species, males require time to replenish their ejaculates following copulation, and subsequent copulations often last for much longer (e.g. Svärd & Wiklund. 1989), indicating that the production of other ejaculate substances to be included in the spermatophore is also costly. Thus, in order to maximize their reproductive success, males in polyandrous species should allocate ejaculate resources adaptively among their matings.

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It has been suggested that lepidopteran males can decide how many sperm to transfer in relation to the quality of their mates (e.g. Wedell & Cook, 1999a). For example, male *Plodia interpunctella* (Hübner, 1813) transferred more sperm when mating with more fecund females (Gage, 1998). In some species, males transferred more sperm to mated females (e.g. Solensky & Oberhauser, 2009). On the other hand, condition of the male itself may also affect ejaculate quality. Males who had waited longer until mating transferred larger spermatophores containing more sperm (e.g. Oberhauser, 1988; Watanabe & Hirota, 1999). In addition, males might increase investment into the current mating when the probability of future matings is low (Clutton-Brock, 1984). Wedell & Cook (1999b) stated that the probability of future matings of a male must decrease with the number of matings he has already achieved.

The aim of the present study is to clarify how male butterflies allocate their ejaculate substances in relation to their own mating status in the polyandrous swallowtail butterfly, *Papilio xuthus* (Linnaeus, 1767), in which females mate on average three times during their lifetime (Watanabe & Nozato, 1986). Due to the amount of spermatophore material and because the number of both types of sperm in the males' reproductive organs increase with days since eclosion (e.g. Riemann *et al.*, 1974; Wedell & Cook, 1999b), we first evaluate the production rate of the spermatophore material and both types of sperm in both virgin and previously mated males. To achieve that, we dissected both males and females soon after copula termination. During mating, males do not always transfer all sperm stored. So we dissected males and females just after the termination of copulation to calculate the real number of both types of sperm produced. Thereafter, by using these data, we examined the relationship between the number of sperm transferred to the female and the number of sperm produced until the mating (i.e. sum of sperm transferred plus the sperm remaining in the male reproductive organs) to assess the effect of a male's total actual sperm reserve on the ratio of sperm transferred.

## Materials and methods

### Mating experiments

In 2008, laboratory-reared adults of *P. xuthus* from the summer generations were weighed on the day of eclosion and given an individual number with a felt-tipped pen on their left ventral hindwing. The sexes were kept separately in flight cages (400×400×450 mm). They were fed on 20% sucrose solution for 10min each day until the first mating.

Virgin males of *P. xuthus* (n=29, one to five days old) were hand-paired with virgin females (one to three days old), as described in detail by Watanabe & Hirota (1999). Once they had copulated, each pair was put into a small cage, and the copula duration was measured. Immediately after the termination of copulation, the females were killed by decapitation and dissected so that the spermatophore mass and the numbers of both types of sperm in the spermatophore could be measured. The males were also killed by decapitation and dissected so that the numbers of both types of sperm that remained in their reproductive organs could be counted. Accordingly, the total number of sperm produced and the rates of transfer of both types of sperm could also be calculated.

To obtain the mated males in the experiment, virgin males (n=16, one day old) were first mated by hand-pairing and then kept in the flight cages until the second mating was induced. They were fed 20% sucrose solution for 10min each day until the second mating. One to five days after the first mating, they were remated with virgin females (one to three days old), again using the hand-pairing method. When the copulation had been terminated by the animals, the male and female were both killed and dissected to measure the spermatophore mass or the number of sperm in the reproductive organs. According to the preliminary observations, because few sperm was found in the duplexes of one-day-old males just after termination of the first copulation, most of the sperm transferred during the second mating that remained in the duplexes of males immediately after the second mating must have been produced later than the first mating.

Dissection of females to remove the spermatophore was started within 20min after the termination of copulation. During this time, no sperm migration from the spermatophore to the spermatheca occurred (Watanabe *et al.*, 2000). The bursa copulatrix was opened, and the intact spermatophore was carefully removed and weighed to the nearest 0.01mg. The numbers of both the eupyrene sperm bundles and free apyrene spermatozoa in the spermatophore were counted. The duplex and vas deferens of each male were also dissected, and the numbers of both eupyrene sperm bundles and apyrene spermatozoa were counted.

### Sperm counting procedure

In the Lepidoptera, eupyrene sperm bundles uniformly contain 256 free eupyrene spermatozoa (Virkki, 1969). They start to unravel in the spermatophore only after the termination of copulation, whereas apyrene sperm bundles unravel in the male as soon as they are released from the

testes (Katsuno, 1977). Eupyrene sperm bundles are clearly visible under the stereoscopic microscope at 40× magnification and appear uniform in size (Fig. 1a). Therefore, we mechanically disrupted the spermatophore, duplex and vas deferens, and counted the number of eupyrene sperm bundles directly under a stereoscopic microscope. Then, the ejaculates from each reproductive organ were washed in a small tube containing a known volume of saline water (Ringer's solution for insects). The tube was gently stirred for 1min to homogenize the spermatozoa suspension. A total of six subsamples (10µl) were removed from each primary sample using an autopipette and were allowed to dry on slides under dust covers. The dry slides were dipped in distilled water for approximately 3s and were then allowed to dry again. Each subsample was examined under dark-field phase-contrast microscopy at 100× magnification to count the number of apyrene spermatozoa (Fig. 1b). The number of apyrene spermatozoa in the spermatophore, duplex and vas deferens was calculated by multiplying the average 10µl sperm count by its dilution factor.


**Figure 1**. A photograph of an eupyrene sperm bundle (a) and an apyrene spermatozoon (b) from a spermatophore of *Papilio xuthus* under the stereo-microscope.

### Statistical analyses

Analyses were mainly performed using SPSS 12.0J for Windows. The copula duration of mated males was compared with that of virgin males in each resting period by using the Mann-Whitney U-test. Data including the spermatophore mass and the numbers of both types of sperm produced (sum of sperm transferred in the spermatophore plus the sperm remaining in the male reproductive organs) were analyzed using ANCOVA, with the male mating history (whether males mated for the first time or had mated previously) as a fixed factor and the number of days until mating (the number of days from eclosion until a male's first mating, or the number of days between a male's first and second mating) as a covariate. The numbers of eupyrene sperm bundles and apyrene spermatozoa transferred in a spermatophore were log-transformed and analyzed using ANCOVA, with male mating history as a fixed factor and the log-transformed numbers of each eupyrene sperm bundles and apyrene spermatozoa produced as covariates. We also tested if the coefficients of the regression lines of the ratio of sperm transferred were different from unity. In log-transformed space, a regression coefficient not significantly different from unity indicates that the ratio of sperm transferred did not change with the number of sperm produced. This analysis was performed using Microsoft Excel 2003. All numerical results are presented as mean±SE.

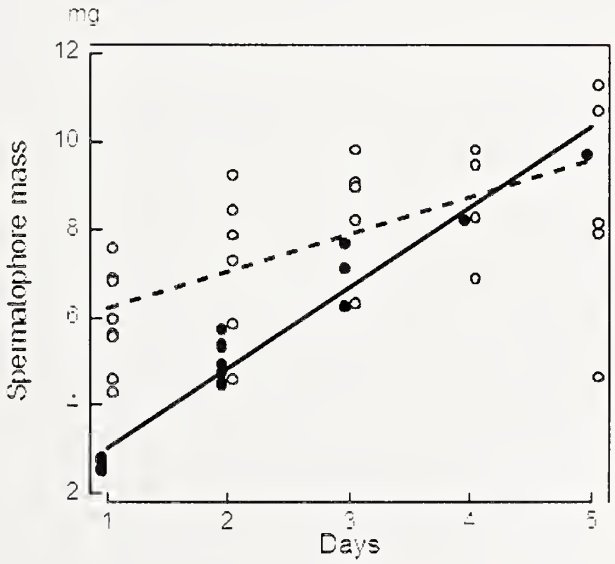


**Figure 2**. Changes in spermatophore mass produced at the first mating by virgin *Papilio xuthus* males (open circles) relative to days after eclosion, and at the second mating by mated males (solid circles) in relation to the number of days after their first mating. The dotted line represents the virgin males (Y=5.34+0.85X, $r^2$=0.35, n=29), and the solid line represents the mated males (Y=1.19+1.83X, $r^2$=0.94, n=16).

## Results

The hand-pairing method was effective in allowing each virgin and mated male to copulate successfully. As shown in Table 1 the copula lasted for approximately 1h in virgin males, and there was no significant difference among the days of resting (ANOVA; $F$=0.534, $p$=0.71). In previously mated males the copula lasted also approximately 1h, and duration was not significantly different from that of virgin males at each day until the focal mating.

A gel-like accessory gland substance and a teardrop-shaped spermatophore containing eupyrene sperm bundles and free apyrene spermatozoa were observed in the bursa copulatrix of the female in each copulation experiment. Males did not retain spermatophore substance in the simplex, while some eupyrene sperm bundles and free apyrene spermatozoa remained in the duplex and vas deferens.

A one-day-old virgin male on average transferred a spermatophore of 5.9±0.4mg (n=8). The spermatophore mass transferred by virgin males increased with days after eclosion, and five-day-old males produced a spermatophore of 9.4±1.3mg (n=6). On the other hand, mated males one day after the first mating transferred a spermatophore of only 2.7±0.1mg (n=4). The spermatophore mass produced by mated males also increased with resting periods. However, the interaction term between male mating history and days was significant (Table 2), indicating that the increase in the spermatophore mass of mated males was significantly higher than that of virgin males. Spermatophore mass of males five days after the first mating was roughly equal to that of five-day-old virgin males (Fig. 2).

The number of eupyrene sperm bundles produced by a male was the sum of the sperm transferred with the spermatophore plus the sperm remaining in the male reproductive organs. This amounted to approximately 41 eupyrene bundles for one-day-old virgin males, while males mated one day after the first mating produced 34 bundles. While the number of resting days significantly affected the number of eupyrene sperm bundles produced, the interaction term between male mating history and days was not significant (Table 2), indicating that the increase in the number of eupyrene sperm bundles produced was not different between virgin males and mated males. Five days after eclosion or first mating, both the virgin and mated males had produced approximately 150 eupyrene sperm bundles (Fig. 3a).

One-day-old virgin males produced approximately 263,000 apyrene spermatozoa, compared to 163,000 for males re-mated one day after the first mating. While the number of resting days significantly influenced the number of apyrene spermatozoa produced, the interaction term between male mating history and days was again not significant (Table 2), indicating that the increase in the number of apyrene spermatozoa produced was not different between virgin males and mated males. Five days after eclosion or initial mating, the virgin and mated males had produced approximately 650,000 and 500,000 apyrene spermatozoa, respectively (Fig. 3b).

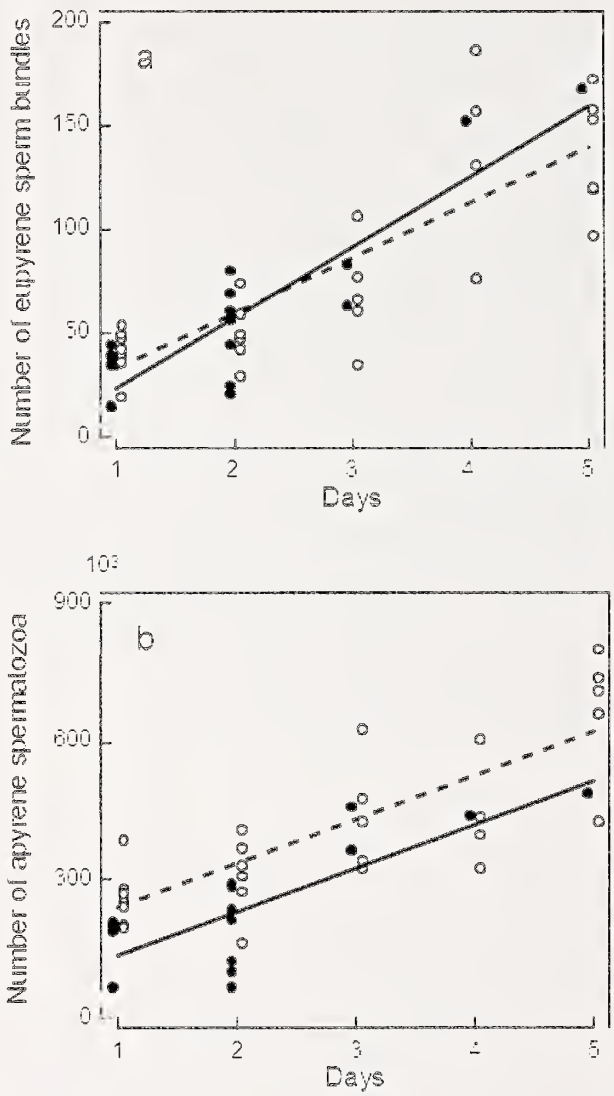


**Figure 3.** Changes in the number of eupyrene sperm bundles (a) and apyrene spermatozoa (b) produced before the first mating in virgin males (open circles) relative to days after eclosion, and before the second mating in mated males (solid circles) in relation to days after their first mating. Dotted line: virgin males (a) Y=7.01+26.44X, $r^2$=0.69; (b) Y=144566+95159X, $r^2$=0.66, n=29); solid line: mated males (a) Y=-11.36+34.16X, $r^2$=0.79; (b) Y=36150+96052X, $r^2$=0.63, n=15).

**Table 1.** Copula duration (min) of virgin males and mated males (mean±SE). For virgin males 'day of resting' means the days since eclosion, while for mated males it means days after the first mating. Figures in parentheses are sample sizes.

| Days of resting | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Virgin males | 60.8±2.7 (8) | 61.7±1.1 (6) | 64.8±7.6 (5) | 65.3±5.2 (4) | 68.0±4.3 (6) |
| Mated males | 57.0±5.0 (4) | 60.7±2.0 (7) | 57.0±0.6 (3) | 59.0 (1) | 91.0 (1) |
| Mann-Whitney U-test | $U$=14.0, $p$=0.81 | $U$=18.0, $p$=0.73 | $U$=6.5, $p$=0.79 | $U$=1.5, $p$=0.80 | $U$=0.0, $p$=0.29 |

**Table 2.** Effects of mating history and days until mating on spermatophore mass and number of both types of sperm produced in *P. xuthus*. Given are *F*-values from an analysis of covariance. Degrees of freedom were df = 1;44 for all variables except for spermatophore mass (where df = 1;45). *p<0.05; ***p<0.001.

| | Source of variation | | |
|---|---|---|---|
| **Response variable** | **Mating** | **Days** | **Interaction** |
| Spermatophore mass | 15.804*** | 46.964*** | 6.177* |
| Number of eupyrene sperm produced | 1.067 | 80.594*** | 1.309 |
| Number of apyrene sperm produced | 2.447*** | 52.855*** | 0.001 |

**Table 3.** Effect of mating history and the number of sperm produced on the number of sperm transferred in *P. xuthus*. Given are *F*-values from an analysis of covariance. Degrees of freedom: 1;44 for both response variables. *$p$<0.05; ***$p$<0.001.

| | Source of variation | | |
|---|---|---|---|
| **Response variable** | **Mating** | **No. sperm produced** | **Interaction** |
| Number of eupyrene sperm bundles transferred | 5.152* | 221.820*** | 5.064* |
| Number of apyrene spermatozoa transferred | 0.516 | 112.415*** | 0.404 |

Out of the 41 eupyrene sperm bundles produced by one-day-old virgin males, approximately 40 (97.6%) were transferred during their first mating, while five-day-old virgin males transferred approximately 95 out of 138 (70.0%) eupyrene sperm bundles produced. The positive relationship between the number of eupyrene sperm bundles produced and the number of eupyrene sperm bundles transferred to the female during copulation indicated that the number of sperm transferred increased with the number of sperm produced (Fig. 4a). However, the regression coefficient was significantly lower than unity ($t$=-4.102, $p$<0.001), indicating that the ratio at which eupyrene sperm bundles were transferred by virgin males decreased as the production of eupyrene sperm bundles increased.

For mated males, a positive relationship was also found between the number of eupyrene sperm bundles produced and the number of eupyrene sperm bundles transferred (Fig. 4a). However, the regression coefficient of mated males was not significantly different from unity ($t$=-0.154, n.s.), indicating that the ratio at which eupyrene sperm bundles were transferred did not change along with the number of eupyrene sperm bundles produced. In addition, the interaction term between male mating history and the number of eupyrene sperm bundles produced was significant (Table 3), indicating that the regression coefficient of mated males was significantly different from that of virgin males.

Out of 263,000 apyrene spermatozoa produced in one-day-old virgin males, approximately 245,000 (94.0%) were transferred in their first mating, while five-day-old virgin males transferred approximately 493,000 out of 665,000 (75.6%) apyrene spermatozoa produced. The number of apyrene spermatozoa transferred increased with the number of apyrene spermatozoa produced (Fig. 4b). The regression coefficient was significantly lower than unity ($t$=-2.593, $p$<0.05), indicating that the ratio at which apyrene spermatozoa were transferred by virgin males decreased with the number of apyrene spermatozoa produced.

For mated males, a positive relationship was also found between the number of apyrene spermatozoa produced and the number of apyrene spermatozoa transferred (Fig. 4b). The regression coefficient of mated males was not significantly different from unity ($t$=-1.064, n.s.), indicating that the ratio at which apyrene spermatozoa were transferred by mated males did not change with the number of apyrene sperm produced. The interaction term between male mating history and the number of apyrene spermatozoa produced was not significant (Table 3).

## Discussion

There was a significant increase in spermatophore mass in both virgin males and mated males with the number of days that had passed until the first and the second mating, respectively. For virgin males, spermatophore mass of five-day-old males was 1.5 times as large as that of one-day-old males. The spermatophore from the second copulation (one day after the first copulation) was less than half the size of the first, as reported by Watanabe & Hirota (1999). Spermatophore mass then increased with the duration of delay until the second mating in mated males, but the rate of increase in these mated males was about double that of the virgin males. As a result, spermatophore mass in mated males five days after the first mating was almost as large as that of five-day-old virgin males. Because females that receive a small spermatophore will remate sooner (Kaitala & Wiklund, 1994), males benefit from transferring a large spermatophore at each mating to ensure high reproductive success. Thus, the rapid increase in spermatophore size after the first mating is likely adaptive as it would allow males to engage in frequent mating in polyandrous mating systems. Comparative studies have also shown that spermatophore size in males of more polyandrous species recovered more rapidly (Svärd & Wiklund, 1989; Bissoondath & Wiklund, 1996).

Constancy of the copula duration might be also an adaptation for female polyandry. In the monandrous congener, *P. machaon*, the copula duration increased 9-fold when mated males mated a second time on the day after the first mating (Svärd & Wiklund, 1986). Such a prolonged copulation must incur a time cost for males. To achieve the frequent matings in a polyandrous mating system, males of *P. xuthus* might not increase duration of the copula, even if they have not transferred a sufficiently large spermatophore yet.

Giebultowicz *et al.* (1988) showed that sperm released from the testis to the duplex followed a daily rhythmic pattern, resulting in an increased number of both types of sperm in the duplex with days until

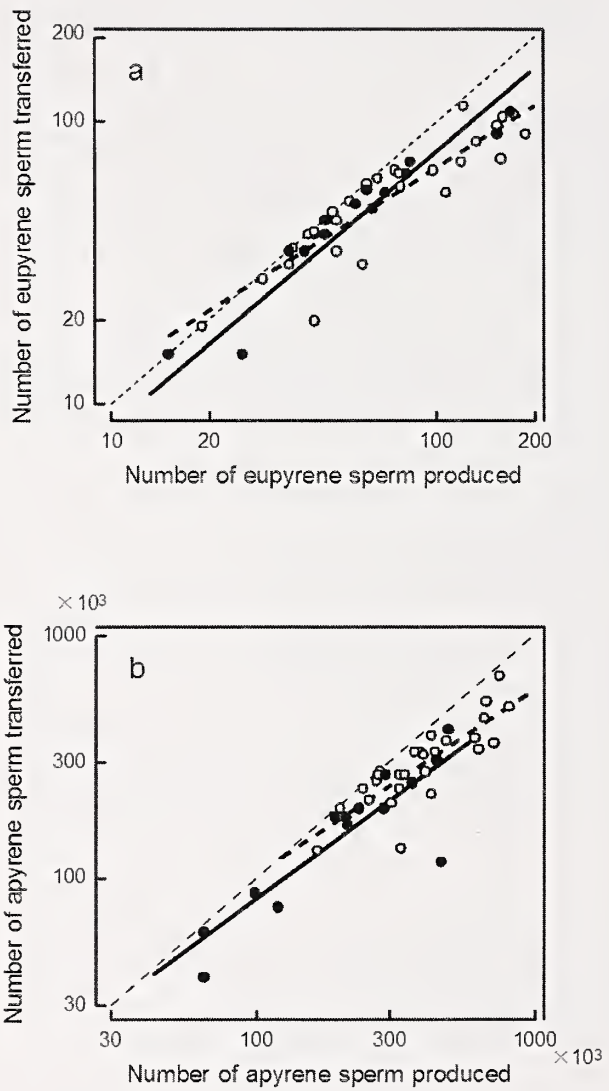


**Figure 4.** Relationship between the number of eupyrene sperm bundles produced (a) and of apyrene spermatozoa produced (b) to the number of eupyrene sperm bundles transferred. Virgin males: open circles with dotted line; previously mated males: solid circles with solid line. (a) virgin: logY=0.39+0.73logX, $r^2$=0.82, n=29; mated: logY=-0.07+0.98logX, $r^2$=0.88, n=15. (b) virgin: logY=1.23+0.76logX, $r^2$=0.71, n=29; mated males: logY=0.62+0.86logX, $r^2$=0.76, n=15.

mating. Sperm is likely accumulated in the duplex with age in males (Hiroyoshi & Mitsuhashi, 1999). In the present study, older males that had mated the same number of times stored more sperm than younger ones, which might be beneficial for older males.

Although the number of sperm transferred increased with the number of sperm produced, the sperm transfer ratio was low in virgin males which produced more sperm. An upper limit on the amount

of sperm transfer might explain the decrease in the ratio of sperm transferred with the number of sperm produced. However, the bursa copulatrix of lepidopteran females can expand to include multiple ejaculates (e.g. Drummond, 1984), and sperm is only a small fraction of the ejaculate. Hence, sperm availability is unlikely to act as major limitation to the number of sperm transferred. Rather, virgin males probably regulate the ratio of sperm transferred in relation to their own sperm reserve for future matings. Because the number of sperm produced was associated with male age, we cannot rule out the possibility that the ratio of sperm transferred decreased with male age. But this is unlikely. Clutton-Brock (1984) pointed out that older males should invest more in the current mating than younger males due to the lower probability of future matings, in agreement with the terminal investment hypothesis.

Mated males transferred sperm at a stable ratio irrespective of the number of sperm produced. Most of the sperm produced by mated males was transferred during the current mating. Although the regression coefficient of the number of apyrene spermatozoa transferred on the number of apyrene spermatozoa produced was not significantly different between virgin males and mated males, males seemed to change their sperm ejaculation tactics after the first mating. Because the probability of future matings must be negatively associated with the number of matings already achieved (Wedell & Cook, 1999b), a mated male attains higher reproductive success by transferring as much sperm as possible to his current mate. Therefore, the difference in the sperm ejaculate tactics between virgin and mated males might correspond to the probability of future matings.

Ejaculate allocation might be affected not only by the probability of future matings, but also by other life-history traits. Cook & Wedell (1996) showed that in *Pieris rapae* (Linnaeus, 1758) mated males transferred a smaller spermatophore than virgin males, although larger numbers of both types of sperm were included. This might be an adaptation to the different risks of sperm competition between the first and the second mating (Wedell & Cook, 1999b). In *P. xuthus*, males accelerated the production speed of spermatophore material after their first mating. In addition, the ratio of sperm transfer was higher in the second mating than in the first mating. These results suggest that males need to transfer a large spermatophore with much sperm in the second mating. Due to the long flight season and their relatively long adult lives (Watanabe & Kobayashi, 2006), the age structure of females in the populations is complex. Thus, males could have a high likelihood of encountering females of various levels of fecundity and with various mating histories. Changes in the rate of production or in the ratio of transfer of ejaculate substances might therefore reflect the low predictability of the mating history of males' future mates.

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NOTE

# The historical occurrence of *Hesperia attalus* (Lepidoptera: Hesperiidae) in New England

Determining historical range limits of species often relies on eyewitness accounts and, in the best possible cases, location data from historical collections. *Hesperia attalus* (W. H. Edwards, 1871), the dotted skipper, is a rare butterfly with a disjunct distribution in small pockets from New Jersey south to Florida and west to Oklahoma, prompting Cech and Tudor (2005) to remark that its "dotted" name better describes its range than its appearance. The species is restricted to open grassy areas, commonly in sand or pine barrens, in eastern states (Schweitzer *et al.*, 2011). Several historical records from New England inform speculation about this species' former range; we reviewed these records in the literature and, when possible, located associated specimens for verification.

Samuel Scudder (1889) was the first to report *Hesperia attalus* occurring in New England, noting that several individuals were collected by R. Thaxter in Belmont, near Boston, Massachusetts. At least nine specimens of *H. attalus* from Scudder's collection survive in the Museum of Comparative Zoology (MCZ) at Harvard University (Table 1). Two specimens are clearly labeled May 12, [18]71 and May 11, [18]71 without a location. The time of year is far too early for adults in any northeastern population, so they are most likely from a more southern, double-brooded population. Two 'type' specimens of *Ocytes seminole* Scudder, 1872 have no collection data on the labels but Scudder (1889) states they are from 'Texas'. These specimens bear the numbers "73" and "74" respectively, while an additional specimen in the MCZ collection has no collection data but bears the number "75", suggesting it may have been in the original series examined. Three specimens collected by Morrison are labeled "Florida". Two of these bear Scudder Coll. labels, but it is likely that all three were in Scudder's collection. A specimen from New Jersey may be one of those referred to in Scudder (1889) when he states he "has seen specimens from New Jersey".

The final specimen in Scudder's collection is labeled "Mass! Thaxter" and presumably is one of the 'several' specimens Scudder refers to as having been collected at Belmont, near Boston, MA. Collection date of the Belmont specimen is not recorded. This late 19th century specimen (Fig. 1: A, C, E) has been examined by AW and found to be consistent with phenotypes from northeastern populations of *H. attalus*. While no geographic variation has been reported in populations of *H. attalus slossonae* (Skinner, 1890), the subspecies of dotted skipper occurring in the eastern United States from New Jersey to Florida, we have noticed subtle but consistent differences between northern and southern populations of the taxon. Adults from univoltine populations in New Jersey average slightly larger than those from bivoltine populations to the south, and both sexes tend to have smaller ventral hindwing spots. Some males from New Jersey completely lack ventral hindwing spots, a trait not seen in adults from further south. Differences between far northern and more southerly populations are pronounced enough that adults from New Jersey are usually identifiable even when locality data are not examined.

Scudder remarked that he knew "nothing concerning ... the seasons of this butterfly", even though he had collection dates from elsewhere in the range. Thus we infer he was referring specifically to northeastern populations – the focus of his book. Scudder also wrote "There is, indeed, no other butterfly of our fauna of which so little is known." These two statements from the regional expert underscore the rarity of *H. attalus* in New England at that time. Extant populations are known today in only one northeastern state (NJ) and only eight states nationally (FL, GA, KS, NC, NJ, OK, SC, TX: Schweitzer *et al.*, 2011; McGuire Center collection). Arthur Shapiro collected a specimen in 1971 on Staten Island, NY; this population (if it was not a wayward stray) almost certainly did not persist, as four decades have passed without another Staten Island or even New York State record despite some directed

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**Table 1**. Specimens of *H. attalus* in the Scudder collection at the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

| Catalog Number | Collector | Date | Location | Notes |
|---|---|---|---|---|
| MCZ-ENT00190769 | | May 12, [18]71 | | |
| MCZ-ENT00190771 | | May 11, [18]71 | | ill. Plate 17, figure 12 Scudder (1889) |
| MCZ-ENT00191301 | | | Texas[1] | Syntype of *Ocytes seminole* Scudder, 1872 |
| MCZ-ENT00008971 | | | Texas[1] | Syntype of *Ocytes seminole* Scudder, 1872 |
| MCZ-ENT00190770 | | | | |
| MCZ-ENT00190761 | Morrison | | Florida | |
| MCZ-ENT00190763 | Morrison | | Florida | |
| MCZ-ENT00190764 | Morrison | | Florida | No Scudder coll. label[2] |
| MCZ-ENT00190766 | | | New Jersey | |
| MCZ-ENT00190768 | Thaxter | | "Mass!" | |

[1] From Scudder (1889), no loc. on label; [2] two preceding Morrison specimens are labeled "73" & "74", and this one "75" so likely a series

searching (Shapiro & Shapiro, 1973; Gochfeld & Burger, 1997; H. Zirlin, pers. comm.; A. Shapiro, pers. comm.). A historical southeastern PA record adjacent to the NJ border also has not been repeated in decades since (Schweitzer *et al.*, 2011).

Farquhar (1934) stated that W.T.M. Forbes collected the Dotted Skipper in Massachusetts; and Forbes (1960) mentioned *H. attalus seminole* (Scudder, 1872) specimens in the collections at Cornell, Harvard, including the Scudder collection, and the British Museum (Natural History; now Natural History Museum, London). An examination of material in the Cornell University Insect Collection revealed no *H. attalus* specimens collected in New England. However, two specimens of the closely related *H. leonardus* (T. Harris, 1862), collected by Forbes in Paxton and Princeton, MA, and possibly a third from Princeton without collector's name, are in the Cornell collection. All the *H. attalus* specimens located in The Natural History Museum, London are from Florida although the hesperiid collection is not completely indexed (B. Huertas, pers. comm.). Thus we were unable to confirm any *H. attalus* records for New England as cited by Farquhar (1934) or Forbes (1960).

The only other, and most widely cited record from New England is a specimen taken by Charles P. Kimball in September 1938 on Nantucket Island, Massachusetts (Jones & Kimball, 1943). This record has persisted in most butterfly guides since that time, either for inclusion in MA, RI and CT as part of the species range (Howe, 1975; Pyle, 1981, Opler & Malikul, 1992) or as a stray (Opler & Krizek, 1984 [location of record on map is incorrect]; Glassberg, 1999; Cech & Tudor, 2005; Schweitzer *et al.*, 2011). The specimen resides in the Nantucket Maria Mitchell Association's insect collection, in somewhat poor condition (Fig. 1: B, D, F). A close examination of the specimen by AW revealed that it is an atypical *H. leonardus*, a common species on the island, and not *H. attalus*.

This worn male specimen shows an enlarged dark area around the dorsal forewing stigma and a largely orangish hindwing consistent with *H. leonardus*, rather than the dorsal hindwing macules appearing dot-like as in eastern *H. attalus*. Ventral spotting on the specimen is somewhat reduced for a typical *H. leonardus* on the east coast and subterminal spots on the hind-wing underside form a continuous band without much spacing between them as *H. attalus* would show. The front angle of the forewings is less acute than in *H. attalus*, especially in a normal male specimen. Overall size is quite small for either species. The date of capture, the 19th of September, is also atypical for *H. attalus*, which has a late July-August flight period in New Jersey, but typical of *H. leonardus*, a common skipper in September on Nantucket (LoPresti, pers. obs.).

Only one of the five *H. attalus* records from New England (all from Massachusetts) could be confirmed in our investigation. None of Forbes' *H. attalus* specimens could be located, perhaps due to misidentifications of the closely related *H. leonardus*. The specimen taken by Charles P. Kimball in 1938 on Nantucket Island, Massachusetts, is now regarded as a somewhat aberrant *H. leonardus*. Thus the only valid record appears to be Roland Thaxter's specimen from Belmont, MA, as reported by Scudder (1889).

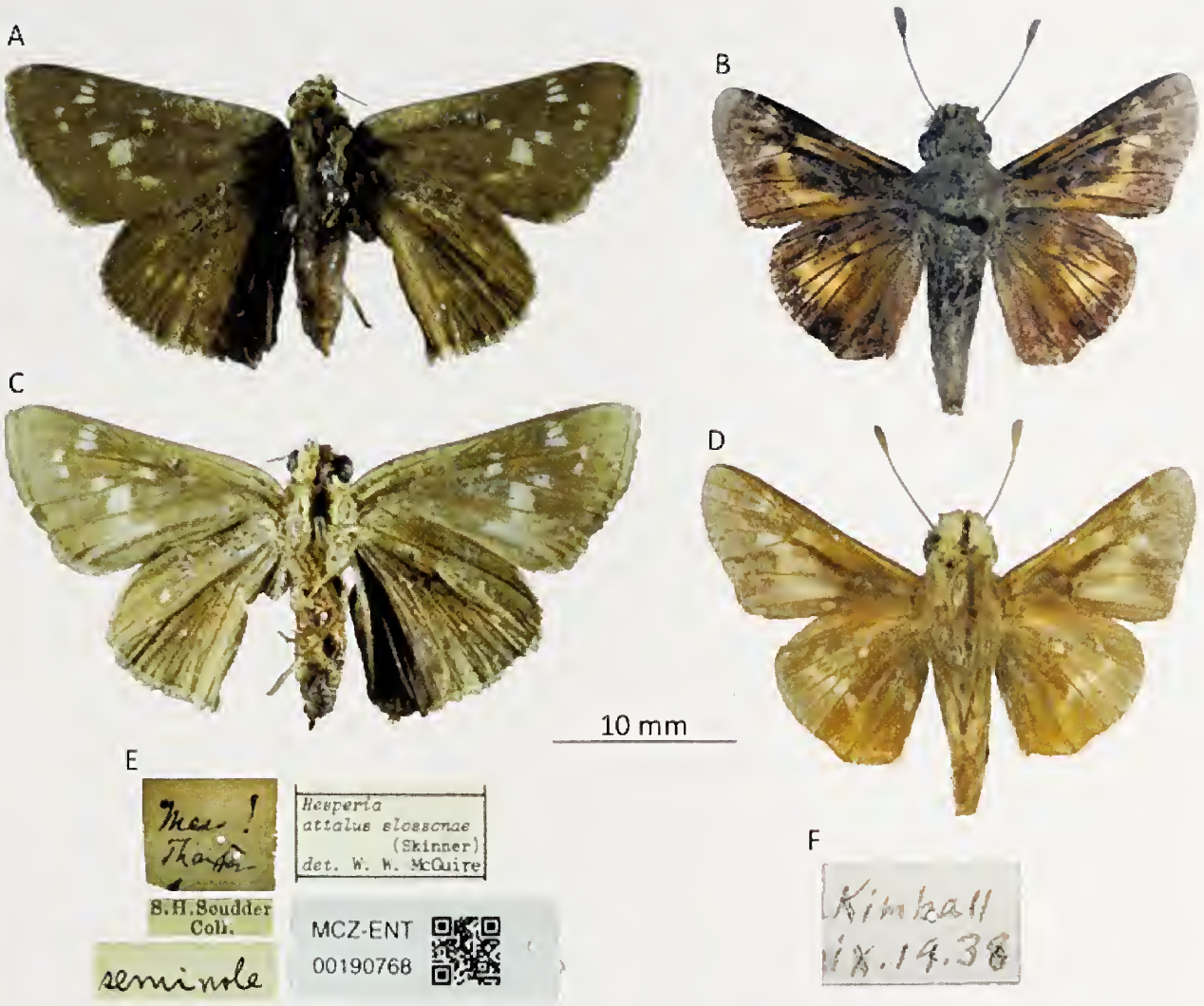


**Figure 1**. Dorsal and ventral views and pin labels of R. Thaxter's *H. attalus* (A, C, E) (copyright President and Fellows of Harvard College) and C.P. Kimball's *H. leonardus* (B, D, F) (Maria Mitchell Association).

Thaxter's upstanding character supports the validity of this record, which is further supported by Scudder's acceptance of the record. Thaxter attended school in West Newton (close to Belmont) and Cambridge before graduating from Harvard with both an A.B. magna cum laude and a PhD. He was a well-respected botanist and mycologist as well as an experienced collector and naturalist with a high regard for scientific accuracy (Clinton, 1935). Between 1875 and 1884, he was actively collecting Lepidoptera in New England and published 10 papers on his observations, mostly in Psyche and The Canadian Entomologist. Although employed as a botanist, he continued working on Lepidoptera, in 1891 publishing a paper on food plants of moths in the families Bombycidae and Noctuidae (Thaxter, 1891). He spent most of his life in New England and published 90 papers and monographs; it seems unlikely that he would allow an inaccurate collecting location record attributed to him (the specimen is correctly identified), to be published or perpetuated.

Thaxter's specimen closely matches the phenotype of specimens from New Jersey, the nearest extant population, consistent with its northeastern provenance. This intriguing record suggests an ephemeral population at that time or a rare incursion of wayward individuals into New England during unusually favorable conditions in the late 1800's. Despite widespread collecting and observations in Massachusetts and elsewhere in New England during the past two centuries, this unique record suggests that *H. attalus* was never common in the region.

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We thank Ryan St. Laurent and Jason Dombroskie (Cornell University), Andrew McKenna-Foster (Maria Mitchell Association), and Blanca Huertas (Natural History Museum, London) for providing data on specimens in their care. Art Shapiro provided information on his Staten Island record and Harry Zirlin advised of many unsuccessful attempts to find *H. attalus* on Staten Island since.

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# A cryptic new *Potamanaxas* (Hesperiidae: Pyrginae: Erynnini) stands out by terminally elongated genitalic valvae

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**Abstract.** *Potamanaxas louisghilli* Grishin, **sp. nov.** is described from Area de Conservación Guanacaste (ACG) in northwestern Costa Rica. Superficially, this species resembles several other *Potamanaxas* taxa with entire pale discal bands on the wings, but is distinguished from them by a row of faint postdiscal forewing spots (not streaks), terminally elongated genitalic valvae, and distinctive COI DNA barcodes. Found feeding on rain forest epiphytic *Cavendishia axillaris* and *Psammisia ramiflora*, the new species is likely to be host-specific to rain forest epiphytic Ericaceae, which are the food plants of all other known species of ACG *Potamanaxas* as well.

**Key words:** cryptic species, biodiversity, caterpillars, skipper butterflies, genitalia, Area de Conservación Guanacaste, Costa Rica.

## Introduction

*Potamanaxas* Lindsey, 1925 (Hesperiidae: Pyrginae: Erynnini) is "a compact genus" (Evans, 1953) consisting of phylogenetically close relatives, characterized by pale discal bands on both wings, frequently disjointed into spots, and genitalic tufts of hair-like scales at the bases of the male valvae (Grishin, 2013c). After recent taxonomic changes and description of several new species (Grishin, 2013a–f), *Potamanaxas* currently includes 28 species (Warren *et al.*, 2014) and this number is likely to change upon further research. Some *Potamanaxas* species are quite rare in collections, resulting in small series of many taxa (e.g., Bell, 1956).

The species-rich specimens from a long-term comprehensive inventory of the non-leaf-miner species of Lepidoptera of Area de Conservación Guanacaste (ACG) in northwestern Costa Rica (Janzen *et al.*, 2009; Janzen & Hallwachs, 2011) are extraordinarily useful for flushing out rarely collected species. Because most of the specimens have been reared from wild-caught caterpillars, knowledge of their traits, food plants, ecology, etc., greatly augments the usual data from adult morphology. Moreover, short sequences (ca. 658 bp) of mitochondrial DNA coding for the C-terminal segment of cytochrome c oxidase subunit 1 (COI), and dubbed "DNA barcodes", are routinely obtained for many specimens (Janzen *et al.*, 2011), adding molecular characters to those of morphology and biology. These DNA barcodes have been remarkable flags, both indicating possible new species, and identifying recognized species (Hebert *et al.*, 2004; Burns & Janzen, 2005; Janzen *et al.*, 2009; 2011; 2012; Burns *et al.*, 2008; 2010; 2013; Grishin *et al.*, 2013a, b; 2014a, b).

Four or five *Potamanaxas* species have been found during these efforts (Janzen *et al.*, 2011; Janzen & Hallwachs, 2014). What was called *Potamanaxas unifasciata* (C. Felder & R. Felder, 1867) in earlier inventory publications is now known to be *Eburuncus unifasciata*, and is not considered here. Each ACG *Potamanaxas* brings a small puzzle to the table and requires a dedicated research project to name. One of these species, known from a series of four reared specimens, is the easiest to approach. While cryptic in wing patterns that resemble those of several other species, it differs prominently from all named taxa with entire (not separated into spots) pale bands on the forewing by having terminally

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elongated genitalic valvae. Here, we formally describe this species, illustrate specimens, and discuss the differences from other *Potamanaxas* taxa, both in facies and in genitalia.

## Material and methods

Adult specimens used in this study were from the following collections: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); McGuire Center for Lepidoptera and Biodiversity, Gainesville, FL, USA (MGCL); Natural History Museum, London, UK (BMNH); Museum für Naturkunde, Berlin, Germany (ZMHB); and American Museum of Natural History, New York, NY, USA (AMNH). All specimens reared from wild-caught caterpillars by the ACG inventory are so indicated by having a specimen voucher code in the format yy-SRNP-x..., where "yy" are the two last digits of a year and "x..." is the serial number of a specimen recorded in that year, from 1 to 6 digits, such as 5289 or 22467. This SRNP code can be searched for on the inventory web site (Janzen & Hallwachs, 2014) and soon, in general internet search engines. Being reared, they are different from the net-caught wild adults that usually populate museums, in that they are on average slightly smaller, owing to the food offered being of lesser quality than the food the caterpillar chooses on its own in the wild.

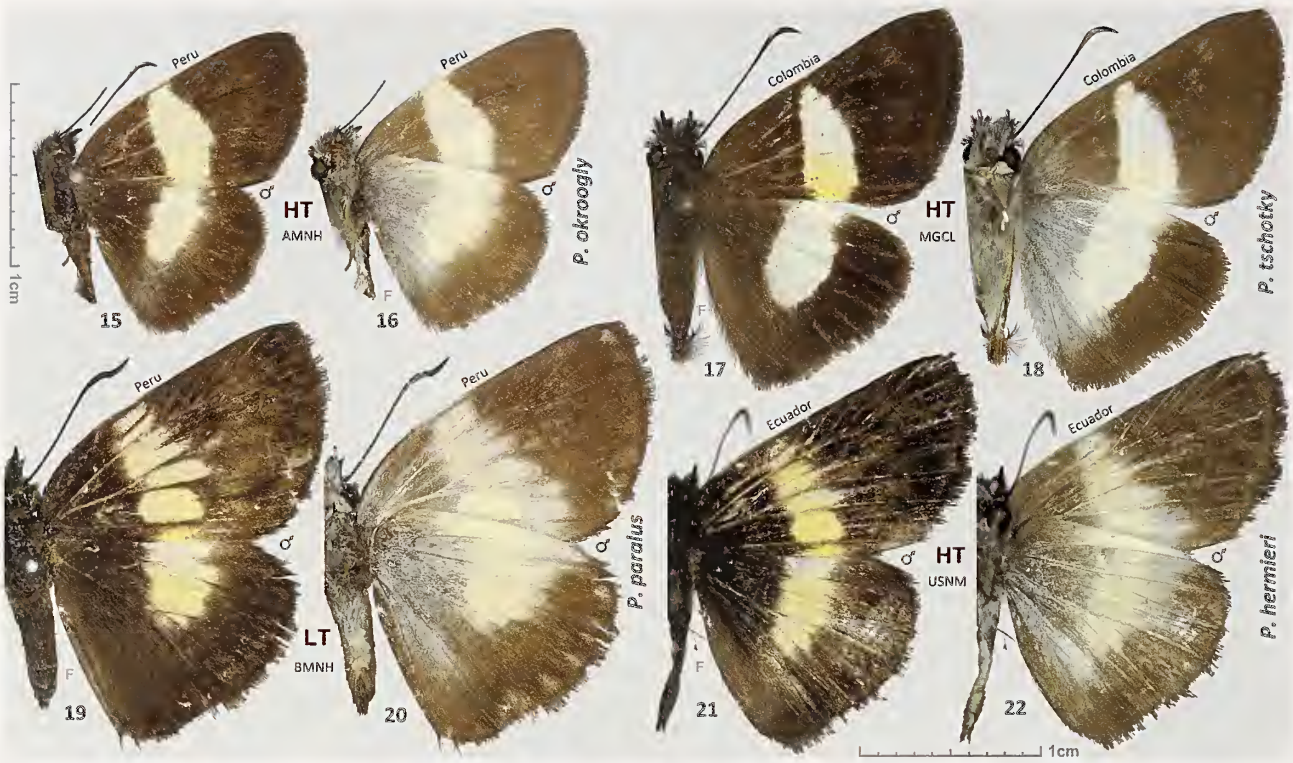
Standard entomological techniques were used for dissection (Robbins, 1991), i.e., the distal part of the abdomen was broken off, soaked for 40 minutes (or until cleared) in 10% KOH at 60°C (or overnight at room temperature), dissected, and subsequently stored in a small glycerol-filled vial on the pin under the specimen. Genitalia and wing venation terminology follows Steinhauser (1981). Length measurements are in metric units and were made from photographs of specimens taken next to a scale and magnified on a computer screen. Photographs of specimens and dry genitalia were taken by the author with Nikon D200 and Nikon D800 cameras through a 105 mm f/2.8G AF-S VR Micro - Nikkor lens; dissected genitalia were photographed in glycerol with the Nikon D200 camera without the lens and through microscopes at 2x, and 5x magnifications. Images were assembled and edited in Photoshop CS5.1. Genitalia photographs were taken in several focus slices and stacked in Photoshop to increase depth of field. DNA sequences were downloaded from GenBank (http://genbank.gov/) or BOLD (http://www.boldsystems.org/). They were aligned by hand (since they matched throughout their length without insertions or deletions), and analyzed using the Phylogeny.fr server (http://www.phylogeny.fr/) with default parameters (Dereeper *et al.*, 2008). Many of these sequences have been reported in Janzen *et al.* (2011) and photos of specimens are available from the Area de Conservación Guanacaste (ACG) on-line database (Janzen & Hallwachs, 2014) and BOLD database (Ratnasingham & Hebert, 2007) to confirm or suggest identifications.

## Results and discussion

Mass rearing of ACG caterpillars of Hesperiidae produced several species of *Potamanaxas* (Janzen *et al.*, 2011), one of which clearly stands out by the morphology of its male genitalia. Terminally elongated genitalic valvae set it apart from all named *Potamanaxas* species with pale entire discal bands on their wings, and resemble only the genitalia of *P. paralus* (Godman & Salvin, 1895) and *P. hermieri* Grishin, 2013. However, these two species differ in having a less robust, longer tegumen relative to the uncus arms, narrower and more elongated saccus, the process of the sacculus positioned farther from the base (Fig. 24b, c), and the forewing pale band more strongly fragmented into spots (Figs. 19–22). COI DNA barcodes suggest that this ACG species might be closely related to *P. paphos* Evans, 1953

**Figures 1–22 (Opposite page).** Type specimens of *Potamanaxas*. **1–6**. *P. louisghilli* **n. sp.** from Costa Rica, ACG: holotype ♂ (**1–2**); paratype ♂, voucher 91-SRNP-132 (**3–4**); paratype ♀, voucher 11-SRNP-31012 (**5–6**), data in text, specimens in USNM. **7–8.** *P. paphos* [holo]type ♂ Ecuador: Paramba, dry season, Apr-1897, 3500', leg. Rosenberg, Rothschild Bequest B.M. 1939-1, specimen No. BMNH(E) #1054150 [BMNH]. **9–10.** *P. melicertes* holotype Panama: "Chiriqui" [Chiriquí Prov., Chiriquí village on the highway, Pacific slope, about 12 km east of David, approx. 8° 23' N, 82° 20' W, per Selander & Vaurie (1962)], leg. Trötsch, Staudinger collection [ZMHB]. **11–12**. *P. thoria* syntype ♂ Ecuador, Hewitson collection 79-69, type H 767, specimen BMNH(E) #1054002 [BMNH]. **13–14**. *P. pammenes* (junior subjective synonym of *P. thoria*) syntype ♀ Nicaragua: "Chontales" [Chontales or Río San Juan Departments, per Selander & Vaurie (1962)], leg. T. Belt, type specimen figured, Godman-Salvin collection 1912–23, type H 766, specimen BMNH(E) #1054001 [BMNH]. Dorsal and ventral surfaces are shown on odd- and even-numbered figures, respectively. Labels are shown for the *P. louisghilli* holotype and are reduced about 2.5 times compared to specimens: the smaller scale bar below one of the labels refers to labels, and the larger scale bar refers to specimens. "F" indicates mirror image (left-right inverted). Images of BMNH specimens are copyright of Trustees of the Natural History Museum, London; used with permission.


HOLOTYPE ♂
Potamanaxas
louisghilli Grishin
Costa Rica
HT
USNM
♂
LEGS AWAY
FOR DNA
NVG120922-46
1cm
1
2
P. louisghilli
PT
3
4
♀
5
6
Ecuador
HT
BMNH
7
8
P. paphos
Panama
HT
ZMHB
9
10
P. melicertes
ST
11
12
P. thoria
Nicaragua
13
14
P. thoria
(=pammenes)




**Figures 1–22 (continued). 15–16.** *P. okroogly* holotype ♂ Peru: Cusco Region, Quispicanchi Province, Marcapata, genitalia slide G967 [AMNH]. **17–18.** *P. tschotky* holotype ♂ Colombia: Valle del Cauca, Rio Anchicayá, elevation 1150 m, 18-Jan-1975, No. CH-473, leg. S. R. & L. M. Steinhauser, A. C. Allyn Acc. 1975-17 [MGCL]. **19–20.** *P. paralus* lectotype ♂ Peru: Cosnipata Valley, leg. H. Whitely, Godman-Salvin Collection 1912-23, type H 772, specimen No. BMNH(E) #1054005 [BMNH]. **21–22.** *P. hermieri* holotype ♂ Ecuador: Esmeraldas, km. 12.5, Lita-San Lorenzo rd., Río Chuchuví, 0° 53.01' N, 78° 30.90' W, 850 m, Jul-2002, leg. I. & R. Aldas, genitalia NVG120922-46 [USNM].

and *P. melicertes* (Godman & Salvin, 1895), which it cryptically resembles in wing pattern, but differs in prominently longer valvae and subtly in having spots vs. streaks in the forewing postdiscal pattern. This species is therefore new, and is described here.

### *Potamanaxas louisghilli* Grishin, new species

(Figs. 1–6, 23, 24a, 25)

**Description:** ***Male*** (n=3, Figs. 1–4) – right forewing length = 16.2 mm in holotype. Forewing twice as long as wide, rounded at apex and tornus, costa convex at the base and apex, straighter mediad, outer margin convex. Dorsal forewing dark, chocolate-brown; conspicuously pale cream-yellow (as opposed to the white on hindwing) discal band from near costa to inner wing margin, separated from costa by a narrow belt of chocolate-brown scales, narrowing towards costa; band entire, not separated into spots by veins; basal band margin typically rounded towards both wing margins; distal band margin more irregular, indented at veins; band mostly uniform in color, slightly yellower along veins; some cream scales on the costa anteriad of the band; a faint row of postdiscal pale spots (not streaks) with two spots between veins $M_1$ and $M_3$ offset distad, and a doublet of pale spots just distad of discal cell. Ventral forewing similar to dorsal in color and pattern, but overscaled with slate-colored scales at the base, discal band barely wider (mostly distad) in most cells and significantly wider in $CuA_2$-1+2A cell, where rectangular slate-colored area absorbs postdiscal pale spots; band sometimes reaching costa, or separated from cream-colored costal area by very narrow line of pale brown scales; postdiscal row of pale spots (not streaks), more prominent than above, faint in some specimens, reduced and offset distad by discal cell (between veins $M_1$ and $M_3$) and a doublet of pale spots distad of discal cell (near the bases of cells $M_1$-$M_2$ and $M_2$-$M_3$), sometimes fused with postdiscal band; row of submarginal pale-brown spots, varying from roundish to triangular, one in each cell. Hindwing nearly triangular, slightly longer than wide, rounded at apex and tornus, somewhat concave around $M_2$ and $CuA_2$ veins and convex between these veins. Dorsal hindwing dark, chocolate-brown; a mostly white discal band a quarter to a third of the wing width runs from costa to vein 2A, constricted and narrower at 1A vein, not bending towards tornus; band entire, not separated into spots by veins, margins of the band somewhat irregular, distal margin more diffuse with brown scales invading the band along the veins; white overscaling around the band near its posterior end; wing overscaled with hair-like slate-violet scales along the band towards tornus; very faint or absent submarginal paler spots in every cell. Ventral hindwing similar to dorsal, but the white band is broader distad, the wing mostly slate basad of the discal band and posteriad to anal margin, the band fused with slate area in cell 2A-3A; a row of submarginal pale-brown spots, one in each cell. Fringes brown, the same color as wing margins above and beneath everywhere, except where the pale band reaches the inner margin of forewing and costa of hindwing, and

along anal margin ventrad fringes cream-white and slate. Head and palpi chocolate-brown with small white spots above, between and behind the eyes and dispersed slate scales on palpi and collar, slate with brown scales beneath, cheeks cream, antennae brown with some slate scales at joints anteriad, a very prominent cream spot anteriad at the base of the club, spot more than half of the club length; nudum brown, of 17 segments. Thorax and abdomen chocolate-brown above, slate beneath; legs with brown, slate and cream-yellow scales, largely brown dorsally, mostly cream ventrally, forelegs with the distal half of tibia mostly white and with a prominent white ring near the distal end of tarsus (3rd and 4th tarsomeres). ***Male genitalia*** (n=2, Figs. 23, 24a) – tufts of scales near the bases of valvae orange-brown, uniformly colored; tegumen as long as wide, less than twice the length of uncus arms, with a small bulge centrally by the uncus; uncus divided, arms on the sides of tegumen, widely separated from each other, about twice as long as wide at the base; gnathos upturned and joint ventrad in the caudal half, spiculose on its surfaces caudad, widely separated from uncus: distance from gnathos ventral side to the base of uncus dorsally exceeds the length of uncus arms; saccus as long as wide, broadly triangular and broadly rounded anteriad; valva with convex costa angled in the middle, cucullus irregularly dentate along the dorsal edge, extending caudad for about the same length as costa, cucullus caudal end narrow, rounded and directed posterodorsad, at the base of dorsal margin cucullus with a triangular tooth-like projection directed anterodorsad, dorsolateral dimension of the valva ("height") is about the cucullus length; sacculus with style-like projection from its very base, projection about twice as long as wide, not widening dorsad; penis slightly shorter than valva length, no cornuti.

***Female*** (n=1, Figs. 3, 4) – right forewing length = 17.4 mm, similar to male but slightly larger, nudum of 17 segments. ***Female genitalia*** (Fig. 25) – lamella postvaginalis with two approximately triangular sclerotized areas along the distal margin with a non-sclerotized break between them in the middle; lamella antevaginalis weakly sclerotized, expanded anteriad with several stronger sclerotized latitudinal ridges; antrum poorly sclerotized, not prominent, narrow; ductus bursae narrow, slightly longer than sterigma; corpus bursae longer that the rest of genitalia, as wide as sterigma.

**Barcode sequence of the holotype**: Genbank Accession JF762690, voucher 07-SRNP-31875, 658 base pairs:

AACTTTATATTTTATCTTTGGAATTTGAGCAGGAATAGTAGGAACT
TCCCTAAGTTTATTAATTCGAACTGAATTAGGTAATCCAGGATCAT
TAATTGGGGATGATCAAATTTATAATACTATTGTTACAGCTCAT
GCTTTTATTATAATTTTTTTTATAGTTATACCAATTATAATTGAG
GATTTGGTAATTGATTAGTGCCATTAATACTAGGAGCCCCAGA
TATAGCATTTCCTCGAATAAATAATATAAGATTTTGACTTTTAC
CCCCCTCTTTAATATTATTAATTTCTAGAAGAATCGTAGAAAATG
GAGCAGGAACAGGTTGAACTGTTTACCCCCCCTTATCTGCCAAT
ATTGCTCACCAAGGTTCCTCAGTAGATTTAGCTATTTTCTCCCT
TCATTTAGCAGGTATTTCTTCTATTCTTGGGGCTATTAATTTAT
CACAACAATTATTAATATACGAATTAGAAATTTATCTTTTGAT
CAAATACCTTTATTTATTTGAGCTGTAGGAATTACTGCTTTACTAT
TACTACTTTCATTACCTGTATTAGCAGGTGCTATTACTATATTAT
TAACAGATCGAAATTTAAATACATCCTTTTTTGACCCAGCAGGAG
GAGGAGATCCAATTTTATATCAACATTTATTT

Sequences of paratypes show differences in 2 positions.

**Types:** ***Holotype*** ♂ (Figs. 1, 2, 23a–c) has the following four rectangular labels: white printed & hand-printed - || Voucher: D.H.Janzen & W.Hallwachs | DB: http://janzen.sas.upenn.edu | Area de Conservacion Guanacaste, | COSTA RICA. | 07-SRNP-31875 ||; yellow printed - || LEGS AWAY | FOR DNA ||; white printed - || NVG120922-46 ||; red printed - || HOLOTYPE ♂ | *Potamanaxas* | *louisghilli* Grishin ||. Holotype data: Costa Rica: Area de Conservación Guanacaste, Guanacaste Province, Sector Pitilla, site Sendero Memos, 10.98171° −85.42785°, 740 m, collected on 29-Mar-2007 in antepenultimate instar feeding on *Cavendishia axillaris* (Ericaceae) by Lucia Ríos, eclosed 2-May-2007, voucher code 07-SRNP-31875, genitalia NVG120922-46, Genbank accession of barcode sequence JF762690. **Paratypes:** Costa Rica: Area de Conservación Guanacaste, Guanacaste Province, Sector Pitilla: 1 ♂ (Figs. 3, 4, 23d–j) site Estacion Pitilla, 10.98931° −85.42581°, 675 m, collected on 12-Mar-1991 in ultimate instar feeding on *Cavendishia axillaris* (Ericaceae), eclosed 7-Apr-1991, voucher code 91-SRNP-132, genitalia No. X-6125 J. M. Burns 2005, Genbank accession of barcode sequence DQ293099; 1 ♂, site Orosilito, 10.98332° -84.43623°, 900 m, collected on 12-Apr-2014 in penultimate instar feeding on *Psammisia ramiflora* (Ericaceae) by Manel Rios, eclosed 25-Apr-2014, voucher code 14-SRNP-30571; 1 ♀ (Figs. 5, 6, 24) site Sendero Memos, 10.98171° −85.42785°, 740 m, collected on 16-Apr-2011 in penultimate instar feeding on *Cavendishia axillaris* (Ericaceae) by Freddy Quesada, eclosed 13-May-2011, voucher code 11-SRNP-31012, genitalia NVG131102-92, Genbank accession of barcode sequence JQ526704.

**Deposition of types:** The holotype and the three paratypes are in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

**Type locality:** COSTA RICA: Area de Conservación Guanacaste, Guanacaste Province, Sector Pitilla, site Sendero Memos, GPS: 10.98171° −85.42785°, elevation 740 m.

**Etymology:** This species is named in memory of Louis G. Hill, a deeply principled Pennsylvania State Senator and Philadelphia Judge and lover of wilderness, whose children are supporters of ACG and the dissemination of ACG insect knowledge, and particularly the taxonomy of the subfamily Campopleginae in the parasitoid wasp family Ichneumonidae, many of which are parasitoids of skipper butterfly (Hesperiidae) caterpillars.

**Distribution and phenology:** Currently, this species is known only from about 10 km$^2$ of ACG mid-elevation Caribbean rain forest (but geopolitically in Guanacaste Province, which is largely dry forest), but it is expected to have a wider distribution throughout Costa Rica and Panama. Specimens with similar genitalia have been recorded from South America as well, and the research on their taxonomic status is on-going. The caterpillars were found in March-April and the adults eclosed in April-May (Janzen & Hallwachs, 2014).

**Diagnosis:** This species belongs to *Potamanaxas* because it has all the traits of the genus as given in the Evans identification key (1953: 6–15, 137). In particular, males have two tufts of hair-like scales near the bases of valvae (Fig. 23e, f), a suggested synapomorphy of *Potamanaxas* (Grishin, 2013a; 2013b; 2013d). By the COI barcodes, it nests within other *Potamanaxas* species (Ratnasingham & Hebert, 2007; Janzen *et al.*, 2011). As suggested by COI barcodes and wing patterns, the new species is most similar to *P. paphos* Evans, 1953 and *P. melicertes* (Godman & Salvin, 1895) (Figs. 7–10), but is confidently distinguished from them by terminally elongated valvae (Figs. 23, 24). *P. louisghilli* could also be confused with other *Potamanaxas* species characterized by pale discal bands on wings, such as *P. thoria* (Hewitson, 1870) (Figs. 11–14, 24d), *P. okroogly* Grishin, 2013 (Figs. 15, 16, 24e), *P. tschotky* Grishin, 2013 (Figs. 17, 18, 24g), *P. paralus* (Godman & Salvin, 1895) (Figs. 19, 20, 24b), and *P. hermieri* Grishin, 2013 (Figs. 21, 22, 24c), but can be distinguished from these species either by wing patterns or male genitalia (Figs. 23, 24).

A combination of the following characters is diagnostic of *P. louisghilli*: **(1)** The yellowish cream—not strong yellow as in *P. flavofasciata* (Hewitson, 1870) and *P. xantholeuce* (Mabille, 1888)—discal band on dorsal forewing is entire and not separated into spots by veins as in most *Potamanaxas* species (see Warren et al. 2014 for illustrations); its margins could be somewhat irregular (e.g., Fig. 1), but not as irregular as in *P. paralus* (Fig. 19), *P. hermieri* (Fig. 21), and *P. xantholeuce*. **(2)** The pale-cream-white discal band on dorsal hindwing spans from the costa to the 2A vein and has irregular margins, not sharply defined as in *P. okroogly* (Fig. 15) and


**Figure 23**. Male genitalia of *Potamanaxas louisghilli* **n. sp**. Genital capsule of the holotype (**a–c**, voucher 07-SRNP-31875, genitalia NVG120922-46, Figs. 1–2) and a paratype (**d–j**, voucher 91-SRNP-132, genitalia No. X-6125 J. M. Burns 2005, Figs. 3–4) in different views: **a, e**. lateral; **b, f**. left ventrolateral; **c, d**. dorsal; **g**. ventral; **h**. right dorsolateral; **i**. posterior; **j**. anterior. Specimens are in USNM.

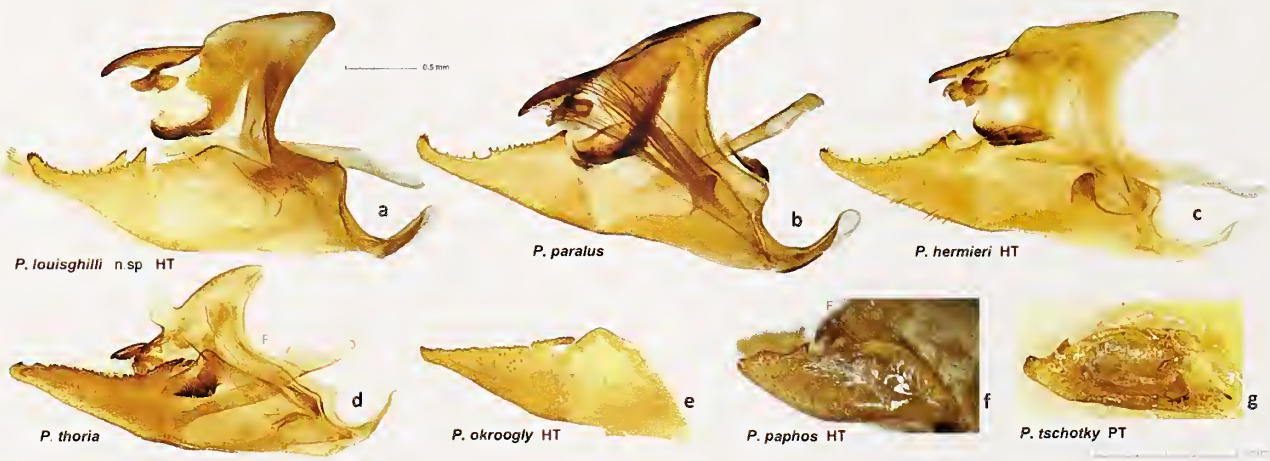



**Figure 24**. Male genitalia of *Potamanaxas*. **a**. *P. louisghilli* holotype, data in text (Figs. 1–2); **b**. *P. paralus*, Peru: Huánuco Region, Tingo María, 23-Jun-1982, 800 m, genitalia #H747 by S. S. Nicolay [USNM]; **c**. *P. hermieri* holotype, Ecuador: Esmeraldas, km. 12.5, Lita-San Lorenzo rd., Río Chuchuví, 0° 53.01' N, 78° 30.90' W, 850 m, Jul-2002, leg. I. & R. Aldas, genitalia NVG120922-46 [USNM] (Figs. 21–22); **d**. *P. thoria*, Ecuador: Imbabura Prov., Ruminahui, 37 km N. Pedro Vicente Maldonado, 0° 16.73'N 78° 59.9'W, 500 m, 9-Mar-2001, leg. D. H. Ahrenholz, genitalia NVG120922-44 [USNM], lateral view; **e**. *P. okroogly* holotype, Peru: Cusco Region, Quispicanchi Province, Marcapata, genitalia slide G967 [AMNH], left valva, interior view (Figs. 15–16); **f**. *P. paphos* [holo]type, Ecuador: Paramba, dry season, Apr-1897, 3500', leg. Rosenberg, Rothschild Bequest B.M. 1939-1, specimen No. BMNH(E) #1054150 [BMNH], lateral view of the abdomen caudal end (Figs. 7–8); **g**. *P. tschotky* paratype, Ecuador, coll. Saunders, Godman-Salvin collection 1912–23, specimen No. BMNH(E) #1054120 [BMNH], left valva, interior view. "F" indicates mirror image (left-right inverted). Images of BMNH specimens are copyright of Trustees of the Natural History Museum, London; used with permission.


**Figure 25.** Female genitalia of *Potamanaxas louisghilli* **n. sp. a.** complete genitalia in ventral view with a scale bar on the left; **b–d** magnified sterigma, ovipositor lobes, and last tergum in posteroventral (**b**), ventral (**c**) and right lateroventral (**d**) views, scale bar on the right. Voucher 11-SRNP-31012, genitalia NVG131102-92, Figs. 5–6, the specimen is in USNM.

*P. tschotky* (Fig. 17); the band does not end posterior to discal cell near $CuA_2$ vein as in *P. hirta* (Weeks, 1901), *P. okroogly* (Fig. 15), *P. tschotky* (Fig. 17), and *P. effusa* (Draudt, 1922), and does not bend towards the tornus as in *P. thoria* (Fig. 11); the posterior end of the band is partly separated from the rest of the band by a constriction along the 1A vein (Figs. 1, 3, 5) and the inner margin of the band is indented at the 1A vein, not smooth as in *P. thoria* (Fig. 11). (3) A row of faint postdiscal forewing spots, not streaks as in *P. paphos* and *P. melicertes* (Figs. 7–10), is complemented with a doublet of spots distad of discal cell; the spots can be seen above (Figs. 1, 3, 5), but are more prominent beneath (Figs. 2, 4, 6); the spots are typically more expressed and elongated than those in *P. thoria* (Figs. 11–14); two spots of the row, those between cells $M_1$ and $M_3$, are offset distad, making room for an additional spot doublet just distad of discal cell (at the bases of cells $M_1$-$M_2$ and $M_2$-$M_3$), this doublet is usually made of the best-developed spots (Fig. 6). (4) Males have a white streak at the front of antennal club and a white ring on foreleg tarsi, as in *P. tschotky* (Grishin 2013d); those are normally absent in other related species, except for the antennal streak in *P. thoria* and *P. okroogly*. (5) Male genitalic valvae are characterized by an elongated and curved dorsad cucullus with a tooth at its base directed anterodorsad (Fig. 23a, e, most similar to *P. paralus* and *P. hermieri*, but different in most other *Potamanaxas* species), and the process on the sacculus stemming from its very base (positioned further posteriad in *P. paralus* Fig. 24b and *P. hermieri* Fig. 24c); the saccus is rather broad, almost triangular with a broadly rounded apex (narrower in *P. paralus* and *P. hermieri*); and the tegumen is more robust and relatively shorter compared to uncus arms than in *P. paralus* and *P. hermieri*.

Characters (1) to (3) are usually sufficient to identify *P. louisghilli* from specimens, live individuals, and their photographs, making dissection unnecessary. However, the structure of male genitalia and its differences from other *Potamanaxas* species are instrumental in substantiating *P. louisghilli* as a species-level taxon and its distinctness from cryptically similar *P. paphos* and *P. melicertes*. Additionally, *P. louisghilli* is clearly different by its DNA barcode from the other recorded species of ACG *Potamanaxas*.

**Immatures and foodplants:** Caterpillars that produced all specimens in the type series were found feeding on *Cavendishia axillaris* and *Psammisia ramiflora*, both in Ericaceae. It is likely to be host-specific to rain forest epiphytic Ericaceae, as are the other species of ACG *Potamanaxas* (Janzen *et al.*, 2011).

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## Editor's note

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# A new species of *Hypolycaena* (Lepidoptera: Lycaenidae) from Arunachal Pradesh, north-eastern India

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**Abstract.** A new species, *Hypolycaena narada*, **sp. nov.**, is described from a series of male specimens from Arunachal Pradesh, north-eastern India. The new species is considerably distinct from other members of the tribe Hypolycaenini, and is easily distinguished from its relatives based on the following combination of characters: (a) slightly shining purple-blue upperside forewing with a dark, diffused androconial patch, (b) underside forewing apex and margin concolorous with the wing, (c) underside wings with narrow discal bands, ending in black costal spots, and (d) coastal black spot near the base.

**Keywords:** Indo-Burma biodiversity hotspot, species discovery, biodiversity surveys, butterfly taxonomy, Hypolycaenini.

## Introduction

The Oriental genus *Hypolycaena* C. & R. Felder, 1862 (Lepidoptera: Lycaenidae: Hypolycaenini) is placed under the subtribe Hypolycaeniti or Hypolycaenina, which is a complex of three allied genera: (1) *Hypolycaena* C. & R. Felder, 1862 (*Wien. Ent. Monatsschr.*, 6: 293). Type-species by selection by Scudder (1875, *Proc. Amer. Acad. Arts Sci., Boston*, 10: 195): *Myrina sipylus* Felder (C.), 1860; type locality: Amboina, Sulawesi, Indonesia (Hemming 1967). (2) *Chliaria* Moore, 1884 (*J. Asiat. Soc. Bengal (II)*, 53(1): 32). Type-species by original designation: *Hypolycaena othona* Hewitson, [1865]; type locality: Northern India (Moore, 1884; Hemming, 1967). (3) *Zeltus* de Nicéville, 1890 (*Butts. India Burmah Ceylon*, 3: 19, 399). Type-species by original designation: *Papilio etolus* Fabricius, 1787; type locality: "Indiis", i.e., India (de Nicéville, 1890; Hemming, 1967). These three genera are weakly separated. Hence, some authors are of the opinion that *Chliaria* and *Zeltus* are synonyms of *Hypolycaena*, and have treated all the species of this group under *Hypolycaena* (d'Abrera, 1986; Ek-Amnuay, 2012). Other authors have treated a combination of the three genera as valid, but treated different, incongruent sets of species under *Hypolycaena* and *Chliaria* (Evans, 1932; Inayoshi, 2015; Io, 2000; Larsen, 2004; Pinratana, 1981; Smith, 1989; 2006; Varshney, 2010). "Hypolycaeniti" was discussed in detail by Corbet *et al.* (1992), who treated the three genera as separate and distinguished between them as given in the generic key below.

Corbet *et al.* (1992) further noted of *Hypolycaena*: "The characters are those of the tribe. The genus, as generally employed to-day, contains a very heterogeneous lot of species. The larva of at least one typical Oriental species feeds on the flowers of orchids, as in the very closely allied *Chliaria*, but the larvae of atypical species have been found on a variety of dicotyledons. Distributed throughout the Ethiopian and Oriental Regions and reaching Australia.", and of *Chliaria*: "Very close to, and doubtfully separable from, *Hypolycaena*, the only structural difference being the more abrupt antennal club, which is somewhat flattened beneath. The male genitalia [...] are of the same pattern as those of the typical species of *Hypolycaena*; they differ only slightly from one species to another and those of the component taxa of the *othona* complex are similar. [...] The genus is distributed from north India to Taiwan and Sundaland." There are 11 species in the *Hypolycaena-Chliaria* complex in the Indo-Malayan Region (Table 1).

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**Generic key to "Hypolycaeniti" (verbatim from Corbet *et al.* 1992).**

| | |
|---|---|
| 1(2) | Underside hindwing without a black spot at the base of space 7 (but an orange bar may be present).....***Hypolycaena*** |
| 2 | Underside hindwing with a black spot at the base of space 7. |
| 3(4) | Hindwing tail at vein 1b twice as long as the tail at vein 2.....***Zeltus*** |
| 4 | Hindwing tail at vein 1b much less than twice the length of the tail at vein 2.....***Chliaria*** |

During recent surveys of butterfly diversity in NE India, a *Hypolycaena* species was recorded from the Namdapha National Park in eastern Arunachal Pradesh by several colleagues. This species did not match descriptions and illustrations of any known species of Hypolycaenina, as compared with the references mentioned above. A comprehensive examination of Hypolycaenina in the British Museum of Natural History (now the Natural History Museum, London), and in the Museum of Comparative Zoology at Harvard University, revealed that this species was distinct and new to science, which is described below. The wing venation nomenclature used below is the numerical system that is widely used in the Asian butterfly literature (Evans, 1932; Cantlie, 1962; Miller, 1970; Corbet *et al.*, 1992; Yata *et al.*, 2010). The terminology used below for wing patterns, morphology and genitalia also follows these standard taxonomic works on Asian butterflies.

## *Hypolycaena narada* sp. nov. (Figs. 1-3, Tables 1-2)

**ZooBank LSID urn:lsid:zoobank.org:act:27693C5C-CF9B-476B-B94D-878A61F4FFB6**

**Holotype:** Voucher code NCBS-PY976. Fig. 1, Table 2. ♂. The type locality is near the village of Bodhisatta, Changlang District, Arunachal Pradesh, NE India (N 27°33′, E 96°23′). Collected by Krushnamegh Kunte, 14 March 2015. Preserved dry, pinned, deposited in the Research Collections Facility at the National Centre for Biological Sciences, Bengaluru (=Bangalore), India.

**Paratypes:** (1) NCBS-PY977. Fig. 1, Table 2. ♂. Collection data same as the holotype, except collected on 8 March 2015. (2) NCBS-PY978. Fig. 1, Table 2. ♂. Collection data same as the holotype, except collected on 8 March 2015. (3) NCBS-PY979. Table 2. ♂. Collection data same as the holotype. (4) NCBS-PY980. Table 2. ♂. Collection data same as the holotype, except collected on 11 March 2015. (5) NCBS-PY981. Table 2. ♂. Collection data same as the holotype, except collected on 11 March 2015. (6) NCBS-PY982. Table 2. ♂. Collection data same as the holotype, except collected on 11 March 2015 and preserved in ethanol for molecular work. (7) NCBS-PY983. Fig. 2 ♂ genitalia, Table 2. ♂. Collection data same as the holotype, except collected on 14 March 2015 and preserved in ethanol for molecular work.

**Description: Holotype:** Forewing length 15mm. ***Upper side***: Both wings dark and slightly shining purple-blue. *Forewing* border sooty-grey, narrow, just a thread at tornus, gradually dilating to 2mm at apex and continuing along the costa almost up to the base. This border sometimes appears pale grey-brown in a side light (Fig. 3B). A dark, diffused androconial discal patch, spanning from the base of v2 to v7 and from cell-end outward for approx. 4mm. *Hindwing* black tails, ending in white tips, at v1 (approx. 6mm long) and v2 (approx. 4mm long), wing margin wavy at v3 and v4. The abdominal fold and costal margin in space 8 dark grey-brown. Dispersed but prominent white scalation in the submarginal terminal area, which is condensed into submarginal white bars in spaces 1b, 1c and 2. A tornal, large, white-crowned black spot adjacent to the white bar in 1b. A black marginal line running the length of the termen, and cilia FW and HW pale grey. HW basal half and space 1c covered in ochreous, hair-like long scales, which are especially visible in a side light.

***Underside:*** Both wings pale grey, shining silvery-grey in bright light. *Forewing* cell-end bar defined on both sides by inner black and outer white lines. Discal band narrow, uneven but contiguous, ochreous in the middle, black- and then white-edged on both the sides, pointed and vanishing near v1, ending in a black spot at v10. A faint grey-brown, wavy, post-discal line from v1 to v6. *Hindwing* cell-end bars similar to the FW, but less well-marked. Two prominent, oblong, white-ringed black spots along the costal margin, of which the basal spot is before the cell-end bar, and the outer spot is beyond the discal band. The discal band narrow, ochreous in the middle, black- and then white-edged on both the sides, and highly broken. It is composed of an oblique line in 1b, a pair of disconnected oblique lines in 1c converging towards each other in the middle, and then a series of largely conjoined spots in spaces 2 to 6, which is dislocated at v4. A tornal, large, white-crowned black spot in 1b, lined towards v1 by sparse, iridescent blue scales. Another large, orange-crowned black spot in space 2. This is followed by grey-centred white rings in spaces 3 to 6, which are inwardly defined by dark grey. A black marginal line running the length of the termen of both wings, with cilia bright pale grey.

Head, thorax and abdomen overall similar to other *Hypolycaena*. Frons white. Labial palps white at the base, black near the tip. Eyes black. Antennae black, narrowly ringed white; club black, tipped reddish similar to *Hypolycaena othona* Hewitson, [1865]. Proboscis ochreous. Thorax white below, purple or purple-brown above. Legs broadly ringed black and white. Abdomen dark brown to almost black above, white below.

**Paratypes, and intraspecific variation:** All the paratypes are largely similar to the holotype, including in the overall colour pattern and forewing length (Fig. 1, Table 2). Variation is seen primarily in the nature of the discal bands on the underside, and the extent of white scalation near the terminal margin on the upper side of hindwing. In the holotype, the discal band on the forewing underside is straight and almost touches v1. This discal band is similarly straight and almost touching v1 in all the paratypes except in NCBS-PY977, in which the discal band is bent inwards at v3, and ends in the middle of space 1c. On the hindwing underside, the discal spot in 3 is not elongated in the holotype and all the paratypes, except in NCBS-PY977, in which it is elongated and points towards the wing base. In the holotype, there are dispersed but prominent white scales in the submarginal terminal area of the hindwing, which are condensed into submarginal white bars in spaces 1b, 1c and 2. Similar white scales and submarginal white bars are present in paratypes NCBS-PY980 and NCBS-PY981. In paratypes NCBS-PY977, NCBS- PY978 and NCBS-PY979, the dispersed white scales are nearly absent, and the submarginal bars in 1a, 1b and 2 are very narrow and therefore somewhat indistinct (this is especially the case in NCBS- PY978).

**Male genitalia:** The male genitalia of the paratype (NCBS-PY983; Fig. 2) are of similar form but quite distinct from the male genitalia of *H. kina celastroides* Corbet, 1938 and *H. tora semanga*

**Table 1**: Overview of the Indo-Malayan *Hypolycaena* (inclusive of *Chliaria*).

| Species | Type locality | Distribution | Polytypism | References |
|---|---|---|---|---|
| *Hypolycaena amabilis* (de Nicéville, 1895) | "N.-E. Sumatra; Java" | Southern Thailand to Borneo and Java | Polytypic | 1, 11-13 |
| *Hypolycaena balua* Moulton, 1911 | Kuching (Sarawak, Borneo, Malaysia) | Peninsular Malaya to Borneo | Polytypic | 2, 11-13 |
| *Hypolycaena erylus* (Godart, [1824]) – Common Tit | Java | Himalaya to Sulawesi, the Philippines, and New Guinea | Polytypic | 3, 11-13 |
| *Hypolycaena kina* (Hewitson, [1869]) – Blue Tit | "Darjeeling" (West Bengal, India) | Himalaya to Indo-China and Malaysia | Polytypic | 4, 11-13 |
| *Hypolycaena merguia* (Doherty, 1889) | | Myanmar to Sumatra, Java and Borneo | Polytypic | 5, 11-13 |
| *Hypolycaena narada* Kunte, sp. nov. – Banded Tit | Bodhisatta (Arunachal Pradesh, India) | NE India, possibly N. Myanmar | Monotypic | – |
| *Hypolycaena nilgirica* (Moore, [1884]) – Nilgiri Tit | "Coonoor, Nilgiris" (Tamil Nadu, India) | Western Ghats and Sri Lanka | Monotypic | 6, 11-13 |
| *Hypolycaena othona* (Hewitson, [1865]) – Orchid Tit | "Northern India" | SW India to Indo-China, Malaysia and Indonesia | Polytypic | 7, 11-13 |
| *Hypolycaena pahanga* Corbet, 1938 | "Malaya" | Peninsular Malaya | Monotypic | 8, 11-13 |
| *Hypolycaena thecloides* (C. & R. Felder, 1860) | Malay Peninsula | Myanmar to the Philippines, Nicobar Islands, Sumatra, Java, Borneo | Polytypic | 9, 11-13 |
| *Hypolycaena tora* (Kheil, 1884) | Nias (Sumatra, Indonesia) | Sumatra, Indonesia, Peninsular Malaya | Polytypic | 10-13 |

References: 1: de Nicéville 1895; 2: Moulton 1911; 3: Godart 1824; 4: Hewitson 1869; 5: Doherty 1889; 6: Moore 1884; 7: Hewitson 1865; 8: Corbet 1938; 9: C. & R. Felder, 1860; 10: Kheil 1884; 11: d'Abrera 1986; 12: Corbet *et al.* 1992; 13: Species index cards in the British Museum of Natural History (Natural History Museum, London).

**Table 2**: Type material of *Hypolycaena narada*, **sp. nov**. All specimens were collected by the author near Bodhisatta, Changlang District, Arunachal Pradesh, India (N 27°33′, E 96°23′), and are now deposited in the NCBS Research Collections Facility, Bengaluru, India.

| Type | Voucher code | Sex | Forewing length | Collection date | Preservation method |
|---|---|---|---|---|---|
| Holotype | NCBS-PY976 | Male | 15 mm | 2015/03/14 | Dry, pinned |
| Paratype | NCBS-PY977 | Male | 15 mm | 2015/03/08 | Dry, pinned |
| Paratype | NCBS-PY978 | Male | 15 mm | 2015/03/08 | Dry, pinned |
| Paratype | NCBS-PY979 | Male | 15 mm | 2015/03/14 | Dry, pinned |
| Paratype | NCBS-PY980 | Male | 15 mm | 2015/03/11 | Dry, pinned |
| Paratype | NCBS-PY981 | Male | 16 mm | 2015/03/11 | Dry, pinned |
| Paratype | NCBS-PY982 | Male | 15 mm | 2015/03/11 | In ethanol |
| Paratype | NCBS-PY983 | Male | 14 mm | 2015/03/14 | In ethanol |

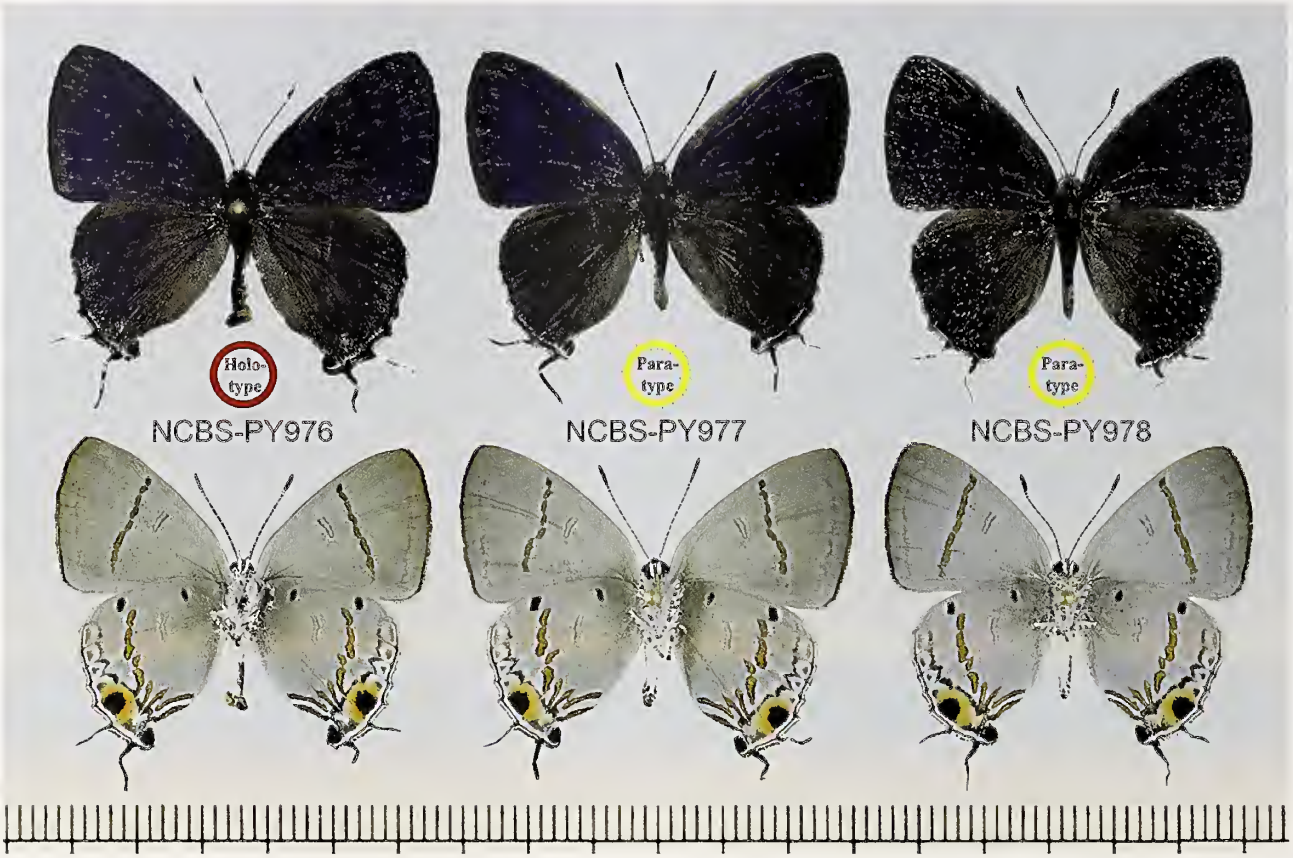


**Figure 1.** Type specimens of *Hypolycaena narada* **sp. nov.**, showing minor phenotypic variation. Specimen details are given in Table 2 and in the text. A millimetre-scale is at the bottom.

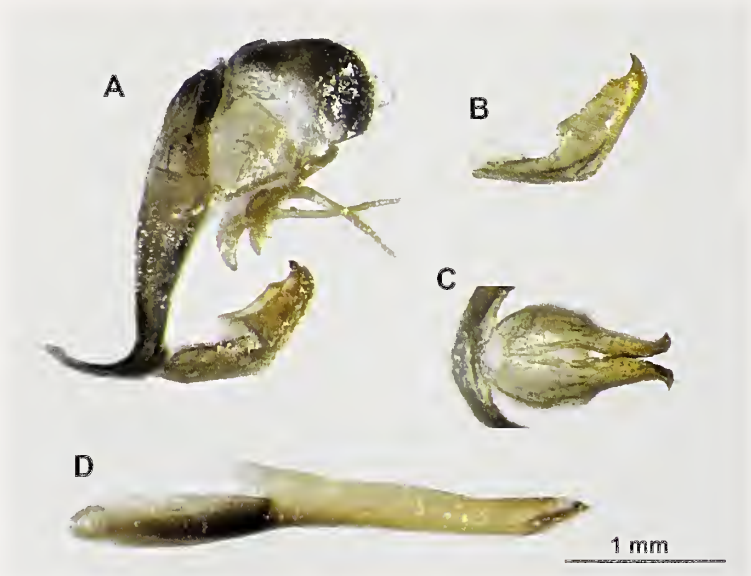


**Figure 2.** Male genitalia of *Hypolycaena narada* **sp. nov.** (paratype, NCBS-PY983). **A**: lateral view, with the aedeagus removed. **B**: right valva, lateral view from outside. **C**: ventral view of the fused valvae. **D**: aedeagus, lateral view. All separated parts of the male genitalia (B-D) are proportional to A. The scale bar is approximate. Dissection and images by Dipendra Nath Basu.

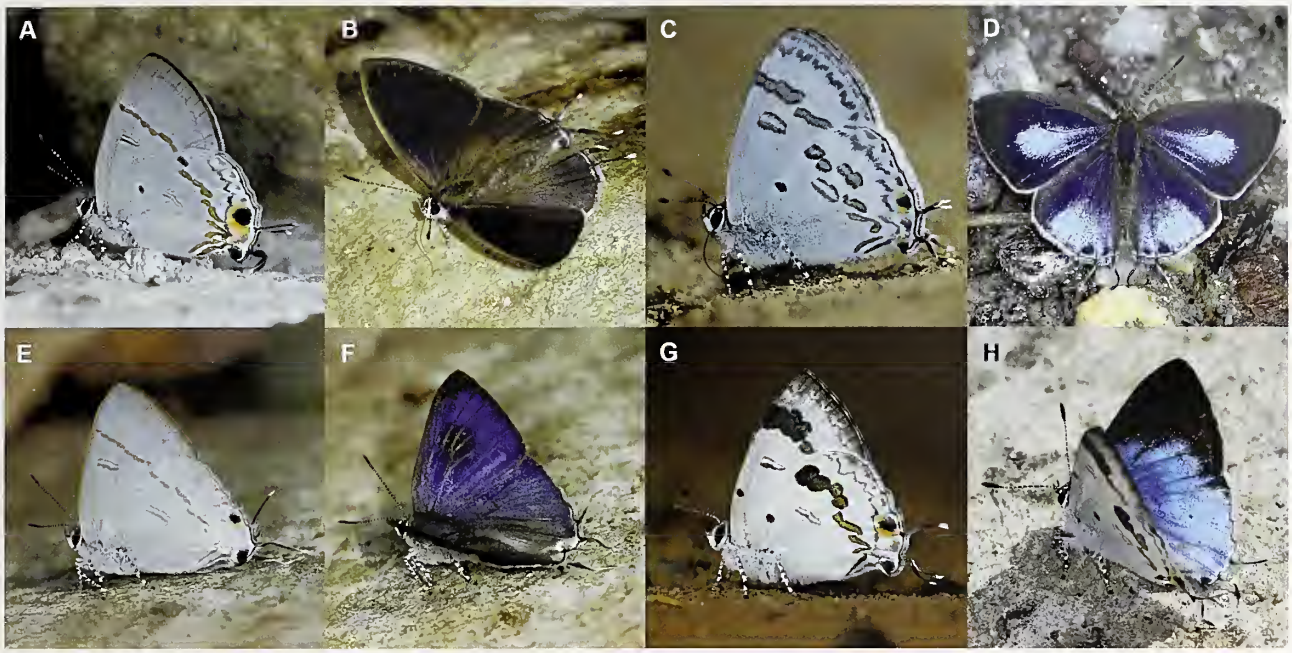


**Figure 3**. Indian *Hypolycaena* co-occurring with *H. narada* at its type locality. **A-B**: *H. narada* male. **C-D**: *H. kina kina male*. **E-F**: *H. erylus himavantus* male. **G-H**: *H. othona othona* male. **A-B**: Lunkai Nullah, Namdapha National Park, Changlang District, Arunachal Pradesh, India. 8 March 2015. **C**: 19th Mile, Namdapha NP. 10 March 2015. **D**: Passingthang, Upper Dzongu, North Sikkim District, Sikkim, India. 4 August 2014. **E-F**: Lunkai Nullah, Namdapha NP. 8 March 2015. **G**: 19th Mile, Namdapha NP. 10 March 2015. **H**: Lunkai Nullah, Namdapha NP. 8 March 2015. These specimens were not collected. (All images by Krushnamegh Kunte, except D by Rohan Lovalekar).

Corbet, 1940 that are illustrated by Corbet *et al.* (1992). The uncus is large and round, similar to that of *H. kina celastroides*. Right and left valvae are fused but deeply cleft, similar to other Hypolycaenina. The shape of the valva (Fig. 2B-C) is distinct from other species of *Hypolycaena*, with the base rounded and the tip elongated, angled and pointed.

**Female:** Female of this species is unknown.

**Diagnosis:** *Hypolycaena narada* is distinguished from other members of *Hypolycaena* and related Hypolycaenina based on tbe following combination of traits: (1) slightly shining, dark purple-blue UPFW (senna-brown, shiny blue or pale purple and blue in related *Hypolycaena*), (2) UPFW with a diffused, dark purple androconial patch in male (as a prominent brand in *H. erylus*, and largely absent in related *Hypolycaena*), (3) UNFW apex and terminal margin concolorous with the wing (ochreous in *balua, tora, merguia* and many similar *Hypolycaena*), (4) UN narrow, more or less contiguous, ochreous discal band, ending in black costal spots (either not narrow, absent or highly broken in other *Hypolycaena*, black costal spots absent in many Oriental *Hypolycaena*). These characters are summarized, and an identification key for Indo-Malayan *Hypolycaena* is provided, below. All the previously known Indo-Malayan *Hypolycaena* are illustrated by d'Abrera (1986), Corbet *et al.* (1932) and Ek-Amnuay (2012) where they may be compared.

**Etymology:** The specific name is based on Narada, a learned Vedic sage from Indian mythology, who was known for his mischief. The name is applied here to the species as a remark on a mischievous—and at the same time wise—prank by a friend, who shall remain unnamed, that led to this species description.

**Distribution**: This species has so far been recorded only from the Changlang District of Arunachal Pradesh in north-eastern India. Within Changlang, it has been recorded from at least four localities: (a) near the village of Bodhisatta, the type locality, (b) Lunkai Nullah (stream), near Bodhisatta and Deban tourist complex (N 27°30.4′, E 96°24.0′), (c) Deban, near the tourist complex (N 27°29.8′, E 96°23.4′), and (d) 19th Mile, on road to Vijaynagar (N 27°28.7′, E 96°24.1′). All four localities fall between 300m and 450m asl. Spot records from the first two localities are my personal observations, spot records from the last three localities are by Rohan Lovalekar, Hemant Ogale, Milind Bhakare, Amol Patwardhan and Rudraprasad Das (www.ifoundbutterflies.org/sp/2946/Hypolycaena-narada). The species quite likely occurs in Assam and other neighboring states in north-eastern India, where suitable habitat is present. The type locality is also very close to northern Myanmar and south-eastern Tibet. However, it remains to be seen whether the tall mountains (over 4,000 m asl) on the eastern and northern sides of the type locality towards Myanmar and Tibet, which may act as dispersal barriers, have restricted the species to India.

**Status, habitat, and habits:** The species appears to be locally common along cool streams flowing through mixed evergreen forests. Males are frequently seen puddling on wet soil and on bird droppings from early morning to late afternoon. They have not been seen taking nectar from flowers yet. They are bold while mud-puddling, which makes it easy to approach and photograph them. Even when they are disturbed, they fly to nearby vegetation, and soon return to mud-puddle. Their flight is fluttering but not very fast, similar to other members of *Hypolycaena*.

**Flight period:** The species appears to be very narrowly univoltine. In the past 20 years, various teams have surveyed butterflies in the Namdapha area practically throughout the

**An updated key to the species of *Hypolycaena* (inclusive of *Chliaria*) from the Indo-Malayan Region, expanded from Corbet *et al.* (1992):**

| | |
|---|---|
| **1 (8)** | UNHW[1] without a black spot at the base of space 7 |
| **2 (7)** | UP ♂ purple or blue, ♀ brown |
| **3 (6)** | UPFW shining deep blue |
| **4** | UPFW ♂ with a prominent brand beyond the cell. Large. ..... ***erylus*** |
| **5** | UPFW ♂ without a prominent brand. Small. ..... ***amabilis*** |
| **6 (3)** | UPFW dark purple, no brand. UNFW terminal margin broadly tinged pale ochreous..... ***merguia*** |
| **7 (2)** | UP brown, UPHW large tornal orange patch..... ***thecloides*** |
| **8 (1)** | UNHW with a black spot at the base of space 7 |
| **9 (16)** | UNFW without a small black costal spot above the cell |
| **10 (13)** | UP blue or purple in ♂, white in ♀, with black borders |
| **11** | ♂ UPFW dark purple, with narrow borders, disc not pale (female unknown)..... ***narada*** |
| **12** | ♂ UPFW with very broad borders, disc pale blue, extending narrowly to the base..... ***kina*** |
| **13 (10)** | ♂ UP deep senna-brown. ♀ UP reddish brown, with a white tornal area HW |
| **14** | UNFW terminal margin largely concolorous with the wing, discal line dark grey, upper spots near the costa much larger..... ***nilgirica*** |
| **15** | UNFW terminal margin broadly tinged pale ochreous, discal line dark ochreous and of nearly uniform width throughout..... ***balua*** |
| **16 (9)** | UNFW with a small black costal spot above the cell |
| **17 (18)** | UNFW apex broadly ochreous, discal band brighter ochreous, usually not or very little broader towards costa..... ***tora*** |
| **18 (17)** | UNFW apex concolorous with the wing, but may be darkened. Discal band dark, the upper spots towards costa prominently broader and disjoined from the lower narrow discal line |
| **19** | ♂ UPFW apical half black, basal half shining pale blue. UNFW discal band dark grey, black or dark ochreous, the upper spots 3 to 4 times broader than the lower discal line. ♀ UP brown with a bluish white area HW ..... ***othona***[2] |
| **20** | ♂ UPFW apical black border more restricted, the remaining wing duller, deeper blue, filling most of space 2 and basal half of 3. ♀ UPFW greyish blue with black borders..... ***pahanga*** |

[1] UP=upper side, UN=underside, FW=forewing, HW=hindwing.

[2] Corbet *et al.* (1992) included *semanga* Corbet, 1940 as a subspecies under *othona*, and did not list *tora* as it was extralimital. However, inspection of the relevant taxa in this species-group revealed that *semanga* is much closer to nominotypical *tora* than it is to nominotypical *othona*, in spite of having female UP blue. Therefore, the key above includes *semanga* and similar taxa as subspecies under *tora* (incidentally, this was Corbet's initial placement) rather than under *othona*, and the distinguishing features are identified accordingly. It is likely that some of the putative subspecies under the currently polytypic *othona* and *tora* will ultimately prove to be distinct species when they are investigated closely.

year, but all the 15 or so sightings of this species have only been in the first three weeks of March. This cannot be because it was overlooked in other months: in the past seven years, the species was not seen in any other month even when we were specifically looking for it during six visits spread almost throughout the year. On the other hand, several individuals of the species were seen on each of the three visits in March (2009, 2014, 2015). From these observations, it appears that the species has a single brood per year, adults emerging in early March and persisting until late March.

**Larval host plants and early stages:** unknown. As noted by others (Corbet *et al.*, 1992; Fiedler, 1992; Robinson *et al.*, 2001; van der Poorten & van der Poorten, 2013), *Hypolycaena* feed as larvae on diverse plants but mostly on dicots, except "*Chliaria*", which feed on orchids.

**Sympatric *Hypolycaena*:** Three species of *Hypolycaena* are currently known to co-occur with this species: *erylus*, *othona* and *kina* (Fig. 3). Their habits and habitat use are similar.

## Discussion

The subtribe Hypolycaeniti *sensu* Corbet *et al.* (1992), split into three genera, viz., *Hypolycaena*, *Chliaria* and *Zeltus*, or Hypolycaeniti/Hypolycaenina as currently organized by other authors in one to three genera, poses a taxonomic problem. As noted by Corbet *et al.* (1992), *Hypolycaena* alone is a large genus that has a very wide distribution from the Ethiopian Region through the Oriental Region up to the Australian Region. If *Chliaria* and *Zeltus* are included under *Hypolycaena*, it comprises morphologically heterogeneous species that also use quite diverse larval host plants, suggesting sufficient divergence within this group to merit recognition of taxonomic subunits. Therefore, it may be appropriate and convenient to organize Hypolycaeniti/Hypolycaenina—comprising nearly 60 species, many of them polytypic—in several allied genera rather than a single large *Hypolycaena*. Whether *Hypolycaena* should be all-inclusive, or should *Chliaria* be distinct and valid if *Hypolycaena* is split into several genera as informed by molecular phylogenetic analyses, remains unresolved in absence of a comprehensive taxonomic treatment of Hypolycaenina. The taxonomic confusion surrounding *Chliaria* will prevail until such a molecular phylogenetic analysis is attempted. At present, I have followed recent trends (d'Abrera, 1986; Inayoshi, 2015) in treating *Chliaria* as a synonym of *Hypolycaena*, and offer an updated key to distinguish between its Indo-Malayan species (see the key above).

As more information on this species accumulates, it will be made available on the *H. narada* species page of the *Butterflies of India* website (Kunte, 2015; www.ifoundbutterflies.org/sp/2946/Hypolycaena-narada).

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## Editor's note

The electronic edition of this article has been registered in ZooBank to comply with the requirements of the amended International Code of Zoological Nomenclature (ICZN). This registration makes this work available from its electronic edition. ZooBank is the official registry of Zoological Nomenclature according to the ICZN and works with Life Science Identifiers (LSIDs). The LSID for this article is: urn:lsid:zoobank.org:pub:4CA3D42A-CE93-4408-9457-463CEFBD4796 Registration date: June 7th, 2015. This record can be viewed using any standard web browser by clicking on the LSID above.

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# Two new species and taxonomic notes on species of *Moeris* Godman, 1900 (Hesperiidae, Hesperiinae, Moncini)

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**Abstract.** *Moeris* Godman, 1900 was described to include two species, being afterwards combined with 10 additional species by Evans (1955). As the male genitalia of several Moncini species have been illustrated and described during recent decades, taxonomic rearrangements reduced the number of species in *Moeris* to four. In the present study two new species are described: *Moeris seth* Mielke, Carneiro & Casagrande **sp. nov.** from southern and southeastern Brazil, and *Moeris nut* Mielke, Carneiro & Casagrande **sp. nov.** from the Ecuadorian and Peruvian Andes. The status of *Moeris menopis* (Schaus, 1902) **stat. rest.** is revalidated; *Moeris strada* Evans, 1955 **stat. nov.** and *Moeris stroma* Evans, 1955 **stat. nov.** are elevated to species level; and a new combination of *Mnasitheus submetallesces* (Hayward, 1940) **comb. nov.** is proposed given the similar pattern of stigma, male and female genitalia to *Mnasitheus* Godman, 1900 species, rather than with *Moeris striga* (Geyer, 1832), the type species of *Moeris*. Also, a neotype of *Moeris striga* and the lectotype of *Moeris menopis* are here designated in order to ascertain their taxonomic status. The female genitalia of the species studied are illustrated for the first time.

**Key words:** Neotropical skipper, Hesperioidea, taxonomy, new combination.

## Introduction

*Moeris* Godman, 1900 was described to include two species with Central American distribution. Evans (1955) added 10 more species to *Moeris*, including further subspecific taxa within *Moeris striga* (Geyer, 1832), *M. vopiscus* (Herrich-Schäffer, 1869) and *M. hyagnis* (Godman, 1900), in all totaling 17 taxa. Evans (1955), besides proposing the synonymy of *Remella* Hemming, 1939, combined these species into *Moeris* based on vague characters that are also widely shared with other Moncini taxa (e.g. length of antennae > ½ of costa; palpi slender, third segment short and conical; mid-tibiae spined).

After observing the contrasting patterns in the male genitalia of different species of *Moeris*, Burns (1990) revalidated the genus *Remella* Hemming, 1939, including only its type species *Hesperia remus* Fabricius, 1798, yet leaving all other species in *Moeris*. According to Burns, male genitalia of *Remella remus* resemble those within a cluster of genera, the "group of *Amblyscyrtes* Scudder, 1872, *Mnasicles* Godman, 1901 and *Callimormus* Scudder, 1872", instead those of the *Moeris* species. *Remella rita* (Evans, 1955), *R. duena* (Evans, 1955), and *R. vopiscus* (Herrich-Schäffer, 1869) were subsequently cited in this new combination due to their resemblance with *Remella remus* (Freeman 1991; Stanford & Opler 1993; Warren *et al.* 1998). Further species were subsequently removed from *Moeris* by Mielke & Casagrande (2002): *M. crispinus* (Plötz, 1882) was considered as a subspecies of *Monca telata* (Herrich-Schäffer, 1869); *M. rivera* (Plötz, 1882) was recognized as a valid species within *Callimormus*; and *Perimeles stollmeyeri* Bell, 1931 was recognized as junior synonym of *Mnasicles hicetaon* Godman, 1901.

Accordingly, *Moeris* was then composed of five species (Mielke, 2004; 2005) until the recent discovery that the type of *Moeris ekka* Evans, 1955 is related to *Wahydra* Steinhauser, 1991 based on forewing brand and genitalia morphology (Henao *et al.* in press). Therefore, integrating all recent taxonomic actions, *Moeris* is currently recognized to contain four species, though much incongruence still remains to be solved.

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The present work aims to contribute to the taxonomic arrangement, in the light of insights from morphological characters of the male and female genitalia of *Moeris striga* (and its subspecies) and of *M. submetallescens*.

## Material and methods

Specimens used in this study are deposited in the following collections: DZUP (Coleção Entomológica Pe. Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil), DD (private collection Diego Dolibaina, Curitiba, Paraná, Brazil), and OM (private collection Olaf Mielke, Curitiba, Paraná, Brazil). Additionally, types of *Moeris* taxa were studied from the Natural History Museum, London, United Kingdom, and IMLA (Fundacion e Instituto Miguel Lillo, Tucuman, Argentina). A species catalogue is available in Mielke (2005).

Genitalia of both sexes were prepared with standard methods and illustrated. Abbreviations used throughout this paper are: DFW - dorsal forewing; DHW - dorsal hindwing; VFW - ventral forewing; VHW - ventral hindwing; m - male; f - female. Size is given as the length of forewing. Species descriptions were made using 3i Interactive Key and Taxonomic Database (Dmitriev & Dietrich, 2008).

## Results and discussion

*Moeris striga* (Geyer, 1832)
(Figs. 1, 8, 15, 22, 28)

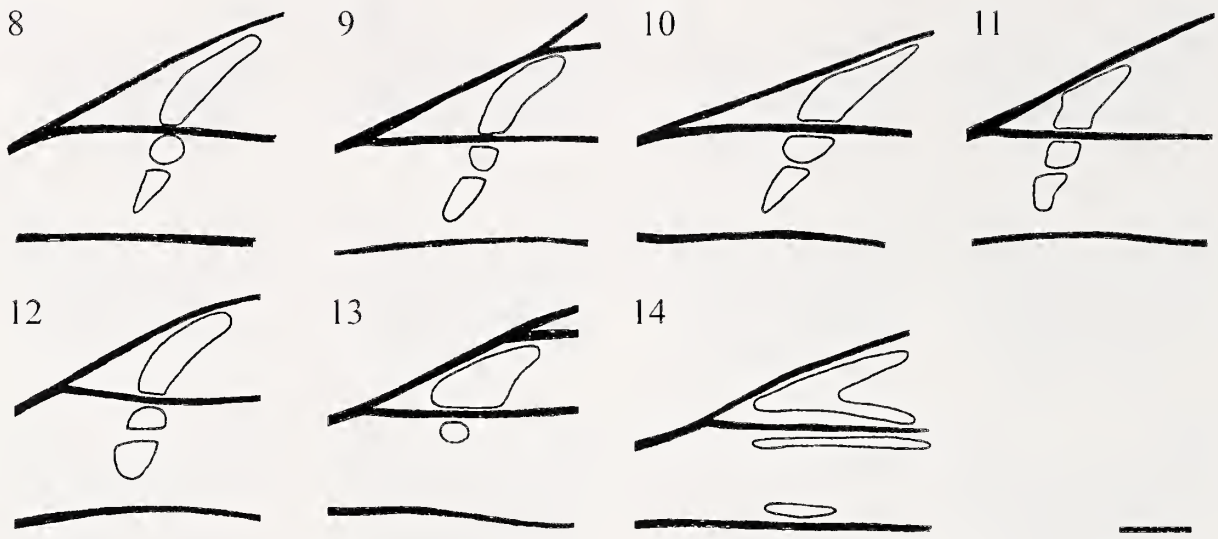
**Diagnosis**: Dorsal and ventral wings as in *Moeris seth* Mielke, Carneiro & Casagrande **sp. nov.**, *M. strada* Evans, 1955 **stat. nov.**, and *M. stroma* Evans, 1955 **stat. nov.** Posterior margin of harpe smooth, but with an inner parallel spined protruding line. Ampulla with dorsal elongated spine and spined dorsal margin. Aedeagus with an elongated, serrated cornutus besides an equally long globular cornutus with a spine on tip; lamella antevaginalis placed anterior of lamella postvaginalis as in *M. seth* Mielke, Carneiro & Casagrande **sp. nov.**, but shorter; posterior margin of lamella postvaginalis thin and bilobed.

**Remarks**: Dorsal wing markings (subapical and cell spots), and ventral wing spots and bands vary in presence, development and shape between specimens, not only in *M. striga*, but also in all other *Moeris* species. Thus, it is difficult to identify these species using only wing characters. On the other hand, the unique male and female genitalia morphology confirms its species status, which was observed after dissecting and analyzing 47 specimens. A neotype designation is necessary in this case, to clarify its taxonomic status, since the diagnostic characters can only be seen in the genitalia. Genitalia, however, were not mentioned in its original description. The type specimen(s) of *Talides striga* Geyer, 1832 is lost, as many types of Geyer's collection (Friedlander, 1987; Pelham, 2008). Although two type specimens of Carl Geyer are reportedly deposited in the Museum für Naturkunde (Berlin, Germany), these specimens (as many other Hesperiidae types) have never been seen by any researcher in Berlin nor in any other museum. As wing markings and original descriptions are not sufficient to provide a reliable species recognition, an actual specimen is necessary to represent the name and provide stability of nomenclature. As the type locality in the original description is stated only as "Rio", we dissected and analyzed 19 specimens from different locations in Rio de Janeiro city, plus 5 other specimens from other cities in Rio de Janeiro state. All of them presented the same characters as described above. Therefore, the neotype of *Moeris striga* is hereby designated with the following labels: / Neotypus / Talides striga Geyer, 1832, D'Alm. det./ Male capt. 10-II-1927, Covanca – Jacarépaguá – Rio [de Janeiro,] Ferreira d'Almeida [leg.] – Rio / Coll. D'Almeida / Topótipo / Nº 11980 / DZ 31.525 / Neotypus Talides striga Geyer, 1832, Carneiro, Mielke & Casagrande det. 2014 /. DZUP.

**Geographical distribution**: lowland Brazilian Atlantic forest, including western semi-deciduous ecosystems and gallery forest among the cerrado.

**Studied material**: **Argentina**: 1m, Misiones, Dos de Mayo 26.II.1989, Foerster leg., OM 22.016 (OM). **Bolivia**: 2m, 1f, La Paz, Caranavi V-VI.1989, VIII.1989, Tello leg., OM 24.216, OM 24.512, OM 23.951 (OM). **Brazil**: 1m, Bahia, Rio de Contas, Jitaúna, DZ 31.588, 26.III.1969, Ebert leg., (DZUP). 1f, Ceará, Viçosa do Ceará, 3 km W, 28.V.2013, Dolibaina & Pessoa leg., DD 001 (DD). 1m, Ceará, Guaramiranga, 13-15.VII.2012, Dolibaina & Lima leg, DD 002 (DD). 1m, 1f, Distrito Federal, Brasília Escola Fazendaria, 3.VI.1977, 6.VI.1977, Gifford leg., DZ 31.354, DZ 31.423 (DZUP). 1f, Distrito Federal, Brasília Fazenda Água Limpa, 25.V.1976, Gifford leg., DZ 31.427 (DZUP). 1m, 1f, Espírito Santo, Baixo Guandu, 16.IX.1966, 5.IX.1971, Elias & Elias leg., DZ 31. 370, DZ 31.508, DZ 31.570 (DZUP). 2m, Espírito Santo, Conceição da Barra 10.IV.1968, 5.V.1968, Elias & Elias leg., DZ 31.608, DZ 31.371 (DZUP). 1m, 1f, Espírito Santo, Linhares 25-30.VI.1975, V.1982, Elias leg., DZ 31.398, DZ 31.610 (DZUP). 1m, Espírito Santo, Linhares, Reserva Sooretama, Mielke & Brown leg., DZ 31.585 (DZUP). 1m, Espírito Santo, Santa Leopoldina 26.VII.1966, Mielke, Brown & Elias leg., DZ 31.287 (DZUP). 1m, Espírito Santo, Santa Leopoldina, Tirol, 25-28. IV.2001, Moser leg., DZ 27.388 (DZUP). 1m, Espírito Santo, Santa Teresa 26.II.1972, Ebert leg., DZ 27.553 (DZUP). 7f, 6m, Espírito Santo, Santa Teresa, 21-27.IX.1966, 4.XII.1966, 6.I.1967, 18.I.1967, 18-24.I.1967, 25.I.1967, 12.V.1967, 5.IX.1967, 13.IX.1967, 3.XI.1967, 22.XI.1967, 20.VII.1969, Elias & Elias leg., DZ 31.788, DZ 31.278, DZ 31.758, DZ 31.620, DZ 31.580, DZ 31.530, DZ 31.471, DZ 31.447, DZ 31.438, DZ 31.418, DZ 31.365, DZ 31.360, DZ 27.339 (DZUP). 1m, Espírito Santo, São Mateus, X.1985, Elias leg., DZ 31.454 (DZUP). 1m, 1f, Maranhão, Imperatriz 18.VI.1974, 3.VII.1974, Mielke leg., DZ 31.281, DZ 31.674 (DZUP). 1m, Maranhão, Açailândia, 23.VIII.1974, Mielke leg., DZ 27.550 (DZUP). 1m, Maranhão, Santa Lucia, Fazenda Terrasse, 2.VIII.1974, Mielke leg., DZ 27.360 (DZUP). 4m, Minas Gerais, Caratinga, 29.I-3.II.2003, Mielke & Casagrande leg., DZ 31.718, DZ 31.715, DZ 31.328, DZ 27.341 (DZUP). 4m, 3f, Minas Gerais, Corinto 2-14.IV.1979, 1-15.VI.1979, 16-30.VI.1979, VII.1979, 16-31.VIII.1979, Elias leg., DZ 31.235, DZ 31.407, DZ 31.458, DZ 31.527, DZ 31.305, DZ 31.558, DZ 31.578 (DZUP). 2m, Minas Gerais, Paracatú, 16.VI.1972, Mielke & Brown leg., DZ 31.397, DZ. 31.340 (DZUP). 1m, Minas Gerais, Poços de Caldas, 22.V.1969, Ebert leg., DZ 31.335 (DZUP). 1m, Paraná, Antonina, Cacatú, 25.IV.1973, Mielke leg., DZ 31.555 (DZUP). 2m, Paraná, Fênix, 29.IV.1987, Mielke & Casagrande leg., DZ 31.761, DZ 31.661 (DZUP). 2m, 2f, Paraná, Guaíra, 8.X.1982, Mielke leg., DZ 31.388, DZ 31.518, DZ 31.348, DZ 31.338 (DZUP). 1m, Paraná, Guaraqueçaba, Tagaçaba, 17.IV.1971, Mielke leg. DZ 31.378 (DZUP). 1m, Paraná, Guaratuba, Limeira, 21.IV.2000, Mielke leg., OM 51.708 (OM). 1m, Paraná, Londrina, 10.IX.1985, Mielke & Casagrande leg., DZ 31.637 (DZUP). 1f, Paraná, Matinhos, I-XI.1967, Moure & Willink leg., DZ 31.268 (DZUP). 1m, Paraná, Terra Rica, Parque Municipal Três Morrinhos, 13.X.2011, Carneiro, Dolibaina & Salik leg., DZ 31.330 (DZUP). 1m, Pernambuco, Garanhuns 14.XI.1960, Ebert leg., DZ 31.768 (DZUP). 1m, Rio de Janeiro, Duque de Caxias,


**Figures 1-7**. Dorsal and ventral views of *Moeris* Godman, 1900 and *Mnasitheus* Godman, 1900, species analyzed in the present study: a. dorsal view of males; b. ventral view of males; c. dorsal view of females; d. ventral view of females. **1**. *Moeris striga* (Geyer, 1832) OM 20.640, OM 17.325; **2**. *Moeris seth* Carneiro, Mielke & Casagrande **sp. nov**. DZ 31.367 (HOLOTYPE), DZ 31.410 (ALLOTYPE); **3**. *Moeris menopis* (Schaus, 1902) **stat. rest**. DZ 27.488, OM 25.611; **4**. *Moeris strada* Evans, 1955 **stat. nov**. OM 41.721, OM 25.653; **5**. *Moeris stroma* Evans, 1955 **stat. nov**. OM 24.820, DZ 31.214; **6**. *Moeris nut* Mielke, Carneiro & Casagrande **sp. nov**. OM 25.659 (HOLOTYPE); **7**. *Mnasitheus submetallescens* Evans, 1955 **comb. nov**. DZ 31.644, OM 29.912. Scale bar: 1 cm.




**Figures 8-14.** Brand format on dorsal forewing of species observed in the present study: **8**. *Moeris striga* (Geyer, 1832); **9**. *Moeris seth* Carneiro, Mielke & Casagrande **sp. nov.**; **10**. *Moeris menopis* (Schaus, 1902) **stat. rest.**; **11**. *Moeris strada* Evans, 1955 **stat. nov.**; **12**. *Moeris stroma* Evans, 1955 **stat. nov.**; **13**. *Moeris nut* Mielke, Carneiro & Casagrande **sp. nov.**; **14**. *Mnasitheus submetallescens* Evans, 1955 **comb. nov**. Scale bar: 1 mm.

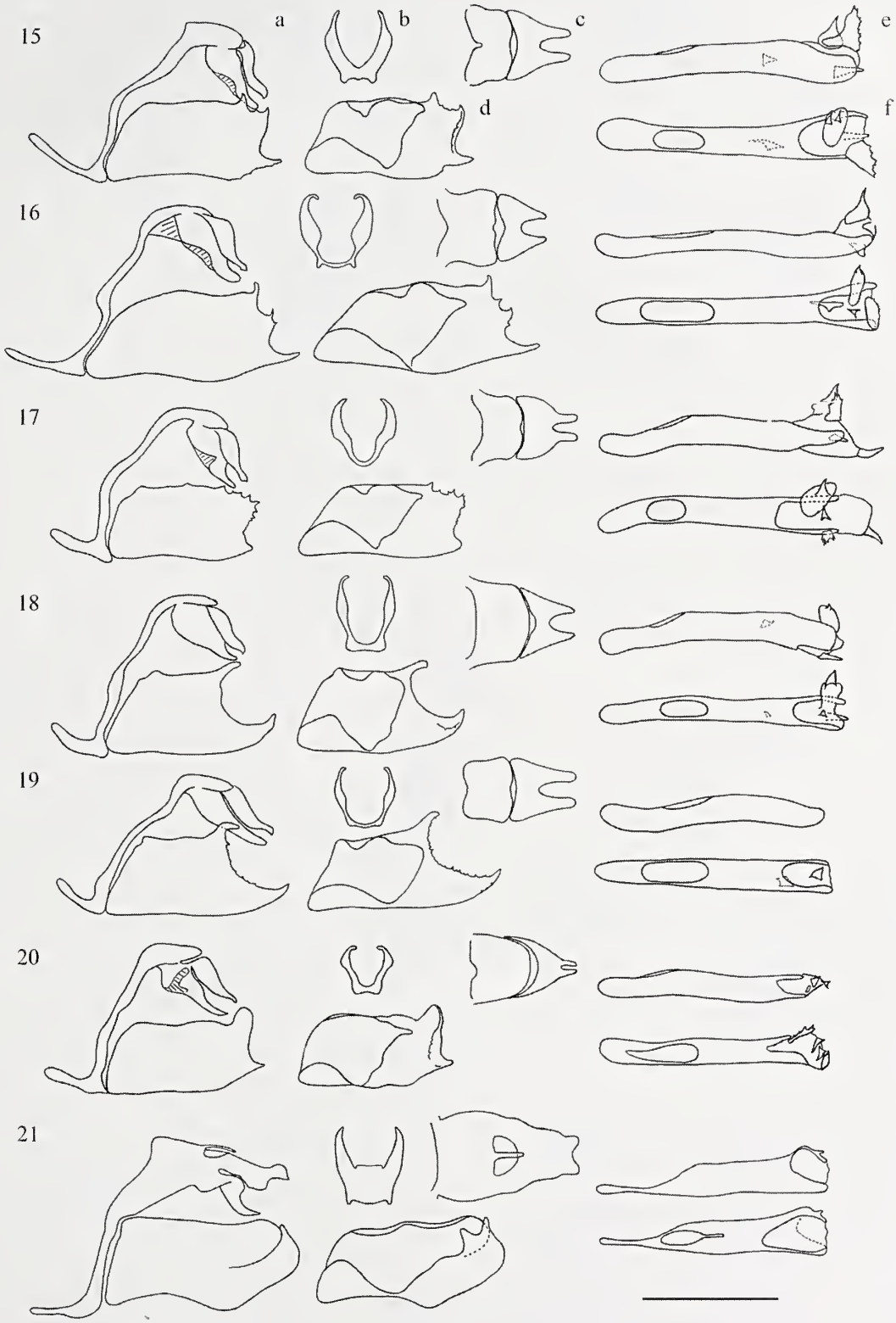
Imbariê, 21.IV.1956, Ebert leg., DZ 31.315 (DZUP). 1m, Rio de Janeiro, Itatiaia, Parque Nacional do Itatiaia, 12.VII.1963, Mielke leg., OM 5.194 (OM). 1m, Rio de Janeiro, Nova Friburgo, Mury, Pico São João, 22-23.I.1996, Mielke & Mielke leg., OM 41.694 (OM). 2f, Rio de Janeiro, Petrópolis, Alto da Serra, 10.V.1964, 27.IX.1964 (Mielke leg.), OM 5.877, OM 6.084 (OM). 1m, Rio de Janeiro, Rio de Janeiro, Covanca, 22.V.1945, D'Almeida leg., DZ 31.735 (DZUP). 3m, Rio de Janeiro, Rio de Janeiro, Covanca, 25.IV.1962, 20.IV.1963, 28.II.1965, Mielke leg., OM 4.405, OM 4.967, OM 6.929 (OM). 3m, Rio de Janeiro, Rio de Janeiro, Jacarépaguá, 10.II.1927,10. IV.1941, 14.VI.1945, D'Almeida leg., DZ 31.525, DZ 31.500, DZ 31.485 (DZUP). 1f, Rio de Janeiro, Rio de Janeiro, Lagoinhas, 9.III.1952, Ebert leg., DZ 31.645 (DZUP). 1f, Rio de Janeiro, Rio de Janeiro, Muriqui, 2.IV.1951, (Ebert leg.), DZ 31.551 (DZUP). 2m, Rio de Janeiro, Rio de Janeiro, Pão de Açúcar, 9.IX.1951, 19.I.1952, Ebert leg., DZ 31.625, DZ 31.695 (DZUP). 1m, Rio de Janeiro, Rio de Janeiro, Sumaré, 9.VII.1968, Brown leg., DZ 31.411 (DZUP). 4m, Rio de Janeiro, Rio de Janeiro, Sumaré, 7.V.1965, 10.IX.1965, Mielke leg., OM 10.602, OM 6.822, OM 7.392, OM 7.393 (OM). 1f, Rio Grande do Sul, Tenente Portela, Parque Estadual do Turvo, 10.XI.1985, Mielke, Araújo & Casagrande leg., DZ 31.221 (DZUP). 1f, Rondônia, Ariquemes, 60 km SE, 17-20.III.1989, Mielke leg., OM 22.333 (OM). 2m, Rondônia, Cacaulândia, 8-19.XI.1994, Mielke leg. OM 36.586, OM 38.369 (OM). 2m, Rondônia, Cacaulândia, Rancho Grande, 17.XI.1991, Mielke leg., OM 28.142, OM 28.118 (OM). 1m, Santa Catarina, Alto Rio dos Cedros, 18.I.1973, Lauterjung leg., DZ 31.300 (DZUP). 1f, Santa Catarina, Balneário Camboriú, 21.I.1984, Mielke leg., DZ 31.368 (DZUP). 1m, Santa Catarina, Florianópolis, Naufragados, 18.X.2003, Mielke & Carneiro leg., DZ 31.617 (DZUP). 1m, Santa Catarina, Joinville, 16-21.I.1981, Ebert leg., DZ 31.635 (DZUP). 15m, 2f, Santa Catarina, Joinville, 28.X.1967, 6.VII.1969, 24.IV.1971, 9.X. 1972, 26.XII.1976, 15.I.1977, 10.II.1978, 2.XII.1978, 24.XII.1978, 29.XII.1987, 4.I.1988, 14.V.1988, 8.X.1988, 18.III.1989, Mielke & Miers leg., DZ 31.787, DZ 31.604, DZ 31.478, DZ 31.488, DZ 31.505, DZ 31.364, DZ 31.318, DZ 31. 475, DZ 31.428 (DZUP); OM 16.846, OM 16.845, OM 18.261, OM 18.263, OM 18.262, OM 18.838, OM 20.640, OM 20.699, OM 51.491 (OM). 4m, 2f Santa Catarina, Joinville, 25.IX.1966, 17.IX.1967, 4.X.1967, 7.X.1967, 9.IX.1968, Miers leg., DZ 31.258, DZ 31.248, DZ 31.468, DZ 31.515, DZ 31.680, DZ 31.774 (DZUP). 12m, 1f, Santa Catarina, Joinville, 27.X.1968,27.III.1970, 20.XI.1970, 24.IV.1971, 22.III.1986, 14.IV.1988, Mielke leg., DZ 31.597, DZ 31.568, DZ 31.574, DZ 31.545, DZ 31.337, DZ 31.460, DZ 31.550, DZ 31.517, DZ 31.377, DZ 31.510, DZ 31.387 (DZUP); OM 17.324, OM 17.325, OM 17.326 (OM). 1f, Santa Catarina, São Bento do Sul, 20.I.1971, Ebert leg., DZ 31.408 (DZUP). 2m, 2f, Santa Catarina, São Bento do Sul, Mato Preto, 11.IV.1971, Mielke leg., DZ 31.264, DZ 31.560, DZ 31.520, DZ 31.225 (DZUP). 1m, Santa Catarina, São Bento do Sul, Rio Vermelho, 1.II.1974, Rank leg., DZ 31.507 (DZUP). 1f, Santa Catarina, São Francisco do Sul, Vila da Glória, 21.I.1982, West & Mielke leg., DZ 27.403 (DZUP). 1m, São Paulo, Araras, 15.V.1966, Ebert leg., DZ 31.420 (DZUP). 1m, São Paulo, Rio Claro, 29.VI.1962, Ebert leg., DZ 31.404 (DZUP). 1f, São Paulo, Rio Claro, 27.IV.1971, Mielke leg., DZ 31.660 (DZUP). 1f, São Paulo, São Paulo, Túnel da Mata Fria, 13.II.1976, Mielke & Casagrande leg., DZ 31.381 (DZUP). 1f, São Paulo, Ubatuba, 14.IX.1980, Ebert leg., DZ 31.405 (DZUP). **Paraguay**: 1m, Alto Parana, Itakyry, General Dias, 15-20.I.1980, Mielke, Mielke & Miers leg., DZ 31.559 (DZUP). **Peru**: 1m, Junin, San Ramon, Hacienda Naranjal, 15-18.X.1989, Mielke & Casagrande leg., OM 23.158 (OM).

## *Moeris seth* Mielke, Carneiro & Casagrande sp. nov.

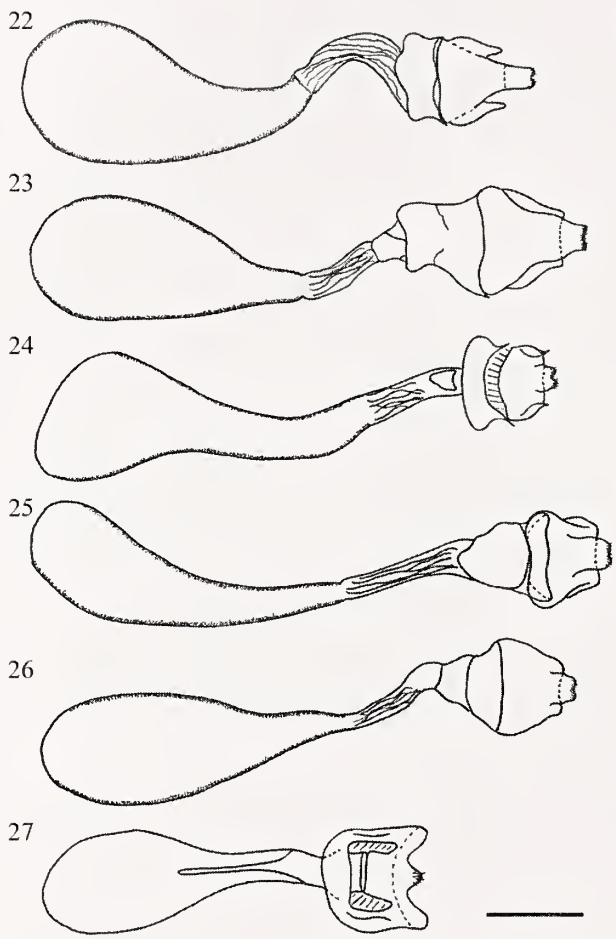
(Figs. 2, 9, 16, 23, 28)

**ZooBank LSID urn:lsid:zoobank.org:act:3AC99C41-CC5D-42BC-8AB8-E3B3E4C32DEE**

**Diagnosis**: Dorsal and ventral wing pattern as in *Moeris striga*, *M. menopis*, *M. strada* and *M. stroma*. However, male and female genitalia present unique characters. Posterior margin of harpe with a spined prominence between its ventro-posterior projection and the dorsal spined projection of ampulla. One of cornuti spines is globular with a spine on tip, as in *M. striga*, but the other serrate




**Figures 15-21**. Male genitalia of *Moeris* Godman, 1900 species analyzed in the present study: a. lateral view of tegumen, saccus, uncus, gnathus and valva; b. ventral view of fultura inferior; c. dorsal view of tegumen and uncus; d. left view of the right valva; e. lateral view of aedeagus; f. dorsal view of aedeagus. **15**. *Moeris striga* (Geyer, 1832). OM 23.158; **16**. *Moeris seth* Carneiro, Mielke & Casagrande **sp. nov**. DZ 31.294 (PARATYPE); **17**. *Moeris menopis* (Schaus, 1902) **stat. rest**. OM 25.605; **18**. *Moeris strada* Evans, 1955 **stat. nov**. OM 41.721; **19**. *Moeris stroma* Evans, 1955 **stat. nov**. OM 26.666; **20**. *Moeris nut* Mielke, Carneiro & Casagrande **sp. nov**. OM 25.659 (HOLOTYPE); **21**. *Mnasitheus submetallescens* Evans, 1955 **comb. nov**. DZ 9.742. Scale bar: 1 mm.




**Figures 22-27**. Ventral view of female genitalia of *Moeris* Godman, 1900 species analyzed in the present study: **22**. *Moeris striga* (Geyer, 1832) DZ 31.235; **23**. *Moeris seth* Mielke, Carneiro & Casagrande **sp. nov**. DZ 31.264 (PARATYPE); **24**. *Moeris menopis* (Schaus, 1902) **stat. rest**. OM 25.635; **25**. *Moeris strada* Evans, 1955 **stat. nov**. OM 25.653; **26**. *Moeris stroma* Evans, 1955 **stat. nov**. OM 44.304; **27**. *Mnasitheus submetallescens* Evans, 1955 **comb. nov**. OM 51.287. Scale bar: 1 mm.

spine is of the same size as other spines and not developed as in the species mentioned. Female sterigma elongated as in *M. striga*, but lamella antevaginalis longer and quadrangular; posterior margin of lamella postvaginalis larger and straight, instead of bilobed.

**Description**: Forewing length: 11–13mm. Eyes red. Vertex dark brown. Antennae longer than 1/2 costa; club short (1/4 shaft); shaft ventrally yellowish to ochreous; nudum 12, extended to the clava. Palpus quadrate (inner edge equal to the transverse width); second segment ventrally yellowish; third segment medium, around half of the second segment length, cylindrical. Thorax dorsally and ventrally dark brown, with distinct ochreous tegula. DFW uniformly brown, except for the reddish scales in the base of costal area and the variably present yellowish subapical and upper cell spots. Brand tripartite, elongated and curved between $CuA_1$ and $CuA_2$, as a rounded spot below $CuA_2$, and coma-shaped $CuA_2$ and 2A. VFW costal area reddish at the base turning slightly yellowish at apex; apical spot markedly yellowish, contiguous with marginal band, which extends and fade beyond $CuA_1$; subapical spots in $R_3$-$M_1$ also yellowish, opaque, and dorsally contiguous with marginal band by a costal yellowish line. DHW homogeneous brown. VHW ground color in variegated yellowish, reddish, black and purple tones spots. Legs yellowish to ochreous; tibial spurs formula 0-1-2; mid and hindtibia spined. Male genitalia: Median apophyses of tegumen absent; fenestra reduced, triangular, wider than long. Saccus lobbed, not reduced nor elongated. Uncus bifid, arms medially separated, separated from each other by a short distance. Gnathos hooked-shaped, with a ventral membranous patch. Valva without posterior median cleft dividing ampulla from harpe; sacculus triangular, shorter than half of valva height; harpe projected posteriorly, ventrally as a short more or less straight spur, while medially lobed with spines; ampulla with a dorsal projection as a spine. Aedeagus cylindrical, shorter than valva + saccus length; coecum shorter than the distal part of aedeagus, dorsally and laterally straight, globular; posterior end laterally projected by parallel truncated projections, therefore dorsally and ventrally hollowed; vesica membranous with four relatively large spines as cornuti, two triangularly spined, one spatular with serrate posterior margin, and the other larger, globular, with a spine on its tip. Fultura inferior directed dorsally and posteriorly; projections thin, extended only laterally of aedeagus; ventrally straight, with antero-ventral lobes. Female abdomen with sensitive spots in pleura above sternum IV, V and VI. 8th tergite with espiracular opening present, ellipsoid, not totally separated from external margin. Female genitalia: Lamella antevaginalis quadrate, projected below anterior part of lamella postvaginalis forming a sclerotized tube hiding the ostium bursae. Lamella postvaginallis elongated, with lateral folding towards the pleura; posterior margin more or less straight, with minute setae clothing. Ductus bursae sinuous, proximally sclerotized, with medio-distal sclerotized grooving and thin lateral signa markings from the end of grooving to the bottom of the corpus bursae.

**Geographical distribution**: Southern Brazilian Araucaria forest, extending to its northern highland enclaves, from Minas Gerais to Rio Grande do Sul.

**Studied material**: HOLOTYPE (DZUP): male with the following labels: / Holotypus / Curitiba[,] PR[Paraná], Brasil[,] 900m 20-II-1968[,] [O.] Mielke leg./ DZ 31.367 /. ALLOTYPE (DZUP): female with the following labels: Curitiba – Paraná[,] 900 metros – Brasil[,] 5-IV.1974[,] O. Mielke leg./ DZ 31.410/. PARATYPES: Paratypus: **Brazil**: 1m, Minas Gerais, Camanducaia, Monte Verde, 18-21. III.1964, Ebert leg., DZ 31.284 (DZUP). 1m, Minas Gerais, Poços de Caldas, 17.XII.1965, Ebert leg., DZ 31.394 (DZUP). 2m, Minas Gerais, Poços de Caldas, Morro São Domingos, 30.III.1965, Mielke leg., OM 6.820, OM 6.821 (OM). Paraná, Almirante Tamandaré, Tanguá, 28.V.1966, Mielke leg., DZ 27.421 (DZUP). 8m, 2f, Paraná, Curitiba, 31.I.1966, 22-IV-1967, 4.V.1967, 22.III.1969, 20.III.1970, 17.II.1971, 5.IV.1974, 13.II.1975, Mielke leg., DZ 27.491, DZ 31.228, DZ 31.291, DZ 31.294, DZ 31.440, DZ 31.430, DZ 31.495, DZ 31.534, DZ 31.664 (DZUP); OM 7.922 (OM). 1m, Paraná, Curitiba, Moure leg., II.1941, DZ 31.455 (DZUP). 4m, 1f, Paraná, Curitiba, Cascatinha, 29.V.1966, 30.XI.1966, 29.I.1967, 22.IV.1967, Mielke leg., DZ 31.451, DZ 31.308, DZ 31.238, DZ 31.628, DZ 31.298 (DZUP). 2m, Paraná, Guarapuava, 13.I.1980, Mielke & Miers leg., DZ 31.577, DZ 31.261 (DZUP). 1m, Paraná, Guarapuava, Rio Iguaçú, 4.II.1976, Mielke & Buzzi leg., DZ 31.567 (DZUP). 1m, Paraná, Tijucas do Sul, Vossoroca, 8.III.1972, Mielke leg., DZ 31.470 (DZUP). 1m, Paraná, Turvo, Britador, 6.I.2010, Dolibaina leg., DD 003 (DD). 1m, Paraná, Turvo, Britador, 24-30.XII.2010, Dolibaina leg., DD 004 (DD). 2m, Paraná, Turvo, Britador, 22.II.2012, Dolibaina leg., DD 005 (DD). 1m, Paraná, Turvo, Britador, 21.XII.2014, Dolibaina leg., DD 006 (DD). 1m, Paraná, Turvo, Britador, 23.XII.2014, Dolibaina leg., DD 007 (DD). 1f, Paraná, Turvo, Salto do Paulinho Rickli, 27.XII.2014, Dolibaina leg., DD 008 (DD). 1m, Rio Grande do Sul, Caxias do Sul, 28.II.1973, Mielke leg., DZ 31.627 (DZUP). 1m, Rio Grande do Sul, Ivoti, 7.IV.2000, Moser leg., DZ 31.251 (DZUP). 1m, Rio Grande do

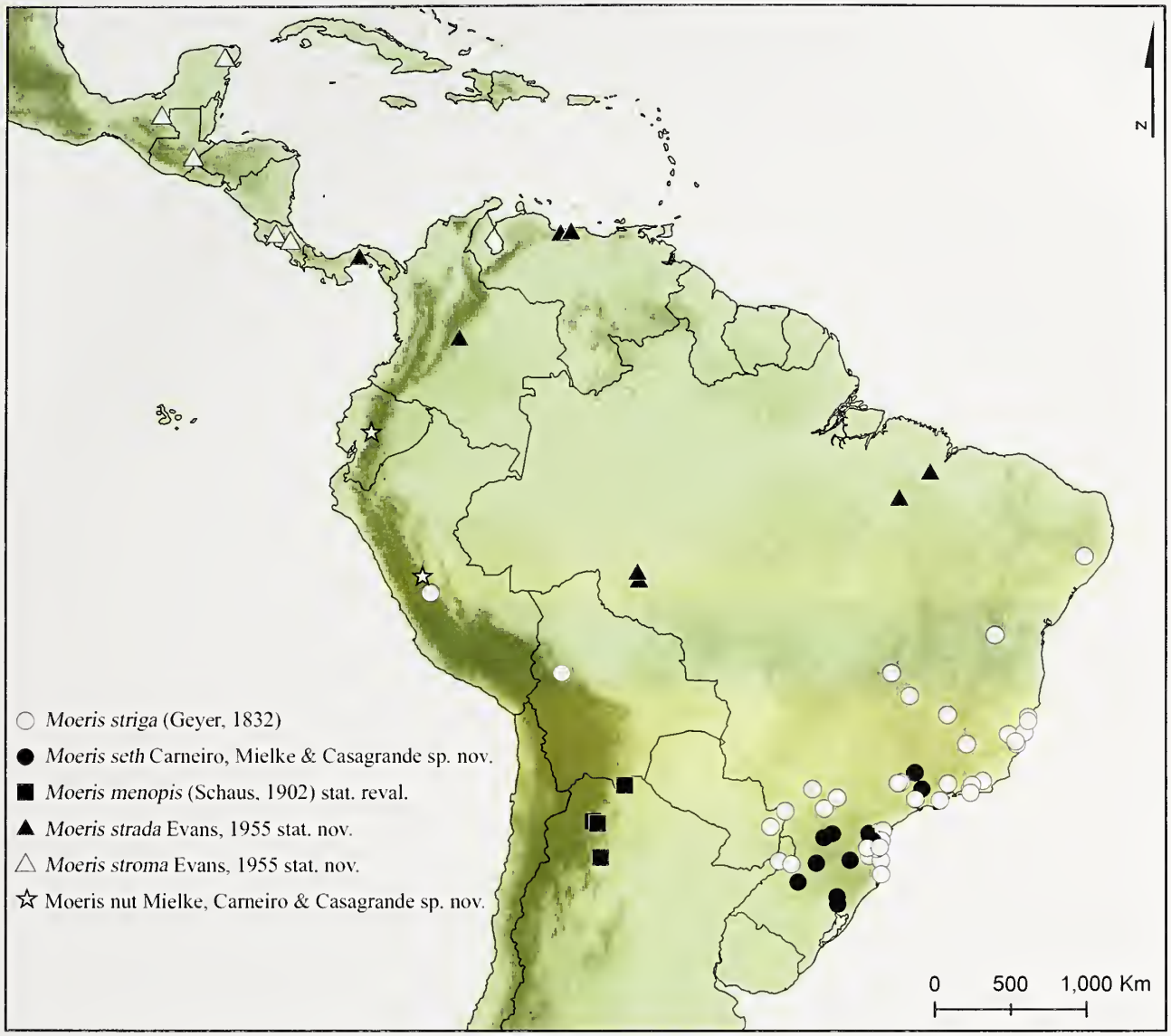



**Figure 28.** Geographical distribution of *Moeris* Godman, 1900 species, as observed in the present study.

Sul, Ivoti, 7.IV.2000, Moser leg., DZ 31.565 (DZUP). 1m, Rio Grande do Sul, Panambi, V.1967, Schaal leg., DZ 31.615 (DZUP). 1m, Santa Catarina, Santa Cecilia, Campo Alto, 13.II.1976, Mielke & Buzzi leg., DZ 31.435 (DZUP). 1m, Santa Catarina, Santa Cecilia, Campo Alto, 12.II.1973, Mielke & Sakakibara leg., DZ 31.517 (DZUP). 5m, 5f, Santa Catarina, Seara, Nova Teutônia, II.1969, I.1972, VIII.1977, I.1982, II.1982, III.1982, IV.1985, I.1986, Plaumann leg., DZ 31.490, DZ 31.358, DZ 31.607, DZ 31.218, DZ 31.457, DZ 31.327, DZ 31.448, DZ 31.694, DZ 31.584, DZ 31.547 (DZUP).

**Remarks**: This species has been misidentified as *M. striga* probably due to the lack of external distinguishing characters between these two species. Their geographical distributions are also similar, though they differ with regard to altitudinal range. *M. seth* has thus far been recorded only from high altitude regions of southeastern and southern Brazil, extending southwards into Uruguay, whereas *M. striga* is a lowland species. Characters in male and female genitalia do not vary in *M. seth* and *M. striga*, facilitating the distinction between the two species.

**Etymology**: The epitheton *seth* comes from the Egyptian mythological god Seth, known as the god of chaos, disorder, and storms.

## *Moeris menopis* (Schaus, 1902) **stat. rest.** (Figs. 3, 10, 17, 24, 28)

**Diagnosis**: Well-marked subapical spots $R_1$-$M_1$ and yellowish large spots in $M_3$-$CuA_2$; harpe with truncated posterior projection and spined dorsal margin; lamella antevaginalis placed anteriorly of lamella postvaginalis; ductus bursae with a conical sclerotized tube posteriorly, close to the antrium.

**Remarks**: This taxa was previously ranked as a subspecies of *M. striga*. However, *M. menopis* also presents unique male and female genitalia patterns, which justifies its species status.

**Geographical distribution**: Northwestern Argentina.

**Studied material**: **Argentina**: 1f, Salta, Quebrada de Ramos,

24.IV.1978, Eisele leg., OM 25.611 (OM). 1f, Salta, Abra Grande, Crán, 10.I-1.III.1967, Golbach leg., OM 25.623 (OM). 1m, Salta, Tartagal, 9.II.1950, Golbach leg., OM 25.641 (OM). 1m, Tucuman, OM 25.605, (OM). 1m, Tucuman, Las Huigeras, 24.I.1970, Mielke leg., DZ 27.488 (DZUP). 1m, 2f, Tucuman, San Miguel de Tucuman, S.P. Colalao, I.1949, Arnan leg., OM 25.635, OM 25.629, OM 25.617 (OM).

### *Moeris strada* Evans, 1955, **stat. nov.**
(Figs. 4, 11, 18, 25, 28)

**Diagnosis**: Dorsal and ventral wings as in *Moeris striga, M. seth* and *M. stroma.* Posterior margin of harpe C-shaped, with the ventral prolongation large as in *M. stroma,* but shorter and slightly more curved dorsally. Aedeagus with a long globular cornuti with a spine on tip as in *Moeris striga,* but all others are distinctly shorter; ductus bursae greatly developed posteriorly as a conical sclerotized tube.

**Remarks**: This taxa was previously ranked as a subspecies of *M. striga.* Though similar in wing pattern to *Moeris striga, M. seth,* and *M. stroma, M. strada* presents unique male and female genitalia patterns which justify its species status.

**Geographical distribution**: Widespread among Amazonian forest to Eastern Panama.

**Studied material**: **Colombia**: 1m, Meta, Villavicencio, 21.IX.1980, Callaghan leg., OM 25.647 (OM). **Panama**: 1m, Panama, Balboa, 27.I.1979, Robbins leg., DZ 31.630 (DZUP). **Venezuela**: 1f, Aragua, Rancho Grande, 20.VIII.1955, Yepes leg., OM 25.653 (OM). 1m, Distrito Federal, Antimano, 15.VIII.1934, Lichy leg., OM 41.721 (OM).

### *Moeris stroma* Evans, 1955 **stat. nov.**
(Figs. 5, 12, 19, 26, 28)

**Diagnosis**: Dorsal and ventral wings as in *Moeris striga, M. seth* sp. nov. and *M. strada* **stat. nov.** Posterior margin of harpe C-shaped, with the ventral prolongation large as in *M. strada* stat. nov., but longer and slightly more straight posteriorly. Aedeagus with equally sized cornuti, besides the elongated ventral spine, a character present in all species of *Moeris* described above; lamella antevaginalis and sclerotized posterior part of ductus bursae both trapezoidal.

**Remarks**: Though similar in wing pattern to *Moeris striga, M. seth* **sp. nov.**, and *M. strada, M. stroma* presents unique male and female genitalia patterns which justify its species status.

**Geographical distribution**: Central America from southern Mexico to Costa Rica.

**Studied material**: **Costa Rica**: 2m, Alajuela, Bajo Rodrigues, 17.II.1990, 14.X.1990, Pagels leg., OM 24.820 (OM). 3m, Cartago, Turrialba, 1-15.X.1971, 1-15.IV.1972, 15-30.IV.1973, Becker leg., DZ 31.361, DZ 31.684, DZ 31.784 (DZUP). **Guatemala**: 1m, Zacapa, La Union, 3.VII.1978, Welling leg., OM 44.690 (OM). **Mexico**: 2m, 1f, Quintana Roo, Nuevo Xcan, 20.VI.1972, IX.1973, 2.VII.1974, Welling leg., DZ 31.654, DZ 31.634, DZ 31.214 (DZUP). 1m, Quintana Roo, Xcan, 23.VII.1967, Welling leg., DZ 31.684 (DZUP). 2f, Tabasco, Tenosique, 13.IX.1962, 19.IX.1962, Welling leg., OM 44.304, OM 44.368 (OM).

### *Moeris nut* Mielke, Carneiro & Casagrande **sp. nov.**
(Figs. 6, 13, 20, 28)

**ZooBank LSID urn:lsid:zoobank.org:act:9B239E5C-6D69-4808-8F8D-408DA2ED5D56**

**Diagnosis**: Brand bipartite and not tripartite or absent as other *Moeris* species. Posterior half of VHW with distinct iridescent bluish scales over reddish and yellowish pattern. *M. padus* Evans, 1955 and *M. hyagnis* (both subspecies) present bluish spots on VHW, but more compact, as longitudinal bands instead of transversal as in *Moeris nut* **sp. nov.** Harpe with a short ventral projection and inner spined protruding line paralael to posterior margin. Ampulla with a lobular dorsal projection.

**Description**: Forewing length: 11.5--12mm. Eyes red. Vertex dark brown. Antennae longer than 1/2 costa; club short (1/4 shaft); shaft basal portion ventrally yellowish to ochreous; nudum 12, on apiculus extended to the clava. Palpus quadrate (inner edge equal to the transverse width); second segment ventrally yellowish; third segment dark brown with sparse yellowish scales, around half of the second segment length, cylindrical. Thorax dorsally and ventrally dark brown, with distinct ochreous tegula. DFW uniformly brown, except for the sparse reddish scales on costal area, yellowish subapical spots in $R_3$-$M_1$ and black brand; brand bipartite, the first section wide, trapezoidal, in $CuA_1$-$CuA_2$, and the second reduced and rounded, in $CuA_2$-2A besides $CuA_2$. VFW costal area with distinct, compact scales, placed in all costal area, reddish at the base turning slightly yellowish at apex; apical spot markedly orange, contiguous with marginal band, which extends and fade beyond $CuA_1$; subapical spots in $R_3$-$M_1$ also orange, opaque, and separated by marginal band by a reddish brown radial band. DHW homogeneous brown. VHW ground color with variegated brownish, reddish and black tones; costal area and posterior half overlaid with bluish scales, except the anal fold, which is uniformly brown. Legs yellowish to ochreous; midtibia spined with pair of spurs; hind tibia spined with two pairs of spurs. Male genitalia: Median apophyses of tegumen absent. Fenestra reduced to a slender space between tegumen and uncus. Saccus not reduced nor elongated, lobed. Uncus symmetric, bifid; arms projected, medially separated from each other by a short distance, without spines on the tips. Gnathos hook-shaped with lateral membranous patch. Valva without posterior median cleft dividing ampulla from harpe; sacculus triangular; harpe projected as a short straight spine posteriorly and as a wide lobular projection dorsally, inner margin with a submarginal spine protruding. Aedeagus as long as valva + saccus length, tubular, straight; coecum globular, shorter than the distal part of aedeagus, dorsally and laterally straight; dorso-posterior and ventro-posterior end of aedeagus hollowed, hollow dorsally V-shaped, ventrally larger and U-shaped; cornuti present as four irregular relatively large spines. Fultura inferior directed dorsally and posteriorly, projections extend only laterally of aedeagus; lateral arms thin; ventrally straight, with reduced antero-ventral lobes. Female unknown.

**Geographical distribution**: Known from only two locations at the western Peruvian and Eastern Ecuadorian Andes.

**Studied material**: HOLOTYPE (OM): male with the following labels: / Holotypus / Pallatanga, Chimborazo, Ecuador, 3200m[,] VIII-1979[,] Lafebre leg. / OM 25.659 / gen. prep. E. Carneiro 2014 /. PARATYPE (OM): 1 male with the following labels: / Paratypus / Pachitea [Peru, Huánuco] / OM 12.045/.

**Remarks**: Known only from two specimens, but the striking differences in brand format and male genitalia confirm it as a distinct species.

**Etymology**: The name *nut* comes from the Egyptian mythological goddess Nut, known as the goddess of the sky.

### *Mnasitheus submetallescens* (Hayward, 1940) **comb. nov.**
(Figs. 7, 14, 21, 27, 28)

**Remarks**: Previously combined to *Moeris,* this species easily recognized by the striking metallic bluish pattern present on the whole VHW. Examination of brand, male and female genitalia precludes its inclusion within *Moeris,* by comparison with its type species, *Moeris striga* (Geyer, 1832). The brand of *Mnasitheus submetallescens* **comb. nov.** is sagittated over the origin of $CuA_2$

and parallel elongated below $CuA_2$ and parallel above 2A. The stigma of all *Moeris* species, when present, is always elongated and perpendicular to $CuA_2$, whose portion below $CuA_2$ is rounded instead of elongated. Valva of *Mnasitheus submetallescens* presents a cleft dividing the ampulla and harpe, which is absent in all *Moeris* species, except for *M. padus*. Additionally, the dorsal bifid pointed projection of inner margin of harpe and the thin coecum of aedeagus are common features of *Mnasitheus* Godman, 1900 species, and are thought to be always absent in *Moeris*. Finally, the female genitalia of *M. submetallescens* present a pair of elongated sclerotized projections besides ductus bursae. This unique character has not been observed elsewhere in Moncini besides *Mnasitheus* species, including *Mnasitheus chrysophrys* (Mabille, 1891), the type species of the genus.

**Geographical distribution**: Widespread among Atlantic forest.

**Studied material**: **Argentina**: 1m, Misiones, Almirante Brown, Reserva Yacutinga, 2-5.III.2007, Mielke & Casagrande leg., DZ 31.215 (DZUP). **Bolivia**: 1f, La Paz, Caranavi, I-II.1990, Tello leg., OM 26.499 (OM). **Brazil**: 1m, Paraná, Foz do Iguaçú, Parque Nacional do Iguaçú, 21-24.IV.1995, Mielke & Casagrande leg., DZ 31.734 (DZUP). 1f, Paraná, Foz do Iguaçú Parque Nacional do Iguaçú, 20-26.VIII.2000, Mielke leg., OM 51.287 (OM). 1m, Rio Grande do Sul, Santa Rosa, 26.XII.1953, Biezanko leg., OM 29.930 (OM). 3f, Santa Catarina, Seara, Nova Teutônia, I.1965, XII.1972, I.1986, Plaumann leg., OM 29.912, OM 29.918, OM 29.960 (OM). **Paraguay**: 1m, Alto Parana, Itakyry, General Dias, 15-20.I.1980, O. Mielke, C. Mielke & Miers leg., DZ 31.644 (DZUP). **Peru**: 1f, Cuzco, Marcapata, OM 12.079 (OM). 2m, Madre de Dios, Pakitza, Parque Manu, 29.IX.1991, 13.X.1991, Mielke leg., DZ 9.742, DZ 27.518 (DZUP). 1m, Madre de Dios, Puerto Maldonado, IX-XI.1992, Tello leg., OM 34.259 (OM). 1m, Madre de Dios, Tambopata, Tambopata Reserve, 26.X.1991, Mielke leg., DZ 31.595 (DZUP).

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## Editor's note

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# Effect of sperm ejection by females on male fertilization success in the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae)

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**Abstract**. Substantial evidence for cryptic female choice (CFC) has been reported in numerous taxa. However, the mechanisms of CFC are not fully established, partly due to the difficulties in disentangling female from male controls on paternity. The loss of sperm in the female sperm storage organs has been observed in several species of Lepidoptera. Due to the complex morphology of the female genitalia, males may not be able to displace sperm in the spermatheca mechanically. Therefore, the sperm of the male previously mated with might only be ejected by the female. To investigate the effect of sperm ejection by females on male reproductive success, we measured the sperm precedence in the swallowtail butterfly, *Papilio xuthus* (Linnaeus, 1767) using sterilized males. In this species, sperm loss from the spermatheca was more likely to occur in females that received a large spermatophore from the current male. The $P_2$ value (i.e. the proportion of eggs fertilized by the second male in a double-mating trial) exhibited a bimodal distribution with peaks at 0% and 100%, indicating that most eggs laid after the second mating were sired by just one of a female's mates. When a female had mated twice, the male that transferred the larger spermatophore was more likely to be the principal sire, irrespective of whether he was the female's first or second mate. Therefore, male reproductive success appears to be affected by the ejection of sperm by the female, indicating that female sperm ejection is the mechanism of CFC in this butterfly species.

**Keywords:** Cryptic female choice; $P_2$ value; sexual selection; sperm usage; spermatophore.

## Introduction

Postcopulatory sexual selection is important for males in polyandrous species, because a male does not always fertilize all the eggs laid by his mates. Hence, male reproductive success does not necessarily increase with the number of matings. Under polyandry males have to compete for fertilization of the eggs even after copulation, a phenomenon termed sperm competition (e.g. Simmons, 2001), which is considered a powerful selective force responsible for shaping male reproductive traits (Harvey & Bradbury, 1994). In addition, female mechanisms that bias paternity toward males with preferred traits, i.e., cryptic female choice (CFC), have also been observed (Thornhill, 1983). Although substantial indirect evidence of CFC has been reported in numerous taxa (e.g. Eberhard, 1991), few studies have conclusively shown paternity bias to be driven by CFC (Fedina, 2007; Pizzari & Birkhead, 2000). Eberhard (1996) suggested that in order to unravel the particular female processes or traits involved in the CFC mechanism, it is necessary to demonstrate that (1) female responses to some conspecific males differ from responses to others, (2) this discrimination between males results in differences in reproductive success of the males, and (3) female biases are associated with particular male characteristics.

Many potential CFC mechanisms such as premature interruption of copulation, lack of sperm transport to storage, the ejection of sperm in storage, lack of ovulation, selective abortion, and others have been proposed (Eberhard, 1996). Of these mechanisms, the loss of sperm in the female sperm storage organ, the spermatheca, has been observed in several species

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of Lepidoptera (e.g. Pair *et al.*, 1977). Lepidopteran males transfer a single spermatophore containing sperm to the bursa copulatrix of the female during copulation (Fig. 1). Sperm in the spermatophore starts to migrate to the spermatheca several hours after the termination of copulation (Watanabe & Hachisuka, 2005). After this migration, sperm is stored in the spermatheca and later used for fertilization. Because sperm cells in the spermatheca by far outnumber eggs in the female's ovarioles, it has been believed that at least a certain number of sperm in the spermatheca survive throughout the female's lifespan. Etman & Hooper (1979) showed that in the cotton leaf-worm, *Spodoptera litura* (Fabricius, 1775; Noctuidae), sperm in the spermatheca of re-mated females began to decrease in numbers, and the spermatheca became empty just after termination of the second copulation and then increased again. Due to morphological restrictions of the aedeagus of lepidopteran males, they cannot remove rival sperm mechanically from the spermatheca (e.g. Drummond, 1984). In addition, because the loss of sperm from the spermatheca occurred before the sperm migration of the current male started (Xu & Wang, 2010), the sperm in the spermatheca cannot have been washed away by the current male's ejaculate. Thus, females apparently control this process. Using the swallowtail butterfly, *Papilio xuthus* (Linnaeus, 1767), Watanabe & Sasaki (2010) demonstrated that sperm loss from the spermatheca was more likely to occur in females that received a large spermatophore from their second male mate. Therefore, lepidopteran females appear to be able to determine whether or not to eject sperm from a former mate on the basis of some traits of her mates.

If females use sperm loss as a means of cryptic mate choice, presence or absence of sperm loss should result in differences in fertilization success of males. However, because sperm loss is an internal process, it is impossible to observe sperm loss and fertilization success in the same female. So, again using *P. xuthus*, we here compared the $P_2$ values (i.e., the proportion of eggs fertilized by the second male in a double-mating trial) between females to which larger spermatophores had been transferred from the second than from the first male, and females to which larger spermatophores had been transferred from the first than the second male. If sperm loss has a significant effect on male fertilization success, the $P_2$ value will be higher in females to which a larger spermatophore has been transferred from the second male, because sperm loss is more likely to occur in females that have received a large spermatophore from the second male.

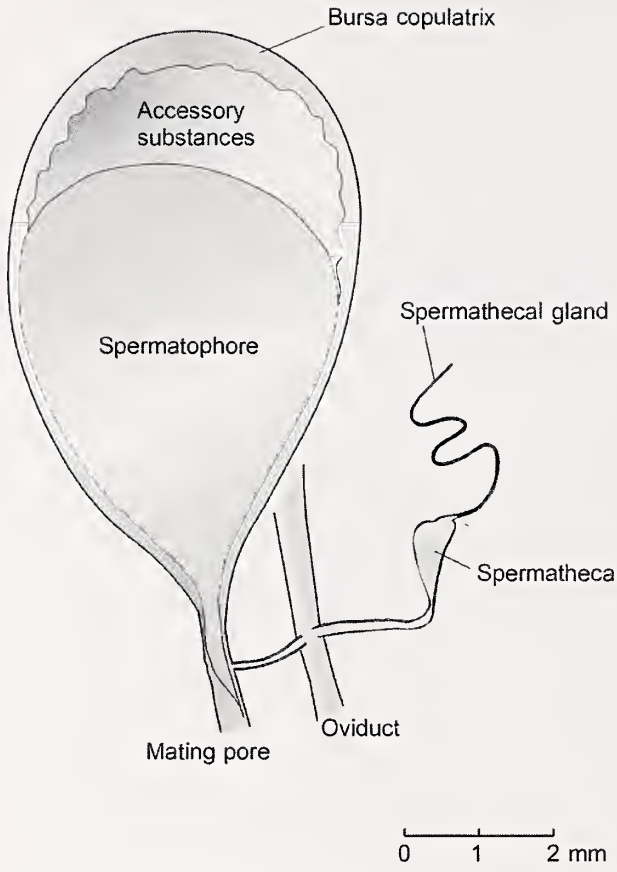


**Figure 1.** A schematic representation of the *Papilio xuthus* bursa copulatrix, including a spermatophore and accessory gland substances, and the spermatheca in a singly mated female (after Watanabe *et al.* (2000)).

## Materials and methods

In the summer of 2010, *Papilio xuthus* females were captured in Ibaraki Prefecture (Japan) to found a captive breeding stock. To avoid diapause, eggs and larvae collected from the females were reared on leaves of the host tree, amur cork, *Phellodendron amurense* Rupr., in the laboratory (room temperature of 28°C and natural long-day photoperiod in July). The laboratory-reared adults were weighed on the day of eclosion (Model AE-240, Mettler, Japan) to an accuracy of ±0.01 mg, and were given individual marks with a felt-tipped pen on their left hind wing. They were kept in net cages (400 × 400 × 450 mm) and fed a 20% sucrose solution for 10 min each day until mating.

The effect of spermatophore size on the pattern of sperm use by females was investigated using females that were mated with a radio-sterilized male and a normal male. We used both virgin and mated males (within 3 days after the first mating) to manipulate the size of

**Table 1**. Body mass of both sexes and the ejaculate mass for each mating group (mean±SD). Statistical tests refer to ANOVA comparisons across the four sets of experimental animals.

| | No. pairs | Female | First male | | Second male | |
|---|---|---|---|---|---|---|
| | | Body mass (mg) | Body mass (mg) | Ejaculate mass (mg) | Body mass (mg) | Ejaculate mass (mg) |
| Set N/N | 8 | 450.8±69.5 | 331.1±42.9 | 15.5±8.1 | 340.5±54.3 | 9.5±7.5 |
| Set S/S | 8 | 448.4±69.3 | 355.3±50.1 | 15.1±3.4 | 304.3±16.2 | 11.3±5.5 |
| Set N/S | 12 | 396.5±65.6 | 324.6±53.4 | 11.8±5.6 | 322.3±58.3 | 8.9±6.3 |
| Set S/N | 11 | 446.4±63.4 | 335.7±58.5 | 10.6±3.2 | 339.5±59.6 | 13.1±5.7 |
| | | $F$=1.58, $p$=0.21 | $F$=0.55, $p$=0.65 | $F$=1.97, $p$=0.14 | $F$=0.92, $p$=0.44 | $F$=0.99, $p$=0.41 |

the spermatophore they were able to transfer to the females. Niihara & Watanabe (2009) showed that a male re-mating within 3 days after a first mating transfer a smaller spermatophore than virgin males. Sterilization of males was carried out by exposure to γ-rays from a $^{60}$Co source (*Gamma cell-220*, Nordion International Inc., Kanata, ON, Canada) at a dose of 250 Gy (dose rate: 100 Gy/min). The dose was based on those used in previous studies with other species (Bissoondath & Wiklund, 1997; Seth *et al.*, 2002). Irradiation was performed on the morning of the males' mating day.

Eighty-nine virgin females were hand-paired on the day after eclosion with normal (N) or sterile (S) males. The body mass of the females before and after mating was measured. Due to the impossibility to measure the actual size of the spermatophore without dissection, we used the increase in female mass after mating as a proxy of the size of the spermatophore transferred (±0.01 mg). After the first mating, females were placed individually in egg-laying cages (400 × 400 × 450 mm) with leaves of the larval host tree in order to deposit their eggs and fed 20% sucrose solution for 10 min each day. Leaves were replaced daily, and all eggs deposited were counted.

Three to four days after the first mating, mated females were mated again with either N males or S males. Females were weighed before and after mating to estimate the size of the second spermatophore they had received. After the second mating, females were again placed individually in egg-laying cages with leaves of the larval host tree in order to deposit their eggs and fed 20% sucrose solution for 10 min each day. Leaves were replaced daily until the females died, and all eggs deposited were counted. Probably because of the laboratory conditions such as limited insolation or limited size of the cages, some females failed to lay substantial numbers of eggs. Therefore, only 39 out of 89 females mated twice, which laid more than 10 eggs after the second mating, were considered in the subsequent analyses. The numbers of females mated successively with two N males (set N/N), with two S males (set S/S), first with an N male, followed by a second mating with an S male (set N/S), and first with an S male, followed by a second mating with an N male (set S/N), were 8, 8, 12 and 11, respectively.

The $P_2$ value was calculated from the viability of eggs (the percentage of eggs that hatched) laid by females in set N/S and set S/N. Because the possibility remains that a certain proportion of eggs fertilized by an N male do not hatch and a certain proportion of eggs fertilized by an S male may hatch, the proportion of eggs fertilized by sperm from the second mating was calculated using the following formula (Sillén-Tullberg 1981):

$$P_2 = 1 - (X_{\text{Set N/S}} - \bar{X}_{\text{Set S/S}})/(\bar{X}_{\text{Set N/N}} - \bar{X}_{\text{Set S/S}})$$

or

$$= (X_{\text{Set S/N}} - \bar{X}_{\text{Set S/S}})/(\bar{X}_{\text{Set N/N}} - \bar{X}_{\text{Set S/S}})$$

where $P_2$ = the proportion of eggs fertilized by the sperm of the second mate, and $\bar{X}_{\text{Set N/N}}$ and $\bar{X}_{\text{Set S/S}}$ represent the average viability of eggs derived after the second mating from a female mating successively with either two N males or two S males, respectively. $X_{\text{Set S/N}}$ indicates the viability of eggs derived after the second mating from a female mating first with an S male, followed by a second mating with an N male. And $X_{\text{Set N/S}}$ is the viability of eggs derived after the second mating from a female mating first with an N male, followed by a second mating with an S male.

The difference in female body mass, indicating spermatophore mass, among the sets was analyzed using ANOVA. The body mass, indicating spermatophore mass, was also compared between the first and the second males in each set by using the Mann-Whitney *U*-test. $P_2$ values were analyzed with generalized linear models with binomial errors of the percentage of offspring sired by the second male to mate. As explanatory variables, we used relative ejaculate mass (log(weight gain of females

**Table 2.** Number of eggs laid (mean±SD) and hatching rate (mean±SE) for each mating group.

| | Before the second mating | | | After the second mating | | |
|---|---|---|---|---|---|---|
| | n | No. eggs laid | Hatching rate (%) | n | No. eggs laid | Hatching rate (%) |
| Set N/N | 6 | 61.3±42.9 | 82.0±7.1 | 8 | 84.4±71.2 | 86.9±3.5 |
| Set S/S | 7 | 85.3±51.9 | 22.4±7.3 | 8 | 27.3±18.6 | 17.9±7.6 |
| Set N/S | 11 | 58.5±34.8 | 75.8±6.2 | 12 | 43.8±35.6 | 66.1±9.0 |
| Set S/N | 9 | 73.0±49.2 | 12.4±9.4 | 11 | 53.2±31.0 | 57.0±12.6 |

n: number of females examined

**Table 3.** Results of the generalized linear model of factors that contribute to variation in $P_2$ values.

| | df | Estimate | SE | $z$ | $p$ |
|---|---|---|---|---|---|
| (Intercept) | | -0.34 | 0.92 | -0.37 | 0.715 |
| Relative spermatophore size | 1 | 2.27 | 1.13 | 2.00 | 0.045 |
| Order of sterilized male mate | 1 | 0.57 | 1.04 | 0.55 | 0.582 |
| Number of eggs laid before the second mating | 1 | 0.00 | 0.01 | -0.09 | 0.929 |

due to the second mating/that of the first mating)) and the mating order of sterilized males (NS or SN). The number of eggs laid between the first and the second mating was also included as an explanatory variable, to identify the effect of the amount of first male's sperm remaining in the spermatheca on $P_2$ values. All statistical evaluations were performed with the R (ver. 2.9.1) statistical package (R Development Core Team, 2009). Unless stated otherwise, all values reported are means ± standard deviation.

## Results

As shown in Table 1, across the sets of tested individuals the body mass of females did not significantly differ. Likewise, neither the body mass of first nor second mating males differed significantly across the sets (first males: 336.0±51.5 mg, n=39; second males: 327.2±52.1 mg, n=39; U=671.0, $p$=0.37). Similarly, the ejaculate mass transferred by the first as well as the second males did not significantly differ across the sets (first males: 12.9±5.5 mg, n=39; second males: 10.7±6.2 mg, n=39; U=623.0, $p$=0.17).

Average viability of eggs laid by females that mated with two N males ($\bar{X}_{\text{Set N/N}}$) was 86.9 %, while that of eggs laid by females that mated with two S males ($\bar{X}_{\text{Set S/S}}$) was 17.9 % (Table 2). By using these values, the $P_2$ value of each female in the sets N/S and S/N, respectively, was estimated.

$P_2$ values ranged from 0% to 100%. No individual showed a $P_2$ value in the range between 30–70% (Fig. 2). Thus, $P_2$ values showed a bimodal distribution, with either the first or the second male taking "sperm priority." First-male priority ($P_2$<30%) occurred in 13 of the 23 twice-mated females, and second-male priority ($P_2$>70%) occurred in the other 10.

The $P_2$ value was higher when the second male transferred a larger spermatophore than the first one (Fig. 3; $z$=2.00, $p$=0.0452). Thus, males who transferred larger spermatophores than their opponents were more likely to take sperm priority. The mating order of the sterilized males did not affect the $P_2$ value ($z$=0.55, n.s.) indicating that sterilization per se did not decrease competitive ability of sperm (Table 3). In addition, the $P_2$ value did not increase with the number of eggs laid before the second mating of females ($z$=-0.09, n.s.).

## Discussion

Watanabe & Sasaki (2010) demonstrated that sperm ejection by female *P. xuthus* occurred when the female received a larger spermatophore from the second male, and they suggested that sperm ejection is the mechanism by which paternity is biased towards preferred males. In the present study, we found that a higher $P_2$ value was achieved when a female received a larger spermatophore from the second male.

Although we used the weight increase in female mass as a proxy for spermatophore size, there seemed to be a strong relationship between sperm ejection and paternity in this species.

$P_2$ values of *P. xuthus* exhibited a bimodal distribution with peaks at 0% and 100%. As suggested by Watanabe *et al.* (2000), once sperm ejection occurs, almost all sperm of the first male in the spermatheca is lost, and the remaining sperm is placed in the background of the spermatheca by the second male's apyrene sperm. Consequently, most eggs laid after the second mating will be fertilized by the second male's sperm. On the other hand, when sperm ejection does not occur, little sperm of the second male can enter the spermatheca, because the spermatheca is fully filled by the sperm of the first male. Watanabe & Hachisuka (2005) pointed out that the spermatheca of the lepidopteran female has a restricted storage capacity. LaMunyon (2000) also reported that the storage capacity of the spermatheca of the tobacco budworm, *Heliothis virescens* (Fabricius, 1777), is approximately the mass of one ejaculate. An alternative to the first male sperm priority hypothesis is that females that received a smaller spermatophore from the second male did not allow the second male's sperm to enter the spermatheca. Sperm migration from the spermatophore to the spermatheca depends largely on the female's musculature (Tschudi-Rein & Benz, 1990), and Curril & LaMunyon (2006) suggested that females of the moth *Utetheisa ornatrix* (Linnaeus, 1758), shunt the sperm of unwanted males to different organs rather than to the spermatheca.

The bimodal distribution of $P_2$ value with peaks of 0% and 100% indicates the low mixing potential of sperm in the spermatheca of female (LaMunyon & Eisner, 1993). Thus, the distribution of $P_2$ value of *P. xuthus* does not seem not to be explained by numerical sperm competition (but see Harvey & Parker, 2000). The small changes in $P_2$ values in relation to the number of eggs laid before the second mating of females also suggests that the amount of the first male's sperm in the spermatheca did not affect the fertilization success of both males.

In the present study, females predominantly used sperm from the male who transferred the larger spermatophore for oviposition. The benefits of producing a large spermatophore have been discussed in many lepidopteran species from the male perspective (Gwynne, 2008). The spermatophore represents a male's mating effort, since a large spermatophore can increase the female refractory period (Sugawara, 1979), resulting in more eggs being inseminated by the male's sperm. The spermatophore also functions as a paternal investment, because

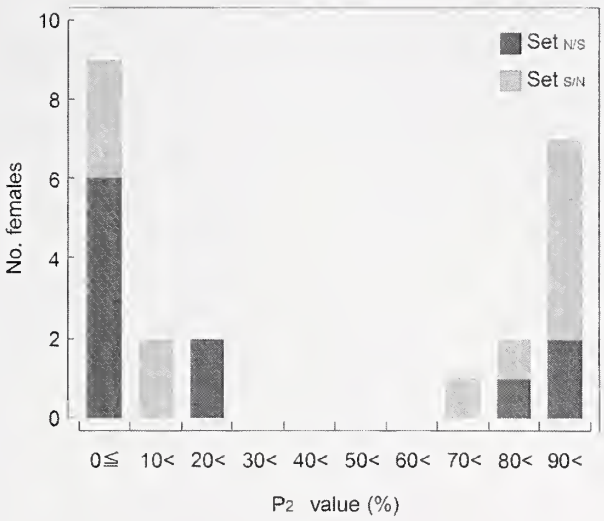


**Figure 2.** Frequency distribution of $P_2$ values in 23 twice-mated *Papilio xuthus* females.

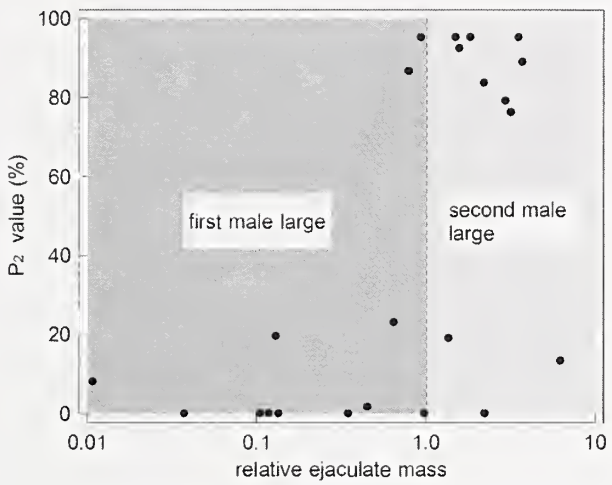


**Figure 3.** Relationship between the relative ejaculate mass (log(weight gain of females due to the second mating/that of the first mating)) and $P_2$ values in 23 twice-mated *Papilio xuthus* females. Statistical results are shown in Table 3.

nutrients contained in the spermatophore are used by a female to increase her longevity (Boggs & Watt, 1981) and reproductive output (Watanabe, 1988). Since the male's ability to produce a large spermatophore is heritable (Wedell, 2006), females gain an indirect benefit by choosing sperm from the male that transferred a larger spermatophore.

The bimodal distribution of $P_2$ values with peaks near 0% and 100% indicate that CFC must have an

impact on male fertilization success in *P. xuthus*. Most eggs will be fertilized by only one male chosen by the female. In addition, ejaculation is costly to the male (Bissoondath & Wiklund, 1997). Males who re-mate within 1–2 days after a previous mating cannot produce as large a spermatophore mass as virgin males (Niihara & Watanabe, 2009). Two to three days of exclusive feeding on nectar are needed for the recovery of the ability to produces a full spermatophore mass (Watanabe & Hirota, 1999). Therefore, males should not re-mate within 1–2 days after a previous mating to avoid selective disadvantages through CFC.

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# The first record of an audible sound produced by a ghost moth, *Phassus* (Hepialidae) from Costa Rica

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**Abstract.** Sound production in lepidopteran adults has been reported in at least 13 families. The majority of these families produce ultrasonic sounds that are inaudible to humans. Here we report the first record of an audible sound produced by a *Phassus* sp. (Hepialidae) from Costa Rica. The sound is clicking or creaking-like, produced as the moth raises its abdomen dorsally (bending the abdomen backwards). The mechanism of this sound production is unknown, but supposed to be a case of stridulation. As the moth raised its abdomen, blue iridescence patches (likely ornamented by structural colors) on the dorsum were observed. The clicking sound is composed of two main parts which varied in frequency and duration. Inferred from the brief observation, the raising of the abdomen and sound production are possibly used for defense against natural enemies.

**Keywords:** Costa Rica, defensive behavior, Lepidoptera, Neotropical, stridulation.

## Introduction

Sound production in adult Lepidoptera is known from at least 13 families, such as Cossidae, Papilionidae, Hesperiidae, Nymphalidae, Pyralidae, Crambidae, Saturniidae, Sphingidae, Uraniidae, Notodontidae, Erebidae, Nolidae, and Noctuidae, all of which are in the infraorder Heteroneura (Lees, 1992 & references therein; Heller & Krahe, 1994; Skals & Surlykke, 1999; Minet & Surlykke, 2003; Smetacek & Smetacek, 2007; van Nieukerken, *et al.* 2011). The sounds are used in courtship, intraspecific and interspecific communication (Surlykke & Gogala, 1986; Spangler, 1988; Alcock *et al.*, 1989; Lees, 1992; Heller & Krahe, 1994; Alcock & Bailey, 1995; Greenfield, 2014 & references therein), and defense against predators (Blest *et al.*, 1963; Møhl & Miller, 1976; Vallin *et al.*, 2005). The characteristics of these sounds and the body parts involved in sound production vary between families, genera, and at species levels, revealing multiple origins of sound production mechanisms (Conner, 1999; Greenfield & Weber, 2000). Human audible sounds, usually between 20 and 5,000 Hz, are documented in some species of the day-flying whistling moths (Noctuidae: Agaristinae) in Australia. Males of *Hecatesia thyridion* Feisthamel produce a percussive sound while in territorial flight (Bailey, 1978; Surlykke & Fullard, 1989) and males of *Platagarista tetrapleura* (Meyrick) produce a whistling sound in flight which is audible from a 20–30 m distance (Common, 1990). Many cracker butterflies *Hamadryas* spp. (Nymphalidae: Biblidinae) produce cracking sounds while in their territorial flights (Monge-Nájera *et al.*, 1998; Yack *et al.*, 2000). During territorial flights at dusk, males of *Letis mycerina* (Cramer) (Noctuidae: Ophiderinae) produce sharp cracking sounds somewhat similar to those of *Hamadryas* spp. (K. Nishida, pers. observation 2003). However, most of the sounds produced in adult Lepidoptera are ultrasonic, generally above human-hearing range between 20,000 and 125,000 Hz (Greenfield, 2014 & references therein). For example, in the family Crambidae, the species *Symmoracma minoralis* (Snellen) (Nymphulinae) produces sound via genital organs (Heller & Krahe, 1994), while in *Ostrinia furnacalis* (Guenée) sounds are produced by rubbing wing scales against the thorax scales (Nakano *et al.*, 2009).

There is a record of an audible sound produced in a defensive context, in the moth *Dudusa sphingiformis* Moore (Notodontidae: Dudusinae). The adult while

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perching raises the abdomen in a jabbing form and produces a rather loud 'crick' or 'creaking' sound that is audible from ca. 50 cm distance (Smetacek & Smetacek, 2007; A. Kubota, pers. comm. 2012).

Sound production in the family Hepialidae (Exoporia: Hepialoidea) was previously unknown (D. Davis, D. Wagner & J. Grehan, pers. comm., 2011). Hepialidae, known as the ghost moths, is one of the most plesiomorphic groups of Lepidoptera. Distributed worldwide, there are approximately 600 described species (Nielsen *et al.*, 2000; van Nieukerken *et al.*, 2011). Mielke & Grehan (2012) recognized 125 species in 30 genera from the Neotropics. Approximately 20 species have been found in Costa Rica (Chacón & Montero, 2007). In the Neotropics hepialids are one of the least studied moth groups, thus there is very little information on their biology and there still remain undescribed species (J. Grehan & C. Mielke, pers. comm., 2011; Mielke & Grehan, 2012). Although many species are nocturnal, and several montane and arctic species are diurnal, the majority appear to be crepuscular in courtship, mating, and laying (dropping) eggs (Wagner & Rosovsky, 1991 & references therein). Hepialid larvae in general are internal or concealed feeders, constructing tunnels in roots, tree trunks, stems, or leaf litter (Grehan, 1989).

One of the major Neotropical genera is *Phassus* Walker, 1856. There are 12 described species, including 6 species in *incertae sedis* position, distributed from Mexico to southern Brazil (Mielke & Grehan, 2012; Grehan, 2013). Three named species have been recorded from Costa Rica, viz. *P. aurigenus* Pfitzner, 1914, *P. costaricensis* Druce, 1887, and *P. huebneri* (Geyer, [1838]) (Nielsen *et al.*, 2000).

Here we describe and illustrate the behavior of sound production in a ghost moth, *Phassus* sp. A spectrogram of the produced sound is provided and a short video clip (13 seconds) of the sound and behavior is available online (see Results section).

## Materials and methods

The observation and recording were conducted in Sector Pittier (09°06'35"N, 82°93'50"W) at 1600 m a.s.l., of La Amistad International Park, Cerro Pittier in Cordillera de Talamanca, Puntarenas Province, Costa Rica, on February 27, 2010. The climate of the habitat (biotic unit) is considered 'tropical very humid with 3 or 4 months of dry season' according to Herrera & Gómez (1993) and the life zone is classified as 'lower montane rain forest' according to Bolaños et al. (1999). This mature montane forest is dominated by large oak trees (*Quercus* spp.) in the canopy, and ferns, palms and small bushes in the understory.

The sound was recorded using a Marantz PMD 661 solid-state digital recorder (accuracy: 16-bit; sampling rate: 44.1 kHz on WAVE format) with a Sennheiser ME66/K6 shotgun microphone. Behavior was videotaped using a Nikon D5000 photographic camera with a Nikkor 55-200mm f/4-5.6G lens. Sound recordings were deposited in Laboratorio de Bioacústica, Escuela de Biología, Universidad de Costa Rica (UCR01419).

We analyzed the sounds using a combination of sonogram, power spectrum, and wave form spectra in Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, USA). This approach allowed us a more accurate measurement of the frequency and duration characteristics (Redondo *et al.*, 2013). We used the following set up to obtain the measurements: a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz (settings: Hann window; 256 kHz sampling, and 50% overlap). In each sound produced we measured: 1) minimum frequency (Hz), 2) highest frequency (Hz), 3) frequency of maximum amplitude (Hz), 4) duration (s), and 5) average entropy (μ: energy). We classified the sound in two parts based on the visual characteristics of the spectrogram. We report measurements as average ± standard deviation.

We were unable to collect, identify, and study the *Phassus* species in detail due to the conditions of our research permit. However from the images we have, the *Phassus* species we observed is quite similar to *P. basieri* Schaus, 1890, *Phassus huebneri* (Geyer, [1838]), or *Phassus triangularis* Edwards, 1885 (Grehan, pers. comm., 2011; Grehan, 2013). Consulting further with J. Grehan and C. Mielke (experts on the family Hepialidae), they suggested that it might also be an undescribed species.

## Results

### Description of the sound production behavior

At approximately 8 a.m., the *Phassus* sp. (n = 1) was found perched on a fern frond using its forelegs, at ca. 50 cm above ground. The middle legs were held loosely in the air and the hind legs were retracted, positioned parallel to its thorax and base of the abdomen (Fig. 1A). When we approached the individual 20 cm or closer, it started to pull itself up vigorously using the forelegs, simultaneously raising its abdomen dorsally, i.e. bending the abdomen backwards (Fig. 1B). The sound production occurred at the beginning, as it raised the abdomen (video link: http://www.kenjinishida.net/videos/phassus.html). The sound was 'zizi' or 'gigi', somewhat like a

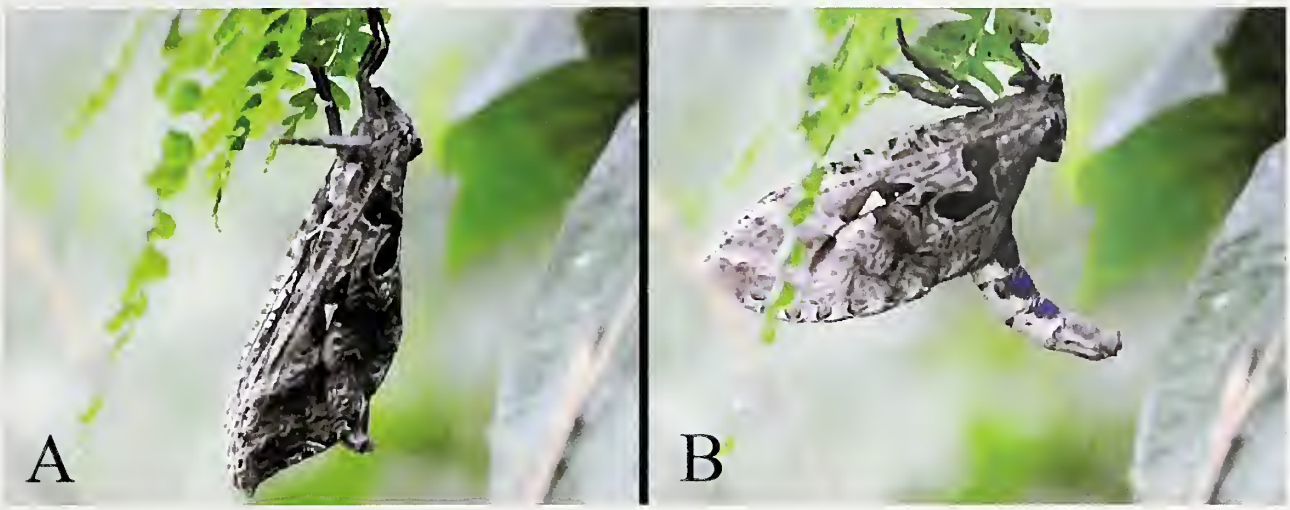


**Figure 1.** **A)** *Phassus* sp. resting on a fern frond, **B)** same individual raising its abdomen as response to the observer's approximation. These images were obtained from the video clip posted online at: http://www.kenjinishida.net/videos/phassus.html.

dull clicking, creaking or 'stridulation'. The raising and lowering of the abdomen lasted about 1 s each and occurred approximately every 2 s. The moth continued to repeat this movement for approximately 2 min. Slight movements of the hind leg tarsi were observed prior to raising of the abdomen.

As the moth raised its abdomen, patches of blue iridescence became visible on the dorsum of each anterior part of abdominal segment. However, depending on the angle, this blue iridescence was not always visible. We were unable to determine the sex of the observed individual.

### Description of the sounds

We measured 23 clicking sounds produced by the *Phassus* moth. Each sound was composed of two parts (Fig. 2). The introductory part was composed of two or three discrete elements, and the duration of each was 0.002 ± 0.0004 s. The main part was a series of close elements (sometimes overlapping each other) and it was difficult to measure each of these. The duration of the main part was 0.08 ± 0.009 s. The minimum frequency was 666.9 ± 113.0 Hz for the introductory part and 393.1 ± 36.9 Hz for the main part. The highest frequency was 9463.1 ± 473.8 Hz for the introductory and 9328.9 ± 823.0 Hz for the main part. The frequency of maximum amplitude was 4021.1 ± 582.9 Hz for the initial and 5103.3 ± 483.7 Hz for the main part. The entropy was 5.86 ± 0.04 μ for the introductory and 5.64 ± 0.04 μ for the main part.

## Discussion

Regarding the behavior of the *Phassus* sp. studied here, a somewhat similar behavior has been observed in *Dudusa sphingiformis* (Notodontidae) which is distributed from the Himalaya towards the Korean peninsula into Tsushima Island, Japan (Jinbo *et al.*, 2013). Smetacek & Smetacek (2007) reported and illustrated the defensive behavior of *D. sphingiformis*. The summary is as follows: when the moth becomes disturbed, it expands its scale tuft on the abdominal apex and starts curling its abdomen like a scorpion's stinger. This movement is continuously repeated and every curling of the abdomen is accompanied with a 'crick' stridulatory sound. This behavior of *D. sphingiformis* was also observed in Bidoup-Nui Ba National Park, Dalat City, Vietnam, and the creaking sound was clearly audible from 50 cm away (A. Kubota pers. comm., 2012).

As we observed in the field, the behavior and sound produced by *Phassus* sp. is somewhat similar to that of *D. sphingiformis*. At this moment, we speculate that the sound is produced via stridulation according to the way the moth moved its abdomen and the type of sound produced. Smetacek & Smetacek (2007) did not explain in detail the mechanism and morphological and/or anatomical structures involved in sound production by *D. sphingiformis*.

Audible stridulatory sounds produced in adult Lepidoptera are fairly uncommon compared to those ultrasonic inaudible stridulatory sounds. Further studies are worth conducting. First of all, to clarify

the mechanism or the source of the sound, it is necessary to conduct careful morphological and anatomical studies of this unidentified *Phassus*, and also of related other *Phassus* species. It would be ideal to manipulate live or recently deceased, soft specimens to locate the specialized organs, e.g. stridulatory files and an amplification mechanism. In other words, examining hard and dried specimens may be less productive in finding and understanding the mechanism. For precise identification of the sound-producing *Phassus* spp., it will be very important to collect and properly preserve specimens because of the similarities in their external characters (Mielke & Grehan, 2012).

Inferring from this first short observation of a *Phassus* sp., the raising of the abdomen with the clicking sound and simultaneous presentation of structural color-like striking blue patches along the dark-colored dorsum probably have a defensive function against some natural enemies such as birds and mammals. It is also necessary to conduct further field observations; collecting and determining the sex may help understand more about the behavior of this *Phassus* sp.

It is worth noting that the blue iridescence on the abdominal dorsum is absent on pinned or dried specimens of *Phassus* spp. (Grehan, pers. comm., 2011). In this case, the blue iridescence could possibly be a type of structural color that is produced internally, i.e. beneath or within the exoskeleton/cuticle with a combination of water/moisture content, like those of many tortoise beetles (Chrysomelidae: Cassidinae) with metallic coloration when alive (Jolivet, 1994; Hull-Sanders, 2003; Vigneron *et al.*, 2007). Thus the blue iridescence is not visible or has gone unnoticed in dry specimens of *Phassus* spp. It is also possible that this iridescence may only occur in this peculiar *Phassus* species. If scales are present on the dorsum of each anterior part of the abdominal segment where the iridescence occurs, it is also worth examining the type of scales to see how the iridescence is produced.

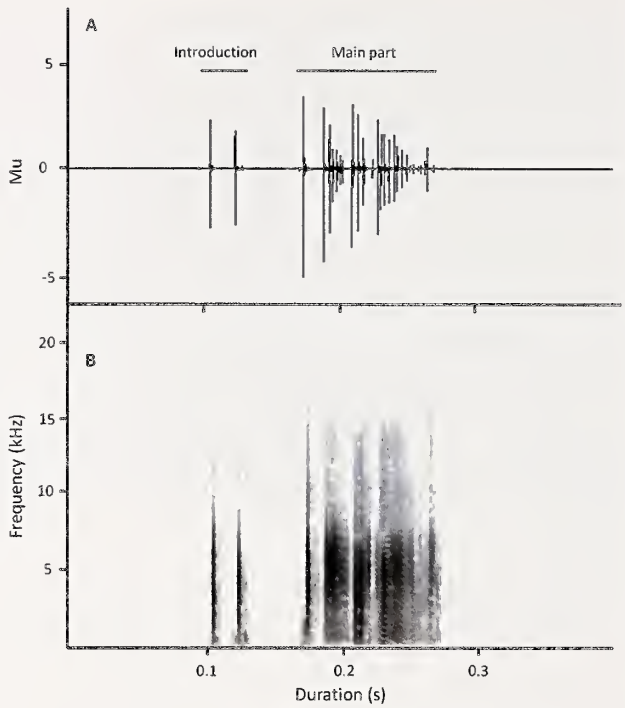


**Figure 2.** Visual representations of the clicking sound, introductory and main part, produced by the *Phassus* sp. A) Oscillogram, B) Sonogram.

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# Two new species of *Euptychia* Hübner 1818 (Lepidoptera: Nymphalidae: Satyrinae) from Mexico and Guatemala

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**Abstract.** Two new species of *Euptychia* are described and illustrated from southern Mexico and Guatemala. *Euptychia neblina* A. Warren & Nakahara **n. sp.**, is described from eleven specimens from cloud forest habitats in the Mexican states of Oaxaca and Chiapas, as well as Baja Verapaz, Guatemala. *Euptychia lacandona* A. Warren & Nakahara **n. sp.**, is currently known from a single female specimen from Chiapas, Mexico. We discuss possible relationships between these two new species and other species of *Euptychia*.

**Key words:** Biogeography, butterfly, cloud forest, endemic, satyr.

**Resumen.** Se describe e ilustra a dos especies nuevas de *Euptychia* del sur de México y Guatemala. *Euptychia neblina* A. Warren & Nakahara **n. sp.** se describe en base a once ejemplares de bosque mesófilo de los estados de Oaxaca y Chiapas en México, junto con Baja Verapaz, Guatemala. *Euptychia lacandona* A. Warren & Nakahara **n. sp.** actualmente se conoce de una hembra de Chiapas, México. Se discuten las posibles relaciones entre estas dos especies nuevas y otras especies de *Euptychia*.

**Palabras clave:** Biogeografía, mariposa, bosque mesófilo, endémico, satírino.

## Introduction

The New World fauna of Satyrinae butterflies remains incompletely documented, and a large number of undescribed species await formal description and classification (Lamas, 2004). This is especially true in the tropics, but several new species of Satyrinae have been described in recent years from the United States and Nearctic regions in Mexico (*e.g.*, L. Miller & J.Miller, 1988; Warren *et al.*, 2008; Cong & Grishin, 2014). While searching for specimens of the recently described *Hermeuptychia intricata* Grishin, 2014 in the collections of the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida (see Warren *et al.*, 2014), specimens representing two undescribed Mesoamerican species of *Euptychia* were encountered from Mexico. Subsequent searches in other institutional collections revealed additional specimens of one of these undescribed species. Herein, we describe and illustrate these two new species of *Euptychia*.

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## Materials and methods

Male and female genitalia were studied using standard techniques, with adult abdomens being soaked in hot 10% KOH for 5-10 minutes, dissected and subsequently stored in glycerol. Female genitalia were stained in diluted chlorazol black before being stored in glycerol. Dissected specimens are indicated below (in Types sections). The terminology for

genital and abdominal structures follows Klots (1956) except for the term 'aedeagus' where we follow Peña & Lamas (2005). Forewing length was measured from the base to the tip of the right forewing using a Vernier caliper. Nomenclature for wing venation follows the Comstock-Needham system as described by Miller (1970: 44), and nomenclature for the areas and elements of the wing pattern follows Peña & Lamas (2005) and Neild (2008). The following collection acronyms are used throughout this paper:

**AMNH** — American Museum of Natural History, New York, USA
**IBUNAM** — Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico
**MGCL** — McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, USA
**USNM** — National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

## Results

***Euptychia neblina* A. Warren & Nakahara, sp. nov.**
(Figs. 1a-d, 2a-g)

"*E. fetna* ?" in d'Abrera, 1988: 761

**ZooBank LSID: urn:lsid:zoobank.org:act:5521C014-0707-48B1-90FB-9A767511740D**

**MALE:** Forewing length 19.1 mm (mean: n = 2)

**Wing venation:** Most of forewing subcostal vein swollen; base of cubital vein barely so; forewing recurrent vein present in discal cell; hindwing humeral vein not developed. **Wing shape:** Forewing subtriangular, costal margin convex, outer margin slightly convex, inner margin straight, but rounded towards anterior thorax near base; hindwing slightly elongate, rounded, outer margin not undulating, inner margin very slightly concave near tornal angle, anal lobe convex, slightly round.

**Dorsal forewing:** Ground colour light brown, distally darker, slightly translucent, thus subtly revealing ventral dark bands and ocelli; trace of ocellus present in dorsal forewing cell $M_1$. **Dorsal hindwing:** Ground color as above; trace of ocellus present in ventral hindwing cell $M_1$; ocellus in cell $Cu_1$, black with one white pupil in center, surrounded by one yellow ring extending across veins $M_3$ and $Cu_2$; dark brown setal patch along cubitus of posterior cell to tornus and to anal margin.

**Ventral forewing:** Ground color paler than dorsal with gray overtones; reddish-brown narrow band extends basally along swollen subcostal vein from radius to wing base; reddish-brown, straight discal band extends from radial vein to vein 2A, across discal cell in a slightly inward diagonal direction; reddish-brown postdiscal band almost parallel to discal band, relatively broad, extends from radial vein (near origin of $R_3$) towards inner margin until reaching vein 2A; forked narrow band along discocellular vein $m_1$-$m_2$ and $m_2$-$m_3$; narrow, dark reddish submarginal band sinuate; marginal band undulating, same color as submarginal band, slightly thinner than submarginal band; ocellus in cell $M_1$, extending across veins $M_1$ and $M_2$, black with one white pupil in center, surrounded by two concentric creamy-yellow rings, yellow rings are outlined in dark reddish brown, outer ring paler, forming small satellite ocellus in cell $M_2$; cell $M_3$ with small ocellus; orange suffusion patch in cell $Cu_1$ extending to cell $M_3$; fringe dark brown at apex shading to fuscous at $M_3$ with dark brown at tornus and along inner (anal) margin, below fuscous mixed with pale gray. **Ventral hindwing:** Ground color base of wing pale whitish gray with a few black scales; reddish dark-brown narrow band about one-third distance from wing base; discal band almost same width as forewing band, nearly straight, posterior one-third very slightly bent inwards; postdiscal band parallel to discal band, concolorous, slightly wider; narrow submarginal band dentate, especially in cells $M_1$, $M_2$ and $M_3$, rather straight towards tornus, same color as forewing submarginal band; marginal band slightly darker than submarginal band, undulating, much thinner than submarginal band; reddish hint at tornus; cells Rs, $M_1$ and $Cu_1$ each with ringed, submarginal ocellus identical to forewing ocellus in cell $M_1$, ocellus in Rs relatively small; cells $M_2$ and $M_3$ each with small ocellus, identical to forewing ocellus in $M_3$; fringe whitish.

**Head:** Eyes sparsely hairy; labial palpi second segment about two times head height, covered with white scales laterally, second and third segment dorsally covered with dark brown scales, ventrally covered with long brown and white hairy scales, third segment one-fourth of second segment in length; antennae approximately 40% of forewing length, color of club uniformly orange.

**Thorax and Abdomen:** Dorsally dark brown with head and thorax similar in coloration; abdomen below pale gray brown.

**Legs:** Foreleg figured in Fig. 2c; meso- and metatibial spurs present.

**Genitalia (Figs. 2a-b):** Tegumen appears subtriangular in lateral view, dorsally flattened, approximately half the length of uncus, with conspicuous posterior projection above uncus; uncus narrow, long, without setae, very slightly hooked, slightly tapered posteriorly, appears subtriangular in dorsal view, cluster of bristle-like structures present on anterior dorsal surface of tegumen; ventral surface of anal tube weakly sclerotized; gnathos absent; combination of ventral arms from tegumen and dorsal arms from saccus straight; appendices angulares absent; saccus approximately two-thirds length of uncus; juxta present; valva sparsely setose, at approximately 30º angle to horizontal; basal half of valva appears somewhat elliptical in lateral view, ventral margin convex, dorsal margin concave, distal half evenly narrow with angular apex, slightly hooked in dorsal view; aedeagus straight, tubular, elongate, approximately 1.5x as long as uncus, posterior third of aedeagus relatively narrow, broadening anteriorly and open anterodorsally, cornuti absent.

**FEMALE:** Forewing length 18.8 mm (mean: n = 3; holotype 18.1 mm)

Similar to male, except as follows: both forewing and hindwing slightly wider and rounder; dorsally paler, dorsal translucence present on both forewing and hindwing, distally darker; dorsal ground color paler; discal band, postdiscal band present on both forewing and hindwing of dorsal surface; submarginal band and marginal band, darker than postdiscal band present on both forewing and hindwing of dorsal surface; orange patch in cells $M_3$ and $Cu_1$ of dorsal forewing. **Legs:** Foretarsus divided into 5 segments, second, third and fourth segments with 2 pairs of spines, outer spine 1.5 times longer than inner spine (Fig. 2d); meso- and metatibial spurs present. **Abdomen:** Weakly sclerotized region between eighth and seventh sternite present in intersegmental membrane. **Genitalia (Figs. 2e-g):** Lamella antevaginalis very well developed, forming a sclerotized region at very base of eighth abdominal segment; ductus bursae membranous; ductus seminalis located close to ostium bursae (posterior end of ductus bursae); corpus bursae roughly oval in dorsal view, without signum, extends to juncture of third and fourth abdominal segment.

**Types.** Holotype female with the following labels: white, printed and handwritten: T. Escalante / Santa Rosa / Comitán /

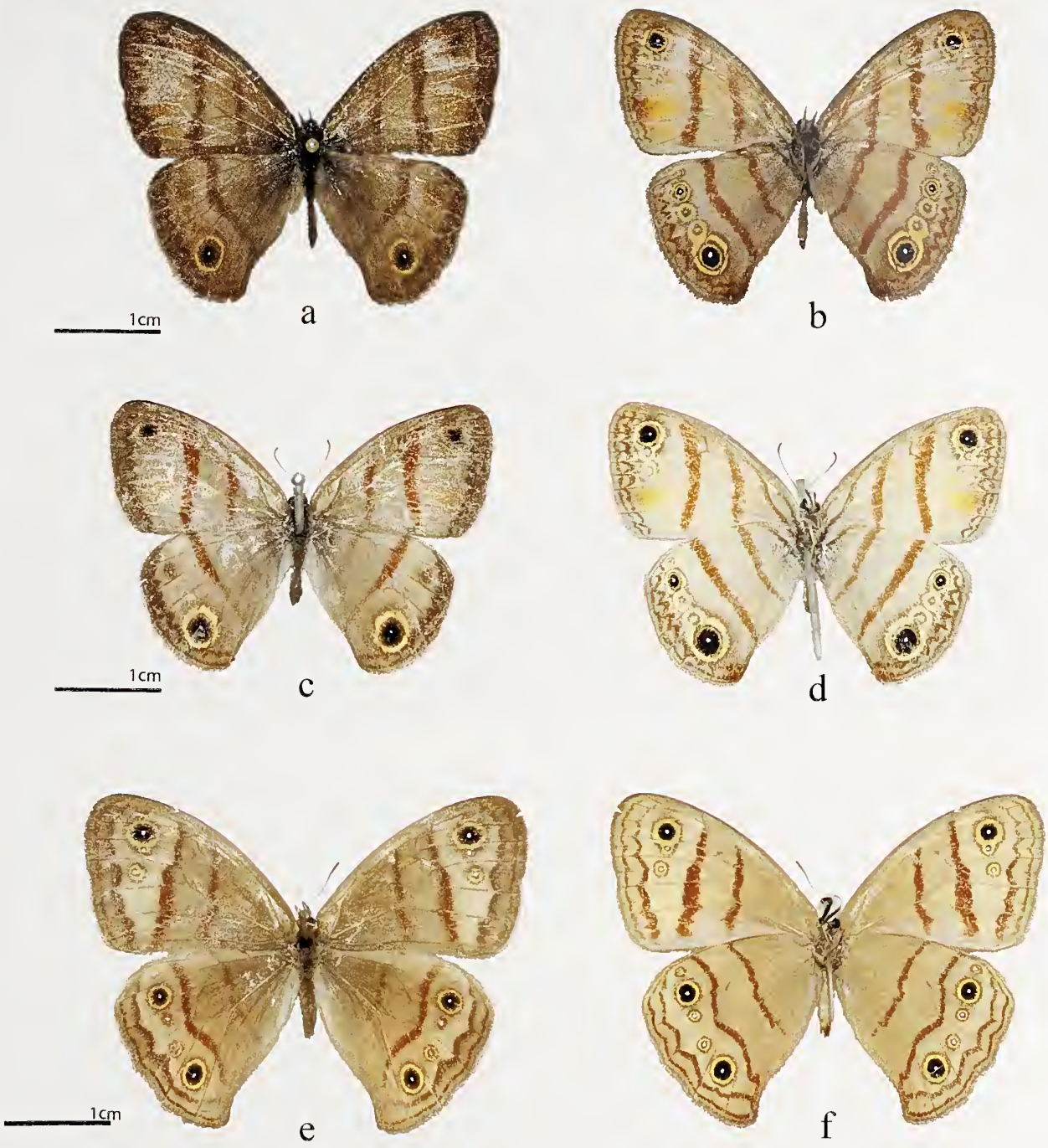


**Figure 1**. Adult specimens of *E. neblina* and *E. lacandona*: **a** dorsal surface of male *E. neblina* (paratype from La Esperanza, Oaxaca, Mexico); **b** ventral surface of *E. neblina* (paratype from La Esperanza, Oaxaca, Mexico); **c** dorsal surface of female *E. neblina* (holotype from Santa Rosa Comitan, Chiapas, Mexico); **d** ventral surface of female *E. neblina* (holotype from Santa Rosa Comitan, Chiapas, Mexico); **e** dorsal surface of female *E. lacandona* (holotype from Bonampak, Chiapas, Mexico); **f** ventral surface of female *E. lacandona* (holotype from Bonampak, Chiapas, Mexico).

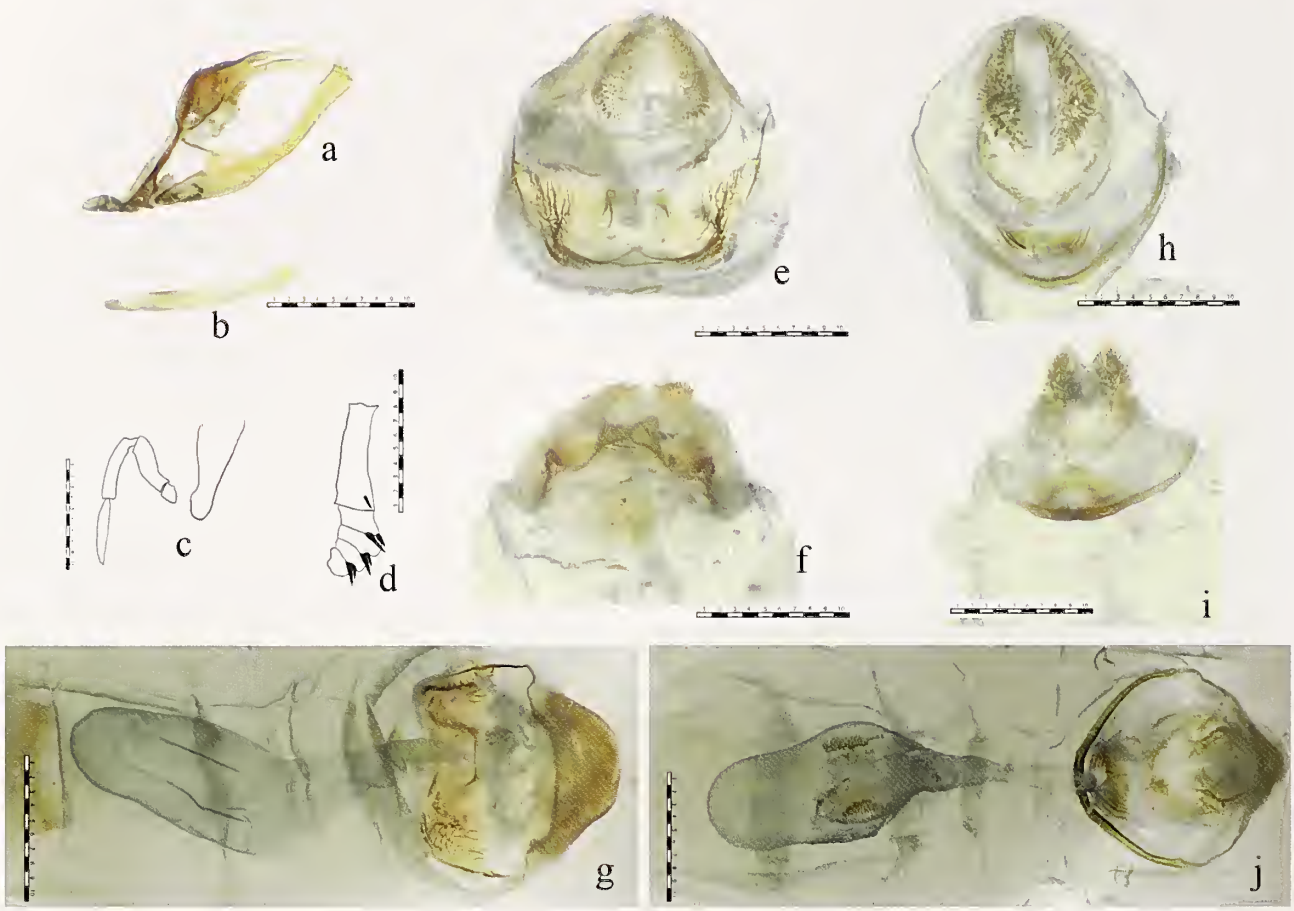


**Figure 2.** Morphological characters of *E. neblina* and *E. lacandona*: **a** male genitalia of *E. neblina* in lateral view (SN-14-155); **b** aedeagus of *E. neblina*; **c** male foreleg of *E. neblina* (SN-14-155); **d** female foretarsus of *E. neblina*; **e** female genitalia of *E. neblina* in front view (SN-15-43); **f** female genitalia (lamella antevaginalis) of *E. neblina* in ventral view (SN-15-43); **g** female genitalia of *E. neblina* in dorsal view (SN-15-43); **h** female genitalia of *E. lacandona* in front view (SN-14-160); **i** female genitalia (lamella antevaginalis) of *E. lacandona* in ventral view (SN-14-160); **j** female genitalia of *E. lacandona* in dorsal view (SN-14-160). Scale bars indicate 1mm.

Chis. [Chiapas] 3-[19]58 /; white, printed and handwritten: Allyn Museum photo / No. 090475-4 /; white, printed: A. C. Allyn / Acc. 1973-48 /; red, printed: HOLOTYPE / *Euptychia neblina* / A. Warren & Nakahara /. The holotype is deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida (MGCL).

Paratypes (3♂, 7♀): 1♀, same data as holotype (genitalic dissection SN-14-156) (MGCL); 1♀, México: Chiapas: Rancho Santa Ana, 27 kms. SE Santa Rosa, 1200m, June 1969, Peter Hubbell (AMNH); 1♂, 1♀, México: Oaxaca: Vista Hermosa, July 1964 A. Díaz Francés (MGCL); 1♂, same locality, 25 March 1978 (IBUNAM); 1♂, México: Oaxaca: La Esperanza, 1750m 21 March 1987, J. de la Maza (genitalic dissection SN-14-155) (MGCL); 4♀♀, Guatemala: Purulhá, July, Schaus and Barnes coll. (genitalic dissection prepared for one specimen: SN-15-43) (USNM).

**Etymology.** Neblina is Spanish for fog or mist, and was chosen for *E. neblina* since all known habitats are in cloud forest habitats, which are frequently dominated by fog and mist.

**Diagnosis.** Diagnostic characters of *E. neblina* which are not shared with other *Euptychia* species are: cluster of bristle-like structures present on anterior dorsal surface of tegumen; absence of signum in corpus bursae. This species resembles *E. hilara* (C. Felder & R. Felder, 1867), but can be distinguished by the following characters: 1) ventral hindwing submarginal band being reddish in *E. neblina*, whereas whitish in *E. hilara*; 2) ventral forewing and hindwing ocelli surrounded by two concentric creamy-yellow rings in *E. neblina*, whereas surrounded by only one ring in *E. hilara*; 3) dorsal margin of tegumen of male genitalia relatively short in *E. neblina*, whereas long in *E. hilara*; 4) projection of the tegumen above uncus present in *E. neblina*, whereas absent in *E. hilara*; 5) lateral sclerotization of the female eighth abdominal segment present in *E. hilara*, whereas absent in *E. neblina*. 6) abdomen of male is relatively longer in *E. hilara* when compared to hindwing inner margin; 7) female foretarsus of *E. neblina* is divided into 5 distinct segments, whereas first and second segment of foretarsus is partially fused in *E. hilara*. In addition, wing pattern of *E. neblina* somewhat resembles that of *E. fetna* Butler, 1870, although adult size of *E. neblina* is relatively larger and specimens of this new species possesses two concentric creamy-yellow rings surrounding ventral forewing and hindwing ocelli.

**Distribution (Fig. 3).** Although rare in collections, *E. neblina* is fairly widely distributed in low- (800-1600m) and

intermediate-elevation (1600-2200m) cloud forest habitats from the southern Sierra Madre Oriental (Sierra de Juárez) of Oaxaca (Vista Hermosa, ca. 1335m; La Esperanza, 1750m, both Mpio. Santiago Comaltepec), through central Chiapas (Santa Rosa, Mpio. Comitán, 1060m; Rancho Santa Ana, 1200m (we were unable to locate this locality, thus it is not shown in Fig. 3), to central Guatemala (Purulhá, Baja Verapaz, ca. 1370m). The habitats in the Sierra de Juárez in Oaxaca include the rainiest sites in montane Mexico (ca. 6000 mm of rain annually). Given this distribution, *E. neblina* should be found at other cloud forest sites between 1060 and 1750m in Oaxaca, Chiapas, and Guatemala, and potentially in Veracruz. Considering the vicariant distributions of other cloud forest species in Mexico, search for *E. neblina* should be conducted in the Sierra de Los Tuxtlas in Veracruz. This region frequently hosts disjunct populations or subspecies of widespread cloud forest taxa, *e.g.*, the pierid *Dismorphia eunoe* (E. Doubleday, 1844), with *D. e. eunoe* in the Sierra Madre Oriental, *D. e. popoluca* Llorente & Luis, 1988 from Los Tuxtlas in Veracruz, and *D. e. chamula* Llorente & Luis, 1988 from Chiapas (Llorente & Luis, 1988). Other examples of disjunctly distributed cloud forest taxa include the nymphalid *Prepona deiphile* (Godart, [1824]) and the riodinids *Mesosemia gaudiolum* H. Bates, 1865 and *M. gemina* J. de la Maza & R. G. de la Maza, 1980, which occur in Chiapas and Los Tuxtlas, Veracruz, respectively. The vicariant biogeographic pattern exhibited by these taxa was discussed by Toledo (1982) and Llorente & Escalante (1992).

## *Euptychia lacandona* A. Warren & Nakahara, sp. nov. (Figs. 1e-f, 2h-j)

**ZooBank LSID: urn:lsid:zoobank.org:act:34FB8BDE-4E67-4D2C-A418-BF84AB562D6C**

**MALE:** Unknown

**FEMALE:** Forewing length 22.3 mm (n = 1)

**Wing venation:** Most of forewing subcostal vein swollen; base of cubitus barely so; forewing recurrent vein present in discal cell; hindwing humeral vein not developed. **Wing shape:** Forewing rounded, subtriangular, costal margin convex, outer margin slightly convex, inner margin slightly concave; hindwing slightly elongate, rounded, outer margin slightly undulating, inner margin slightly concave near tornal angle, anal lobe convex, slightly round.

**Dorsal forewing:** Ground colour light brown and slightly greyish, postmedian paler compared to remainder of wing, translucent thus revealing ventral reddish bands that are darker on the dorsal surface, and ocelli; wing pattern of dorsal surface similar to ventral surface (see below), except for submarginal and marginal band being darker, somewhat broader or thicker than on the ventral surface. **Dorsal hindwing:** ground colour same as forewing; wing pattern of dorsal surface similar to ventral surface (see below), except for submarginal and marginal band being darker.

**Ventral forewing:** Ground colour creamy white, basal two-thirds darker with subtle ochre overtones; both wings with several reddish transverse bands; reddish-brown, discal band extends from radial vein (near origin of $R_1$) to vein 2A, across discal cell, slightly outward diagonal direction below cubital vein; reddish-brown postdiscal band almost parallel to discal band, extends from radial vein (near origin $R_4$ - $R_5$) towards inner margin until reaching vein 2A, almost same width as discal band above vein $M_3$, broadens below this vein; narrow submarginal band sinuate, same colour as discal and post discal band, broadens towards tornus; marginal band very weakly undulating, same color as submarginal band, almost same width as submarginal band above vein $M_2$, thinner than submarginal band below this vein; ocellus in cell $M_1$, extending across veins $M_1$ and $M_2$, black with one white pupil in center, surrounded by creamy-yellow ring, forming small satellite ocellus in cell $M_2$; cell $M_3$ with small ocellus; fringe brownish. **Ventral hindwing:** Reddish discal band slightly narrower than forewing discal band, extends from costal margin to anal margin, fading between veins 2A and 3A, very slightly bent outwards in discal cell; postdiscal band parallel to discal band, concolorous, almost same width as discal band, curved outward between veins $M_2$ and $Cu_2$; submarginal band undulating, less wavy towards tornus, same color as forewing submarginal band, somewhat fused to post discal band both anteriorly and posteriorly; marginal band concolorous with and slightly thinner than submarginal band, weakly undulating; cells $M_1$ and $Cu_1$ each with submarginal ocellus with creamy-yellow ring, identical to forewing ocellus in cell $M_1$; cells Rs, $M_2$ and $M_3$ each with small ocellus identical to forewing ocellus in cell $M_3$; fringe fuscous, darker at tornus.

**Head:** Eyes sparsely hairy; labial palpi second segment about two times head height, covered with white scales laterally, second and third segment dorsally covered with brown scales, ventrally covered with long brown hairy scales; antennae approximately 55% of forewing length, dorsally darker than ventral side, divided into 35 segments, whitish scales scattered on each segment, anterior 3 segments of club significantly darker.

**Thorax and Abdomen:** Thorax dorsally dark brown; abdomen dark brown on first abdominal segment and second abdominal segment shading to pale brown with darker end segments; ventral abdomen creamy white with ochre first to fourth abdominal segments shading to creamy white with reddish brown on eighth abdominal segment.

**Legs:** Meso- and metatibial spurs present.

**Genitalia (Figs. 2h-j):** Lamella antevaginalis sclerotized; sclerotized 'ring' between seventh and eighth abdominal segment, apparently independent from lamella antevaginalis; ductus bursae membranous; origin of ductus seminalis close to ostium bursae (posterior end of ductus bursae); corpus bursae somewhat like 'pear–shaped' in dorsal view, extends to third abdominal segment, with two short signa, spines of signa developed.

**Types.** Holotype female with the following labels: white, printed and handwritten: T. Escalante / Bonampak / Chis [Chiapas] / VII-[19]64 /; printed and handwritten: Allyn Museum photo / No. 090475-9 /; white, printed: A. C. Allyn / Acc. 1973-48 /; white, printed: Genitalia vial / SN-14-160 / S. Nakahara /; red, printed: HOLOTYPE / *Euptychia lacandona* / A. Warren & Nakahara /. The holotype is deposited in the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida (MGCL).

**Etymology.** This species is named for the Lacandon Forest (Selva Lacandona), situated primarily in Chiapas, Mexico, and adjacent parts of Guatemala.

**Diagnosis.** The primary diagnostic character of *E. lacandona* is the developed spines of signa, which are apparently more prominent than in any other *Euptychia* species. Externally it can be distinguished from other *Euptychia* species by its relatively large adult size and narrow reddish bands on ventral forewing and hindwing. However, the latter character is, to some extent, similar to those of *E. fetna* and *E. rubrofasciata* L. Miller & J. Miller, 1988. *Euptychia lacandona* can be distinguished from *E. fetna* by its absence of orange patch in ventral forewing cells $Cu_1$ and $Cu_2$. *Euptychia lacandona* can be distinguished from *E. rubrofasciata* by its zigzagging ventral hindwing submarginal band.

**Distribution (Fig.3).** To date, *E. lacandona* is known only from the type locality, Bonampak, Mpio. Ocosingo, Chiapas, Mexico, at an elevation of about 462m. This site is comprised of lowland tropical rain forest. However, this species is most likely distributed in other extremely humid regions of the Lacandon Forest in Chiapas and Guatemala.

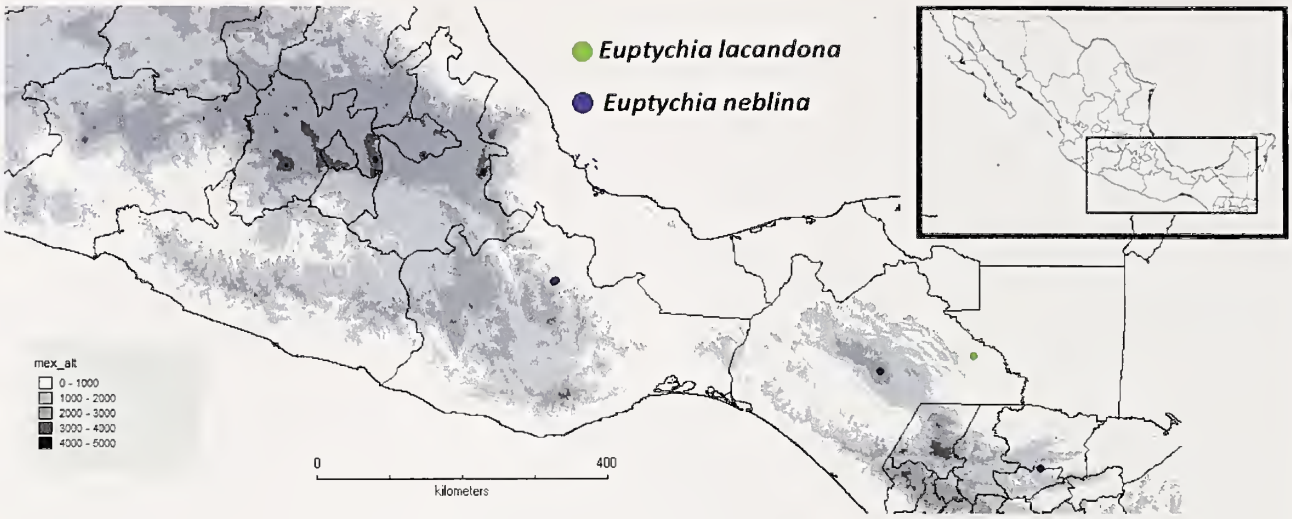


**Figure 3.** Map showing collecting localities for *E. neblina* (blue dots) and *E. lacandona* (green dot).

## Discussion

*Euptychia neblina* is described in the genus *Euptychia* because of the following characters: 1) presence of projection of the tegumen above the uncus in the male genitalia; 2) presence of the forewing recurrent vein in the discal cell; 3) absence of the basal swelling of forewing cubital vein; 4) presence of the sclerotized region of the eighth abdominal segment, located at the very basal side of the eighth abdominal segment; 5) absence of the lateral sclerotization of the eighth abdominal segment of female; 6) origin of ductus seminalis at the posterior end of ductus bursae; 7) reduced humeral vein. All of these characters (1) – (7) are shared by *Euptychia* species and are absent or rarely seen in other euptychiine butterflies. Although state (1) is considered to be a diagnostic character for the genus (Freitas *et al.*, 2012, 2013; Neild *et al.*, 2014; Nakahara *et al.*, 2014), it appears that this structure is absent in some *Euptychia* species (S. Nakahara, unpubl. data). The male of *E. lacandona* remains unknown, thus we were unable to verify state (1), however, the presence of characters (2), (3), (4), (5), (6) and (7) seems sufficient to place this taxon in *Euptychia*.

As mentioned in the diagnosis, the wing pattern of *E. neblina* is similar to that of *E. hilara*, although evidence from morphology, host plant records and molecular data suggests that *E. hilara* should be excluded from the genus *Euptychia*, and a new genus will be described for *E. hilara* (Nakahara *et al.*, in press). Thus, *E. hilara* is probably not a close relative of *E. neblina* and the wing pattern similarities are likely a result of convergent evolution. Based only on wing pattern, *E. neblina* is also similar to *E. fetna*, which is a widely distributed species in Central America. However, the male and female genitalia of *E. neblina* and *E. fetna* are dissimilar in many ways (*e.g.*, projection of the tegumen being very short in *E. fetna*) implying that they are not closely related to each other. In fact, the male genitalia of *E. neblina* is apparently most similar to *E. meta* Weymer, 1911, in terms of its valva shape, relatively long and narrow uncus and the presence of the weakly sclerotized anal tube. Interestingly, these two species both possesses two concentric creamy-yellow rings surrounding ventral forewing and hindwing ocelli. Despite its morphological similarity to some *Euptychia* species, *E. neblina* possesses three interesting characters which are not or rarely seen in other members of the genus: cluster of bristle-like structures present on anterior dorsal surface of tegumen, absence of signa in the corpus bursae, and presence of the meso- and metatibial spurs. The bristle-like character on tegumen is apparently homologous to that reported in *Forsterinaria emo* Zubek, Pyrcz & Boyer, 2013 (Zubek *et al.*, 2013) and needs further investigation when materials become available. The absence of the tibial spur was first reported in *E. mollina* Hübner, 1818, the type species of the genus, in Miller (1968). This spur is apparently absent in many *Euptychia* species except for the two new species described herein, as well as *E. rubrofasciata* and *E. hilara*.

*Euptychia lacandona* is superficially similar and perhaps closely related to *E. rubrofasciata*, judging from its wing pattern and the female genitalia

description in the original description (L. Miller & J. Miller, 1988). We were unable to examine the female genitalia of *E. rubrofasciata* in good condition, but based on the original description of this taxon, the shape of the lamella antevaginalis and its separation from the sclerotized ring correlates well with those of *E. lacandona*. However, due to the fact that *E. rubrofasciata* is a unique member of the genus *Euptychia* based on morphology and biogeography, further study on *E. lacandona* including discovery of the male is necessary in order to reveal its systematic placement. Given that *Euptychia* species are not usually abundant, special collecting efforts should be made to detect the presence of both newly described species at additional sites.

## Acknowledgements

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## Editor's note

The electronic edition of this article has heen registered in ZooBank to comply with the requirements of the amended International Code of Zoological Nomenclature (ICZN). This registration makes this work available from its electronic edition. ZooBank is the official registry of Zoological Nomenclature according to the ICZN and works with Life Science Identifiers (LSIDs). The LSID for this article is: urn:lsid:zoobank.org:pub:6A9B2612-3F32-49B1-B2F6-CECC6F91BE24. Registration date: July 26th, 2015. This record can be viewed using any standard web browser by clicking on the LSID above.

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# A remarkable new *Euptychia* Hübner, 1818 (Lepidoptera: Nymphalidae: Satyrinae) from the Amazon basin of Peru and Colombia

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**Abstract.** A new species of *Euptychia* Hübner, 1818, from the Amazon basin of Peru and Colombia, *E. juanjoi* **sp. nov.**, is described. This new species has a unique wing pattern compared to other members of the genus, but is placed in *Euptychia* due to the presence of a projection of the tegumen over the uncus in the male genitalia.

**Resumen.** Se describe una nueva especie de *Euptychia* Hübner, 1818, de la cuenca del Amazonas de Perú y Colombia, *E. juanjoi* **sp. nov.** Esta nueva especie muestra un patrón alar único comparado con los otros miembros del género, pero se incluye en el género *Euptychia* por la proyección del tegumen sobre el uncus en la genitalia del macho.

**Key words:** Euptychiina, new species, taxonomy, white-sand forest.

## Introduction

*Euptychia* Hübner, 1818 is a speciose genus within the poorly known nymphalid subtribe Euptychiina and represents the oldest generic name for the subtribe (Lamas & Nakahara, 2015). After its description, *Euptychia* was treated by some authors (e.g. Butler, 1867; Weymer, 1910-1911) in a broad sense to include many other euptychiine species which are now no longer placed in *Euptychia* (Lamas, 2004). The catalogue by Lamas (2004), which has been considered as the basis of current Neotropical butterfly classification, listed 13 valid and 16 undescribed species in *Euptychia*. Some of these latter species have been described in the meantime such that currently this genus includes 18 valid species (Brévignon, 2005; Freitas *et al.*, 2012; 2013; Neild *et al.*, 2014; Nakahara *et al.*, 2014), although it appears that there are more taxa awaiting description than it has been previously thought.

This article is part of a series in which the first author, together with various colleagues, intends to describe *Euptychia* species from the Amazon basin and the Guianas. General information about the genus, including distribution and diagnostic characters, can be found in the first paper of the series (Neild *et al.*, 2014). During the preparation of this particular paper, the first author (SN) noticed a unique euptychiine species illustrated in Pinzón (2009), which seemed to be a member of *Euptychia* based on its wing pattern. The first author subsequently dissected two male specimens of this taxon and confirmed that it was an undescribed species, as had been previously recognized by Lamas *et al.* (2003) and Lamas (2004). This species is described herein and placed in the genus *Euptychia*.

## Material and methods

We examined the morphology of *Euptychia* specimens from the following three collections:

**JFLC** — Jean François Le Crom collection, Bogotá, Colombia
**MGCL**—McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Florida, USA

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**Figure 1.** *Euptychia juanjoi* **sp. nov**. Top row (dorsal view on left, ventral view on right): male holotype with labels; bottom row (dorsal view on left, ventral view right): female allotype with label. Scale bar = 10 mm.

**MUSM** — Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru

The abdomens of two Peruvian males in MUSM were dissected to observe genitalic structures. Female genitalia could not be examined because the abdomen of the only female specimen was damaged. Abdomens were prepared using standard techniques, being soaked in hot 10% KOH for 5-10 minutes before dissection and stored in glycerol after dissection. External morphology and dissections were studied using a stereomicroscope and photographed using digital cameras. Terminology for genital and abdominal structures follows Klots (1956), except for the term 'aedeagus', where we follow Peña & Lamas (2005). Nomenclature for wing venation follows the Comstock-Needham system described by Miller (1970), and areas and elements of the wing pattern follow Peña & Lamas (2005) and Neild (2008).

## Taxonomy

***Euptychia juanjoi* Le Crom, Nakahara & Lamas, sp. nov.**
(Figs. 1, 2)

*Euptychia* sp. n. 4: Lamas *et al.*, 2003: 12.
*Euptychia* [n. sp.] Lamas, MS: Lamas, 2004: 219 [# 1319].
*Euptychiina* sp.: Pinzón, 2009: 4.

**ZooBank LSID: urn:lsid:zoobank.org:act:6F950E4B-7D24-4F57-B31D-E91EA81708FC**

**Wing venation:** Most of forewing subcostal vein swollen; base of cubitus barely so; forewing recurrent vein present in discal cell; hindwing humeral vein not discernible. **Wing shape:** Forewing subtriangular, costal margin convex, outer margin almost straight, inner margin straight, but rounded towards thorax near base; hindwing slightly elongate, rounded, outer margin slightly rounded, inner margin slightly concave near tornus, anal lobe convex, slightly round.

**Dorsal surface:** Ground colour light lilac-brown, distally darker, translucent, thus subtly revealing ventral dark bands and

ocelli (Fig. 1). **Ventral surface:** ground colour pale greyish-brown, slightly darker on hind wing; both wings with several dark-brown transverse bands with subtle orange overtones.

**Ventral forewing:** Dark-brown band extends basally along swollen subcostal vein from radius to wing base; dark-brown, straight discal band extends from radial vein, crossing discal cell in a slightly inward diagonal direction, running in a slightly outward diagonal direction below cubital vein, and fading away before touching vein 2A; dark-brown postdiscal band almost parallel to discal band, relatively broad except between radial vein and recurrent vein in discal cell, extending from radial vein towards inner margin until reaching vein 2A; narrow disjunct band along discocellular veins $m_1$-$m_2$ and $m_2$-$m_3$; postmedial band dark-brown, somewhat broad, faint, extending along postmedian; thin, dark grey submarginal band sinuate; marginal band undulating, same color as, but slightly thinner than, submarginal band; fringe brownish; ocellus in cell $M_1$, extending across veins $M_1$ and $M_2$, black with one white pupil in center, surrounded by two concentric creamy-yellow rings, outer ring forming small satellite ring in cell $M_2$; cell $M_3$ with whitish reniform marking containing smaller, concolorous reniform marking with dark-grey border; orange patch in cell $Cu_1$.

**Ventral hindwing:** Regular dark-brown band near the wing base; discal band equally wide as forewing band, slightly paler, nearly straight; postdiscal band parallel to discal band, concolorous, slightly wider, with orange markings of varying length along posterior third; broad, faint, indistinct dark band covering most of median and postmedian; submarginal band undulate, gradually broadening towards tornus, posterior one-third orange, occasionally fused to postdiscal band in cell 2A; marginal band black, undulate, much thinner than submarginal band; fringe brownish; cells $M_1$ and $Cu_1$ each with a ringed, submarginal ocellus identical in appearance to forewing ocellus; ocellus in cell $M_1$ extends across vein $M_2$; cell $M_3$ with creamy-yellow reniform marking; oblong, creamy-yellow ring spanning most of submargin, enclosing both ocelli and reniform marking.

**Genitalia** (Fig. 2): Tegumen appears subtriangular in lateral view, dorsally flattened, approximately half the length of uncus, with conspicuous posterior projection above uncus, visible in lateral view; uncus narrow, long, without setae, slightly hooked, tapered posteriorly, appears subtriangular in dorsal view; gnathos absent; combination of ventral arms from tegumen and dorsal arms from saccus straight; appendices angulares absent; saccus approximately two-thirds length of uncus, juxta present; valvae sparsely setose, at approximately 30º angle to horizontal; basal half of valva appears subrectangular in lateral view, ventral margin convex, dorsal margin concave, distal half narrow with rounded apex, interior portion of apex projects inwards in a hook-like shape; aedeagus straight, elongate, approximately as long as valve, tubular, posterior third relatively narrow, broadening anteriorly and open anterodorsally, cornuti absent; ventral surface of anal tube weakly sclerotized.

Female is similar to male, except as follows: Both forewing and hind wings slightly wider and rounder, ground color paler, ventral bands reddish.

**Holotype:** ♂ (FW length: 15.5 mm) Peru: Loreto, Z[ona] R[eservada] Allpahuayo-Mishana, 170m, 03º58'S, 73º25'W, 1.XII.2001, J.J. Ramírez, Varillal seco II, 0357/7325 (genitalic dissection vial prepared: SN-14-3), in MUSM.

**Allotype:** ♀ (FW length: 12 mm) Colombia: Amazonas, Corregimiento La Pedrera, Caño Curare, Comunidad de Curare, Resguardo Indígena Curare-Los Ingleses (1°18'50"S, 69°45'18"W), J. Pinzón leg., 23.-25.IX.2004, in JFLC.

**Paratypes:** FW length: 13.5-15.5mm (n=4). 1♂: Peru: Loreto, Río Aguas Negras, 150m, 0º31.38'S, 75º15.41'W, 3.III.1994, R. K. Robbins (MUSM); 1♂: Peru: Loreto Z[ona] R[eservada] Allpahuayo-Mishana, 170m, 0358/7325, 30.XI.2001, L. Campos (MUSM); 1♂: Peru: Loreto, Z[ona] R[eservada] Allpahuayo-Mishana, 170m, 03º58'S, 73º25'W, 30.XI.2001, J.J. Ramírez, Varillal seco II, 0358/7326 (genitalic dissection vial prepared: SN-14-20), (DNA sample SN-MUSM-06) (MUSM); 1♂ same data as holotype (MUSM).

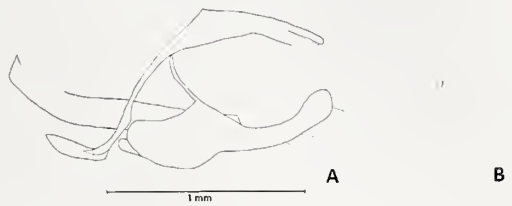


**Figure 2.** Male genitalia of paratype of *E. juanjoi* **sp. nov. A**: lateral view (dotted line of the uncus is drawn based on a second specimen); **B**: anterior end of valvae in dorsal view.

**Etymology.** We dedicate this specific epithet to Juan José Ramírez, who is the third author's good friend and who collected three specimens of this taxon, including the holotype. Juan José Ramírez is from Iquitos, Peru, where the holotype is from, and his nickname is 'Juanjo'.

**Diagnosis.** *Euptychia juanjoi* can be distinguished externally from other *Euptychia* species by the presence of the whitish reniform marking in cell $M_3$ of ventral forewing. In addition, the combination of the following characters can also be used: 1) orange patch in cell $Cu_1$ of ventral forewing; 2) creamy yellow ring surrounding ocellus in cell $M_1$ of ventral forewing, also forming a satellite small ring in cell $M_2$; 3) creamy-yellow ring surrounding ocellus in cell $M_1$ of ventral hindwing, forming a satellite small ring in cell $M_2$ and in cell Rs; 4) creamy yellow ring surrounding ocellus in cell $Cu_1$ of ventral hindwing, forming a satellite reniform ring in cell $M_3$ with an inner creamy yellow reniform marking; 5) orange patch on tornus of ventral hindwing.

**Variation.** The size of the ventral hindwing tornal orange patch varies; two specimens have this orange patch fused to the orange patches covering the postdiscal band, whereas the orange patches remain separate in the remaining four specimens in the type series. Also, the degree to which the tornal orange patch covers the submarginal band varies.

**Distribution** (Fig. 3). Currently known from three localities in the Amazon basin: Allpahuayo-Mishana Reserve and Río Aguas Negras, both in Loreto, Peru, and Amazonas Department, Colombia. Four specimens were collected in the Allpahuayo-Mishana Reserve, one in the Río Aguas Negras, and two in Colombia. However, one of the Colombian specimens was not analysed and is thus excluded from the type series because the collector indicated it was in very poor condition (J. Pinzón, pers. comm.) Because only three sites are known to us, we are unable to accurately estimate the full geographical range of *E. juanjoi*.

**Habitat.** J.J. Ramírez (pers. comm.), who collected the holotype in the Allpahuayo-Mishana Reserve (Peru), reports that, although this white-sand forest is usually an open space with sunlight and sparse leaf litter, there are some areas with tall trees that consequently receive very little sunlight. *Euptychia juanjoi* flies in these areas, in the lower understory, and may sometimes be confused with species of *Mesosemia* (Riodinidae) when on the wing. The Río Aguas Negras locality includes a mosaic of inundation forest and white-sand forest similar to that found in Allpahuayo-Mishana. Regarding the Colombian specimens'

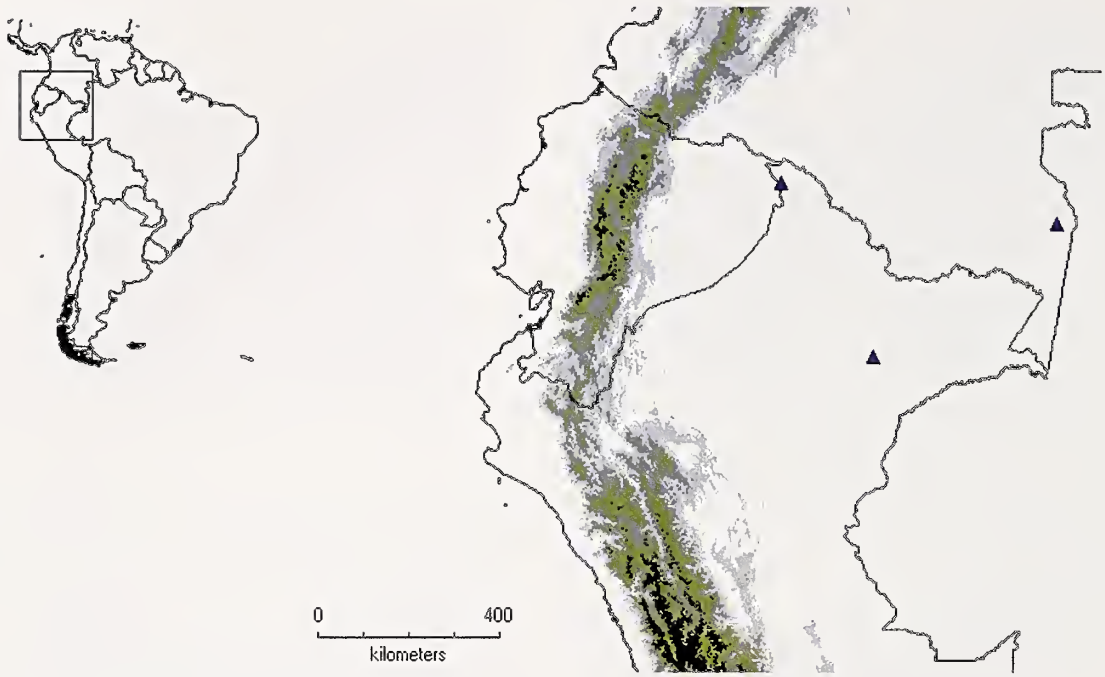


**Figure 3.** Map showing collecting localities (blue triangles) for *E. juanjoi* **sp. nov.**

locality, J. Pinzón (pers. comm.) notes that the forest is typical of black-water inundation forests of the Amazon basin, with a relatively tall closed canopy (~15m) and thick litter layer on the ground. One of the specimens was collected on the northern edge of the river while the other was collected on the southern edge; the northern edge was quite swampy with very soft ground at some places and very thick understory, whereas the southern edge was on higher ground with much sparser understory, but still had a closed overstory (~20 m tall).

## Discussion

This species should be placed in *Euptychia* based on the presence of a conspicuous projection of the tegumen above the uncus, of a recurrent vein in the discal cell of the forewing, and by the absence of basal swelling of forewing cubital vein. The first character is considered diagnostic by several authors (Freitas *et al.*, 2012; Neild *et al.*, 2014; Nakahara *et al.*, 2014), and the latter two are shared by all members of the genus although they are present in some other euptychiine species too (S. Nakahara, pers. observ.). We were not able to examine the female genitalia of *E. juanjoi*, although it would be very interesting to see whether this species has a sclerotized 'ring' located at the base of the 8th abdominal segment, developing from the lamella antevaginalis. This character has been seen in the female genitalia of all *Euptychia* species that exhibit a tegumen projection in the male genitalia. In addition, female genitalia of *Euptychia* species appear to lack the prominent sclerotized region of the 8th abdominal segment (located on both sides) which is seen in many other females of euptychiine species. Assessing the status of this character in a female *E. juanjoi* would support its placement in *Euptychia*.

It is very difficult to determine the systematic placement of *E. juanjoi* within *Euptychia* because this species does not closely resemble any other in the genus; it has numerous characters that are hardly seen in other congeners. Despite its unique appearance, the male genitalia of *E. juanjoi* somewhat resemble that of *E. meta* Weymer, 1911, which is a more widespread species known from the eastern Andes and also from the western Andes and Central America. Both species have similarly shaped valvae, with a rectangular basal half and slightly curved, narrow distal half.

Concerning the Colombian locality of *E. juanjoi* the collector notes that "This is a 'small' black-water river that flows from the south into the Caquetá (Japurá) river about 20km upriver west from the town of La Pedrera and perhaps a couple of kilometers from the native community of Curare. The area where I collected these specimens was a few kilometers up the Caño Curare within a km from the river margins during the dry season, most of which is flooded during the rainy season, particularly close to the Caqueta" (J. Pinzón, pers. comm.). The Allpahuayo-Mishana reserve, where four Peruvian specimens are from, is

classified as a white-sand forest growing on white-sand soil, locally known as 'Varillal', which is recognized as a distinctive forest type (Lamas *et al.*, 2003; Fine *et al.*, 2010). Floristic study of this white-sand forest revealed that its plant diversity is significantly lower compared to non white-sand forests, although 61% of white-sand forest plants are considered endemic or facultative specialists (Fine *et al.*, 2010). Similar results have been reported by comparing the white-sand forests to *terra firme* forests in the upper Rio Negro, Brazil (Stropp *et al.*, 2011). Although there have been several new discoveries of avifauna in this white-sand forest (e.g. Alvarez & Whitney, 2001), its butterfly fauna is still incompletely known. Similar white-sand forests are also present in Colombia, Venezuela, Brazil, and the Guianas (Fine *et al.*, 2010). It will therefore be interesting to see whether *E. juanjoi* also occurs in these other white-sand forests and to further investigate the butterfly fauna of this unique forest type.

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## Editor's note

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# Description of a new species of *Myelobia* Herrich-Schäffer (Lepidoptera, Pyralidae s.l., Crambinae) from Nicaragua feeding on cultivated bamboo, *Guadua aculeata* Rupr. ex E. Fourn. (Poaceae)

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**Abstract.** *Myelobia nicaraguensis* Landry & Maes, **sp. n.** was discovered in eastern Nicaragua. The caterpillar feeds on *Guadua aculeata* Rupr. ex E. Fourn. (Poaceae). Brief biological notes and detailed descriptions of the adult, larva, and pupa are provided as well as part of the CO1 mtDNA barcode. This represents the first pupal description and the first detailed and illustrated description of the larva for the genus *Myelobia*. The immatures of *M. nicaraguensis* and *M. smerintha* are briefly compared and the systematic position of *Myelobia* within the Crambinae is also discussed.

**Resumen.** *Myelobia nicaraguensis* Landry & Maes, **especie nueva**, fue descubierta en el este de Nicaragua, en la región de El Rama. La larva se alimenta de *Guadua aculeata* Rupr. ex E. Fourn. (Poaceae). Se incluyen algunas notas sobre la biología de la especie, además de la descripción de larva, pupa, adulto y el código de barras de ADN CO1. La descripción de la pupa y la descripción detallada e ilustrada de la larva son las primeras del género *Myelobia*. También se comparan los estadios inmaduros de *M. nicaraguensis* y *M. smerintha* y se discute la posición sistemática de *Myelobia* adentro de la subfamilia Crambinae.

**Key words:** Lepidoptera, Pyraloidea, Pyralidae, Crambinae, *Myelobia*, new species, *Myelobia smerintha* (Hübner), Nicaragua, bamboo, egg, larva, pupa

## Introduction

Bamboo has recently been used in Nicaragua to recover exhausted or totally deforested cattle ranches. At the same time, there is an economic interest in producing wood from bamboo to cover soil and rapidly create a newly forested landscape. Besides protection of the soil against erosion, bamboo provides shelter for some wild animals because this crop is grown organically.

One of the most important concerns in any organic production system is the control of pests. At present, three main species of Lepidoptera were reared from larvae attacking the bamboo *Guadua aculeata* Rupr. ex E. Fourn. (Poaceae), a native species, in the El Rama area of Nicaragua. A leaf-rolling pyralid was identified as *Salbia* (Pyralidae: Spilomelinae), probably an undescribed species (A. Solis, pers. comm.). Two borers were also reared, a stem borer in the family Pyralidae attacking mostly woody stems and branches, and a shoot borer in the family Erebidae damaging the young emerging culms. The erebid species was identified as a probable new species in the genus *Scolecocampa* Guenée (Erebidae: Scolecocampinae; M. Pogue, pers. comm.). At least one species in this genus is known to be a pest on sugarcane in Mexico (Pogue 2002), a plant related to bamboo. This suggests the new species from Nicaragua with similar biology might become a potential pest of bamboo in the future. The third species, a stem borer, was the most common of the Nicaraguan bamboo feeders. It was identified as a new member of the Neotropical genus *Myelobia* Herrich-Schäffer (Pyralidae: Crambinae) by BL based on a comparison with the types or

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original descriptions of the 21 known species of *Myelobia*. Species of the genus are known to feed on sugarcane and other related grasses such as *Chusquea*, *Gynerium* and *Merostachys* (Zhang, 1994). Because *Myelobia smerintha* (Hübner, 1821) feeds on bamboo and occurs from Mexico (Dyar, 1917) to South America (S. Passoa, unpubl. records), and very likely in Nicaragua, it potentially could be confused with *M. nicaraguensis*. Therefore, we provide comparative notes on this species as well. Detailed morphological information on other *Myelobia* species is not available. We have not encountered larvae of *Splendeuptychia kendalli* Miller, 1978 (Nymphalidae: Satyrinae), the only butterfly recorded from *G. aculeata* in Latin America by Beccaloni *et al.* (2008). Caterpillars in the families Erebidae, Noctuidae, Hesperiidae, Nymphalidae, and Pyralidae (not identified further) eat *Guadua paniculata* Munro in Costa Rica (Janzen & Hallwachs, 2009).

## Material and methods

Adults of *M. nicaraguensis* were reared from branches of *Guadua aculeata* bamboo, most of which were between 1 and 2 cm in diameter. All branches were cut in sections of 15 to 25 cm length containing at least one complete stem internode. We used segments already occupied by a larva, but also allowed caterpillars to enter undamaged fresh stems when needed. All the branches were set in a big plastic box with cloth on the bottom to permit 100 % humidity while avoiding the accumulation of standing water in the bottom of the box. This container was checked each morning and afternoon for adult emergence. Once we were familiar with the adult coloration, we collected additional moths with a mercury vapour lamp.

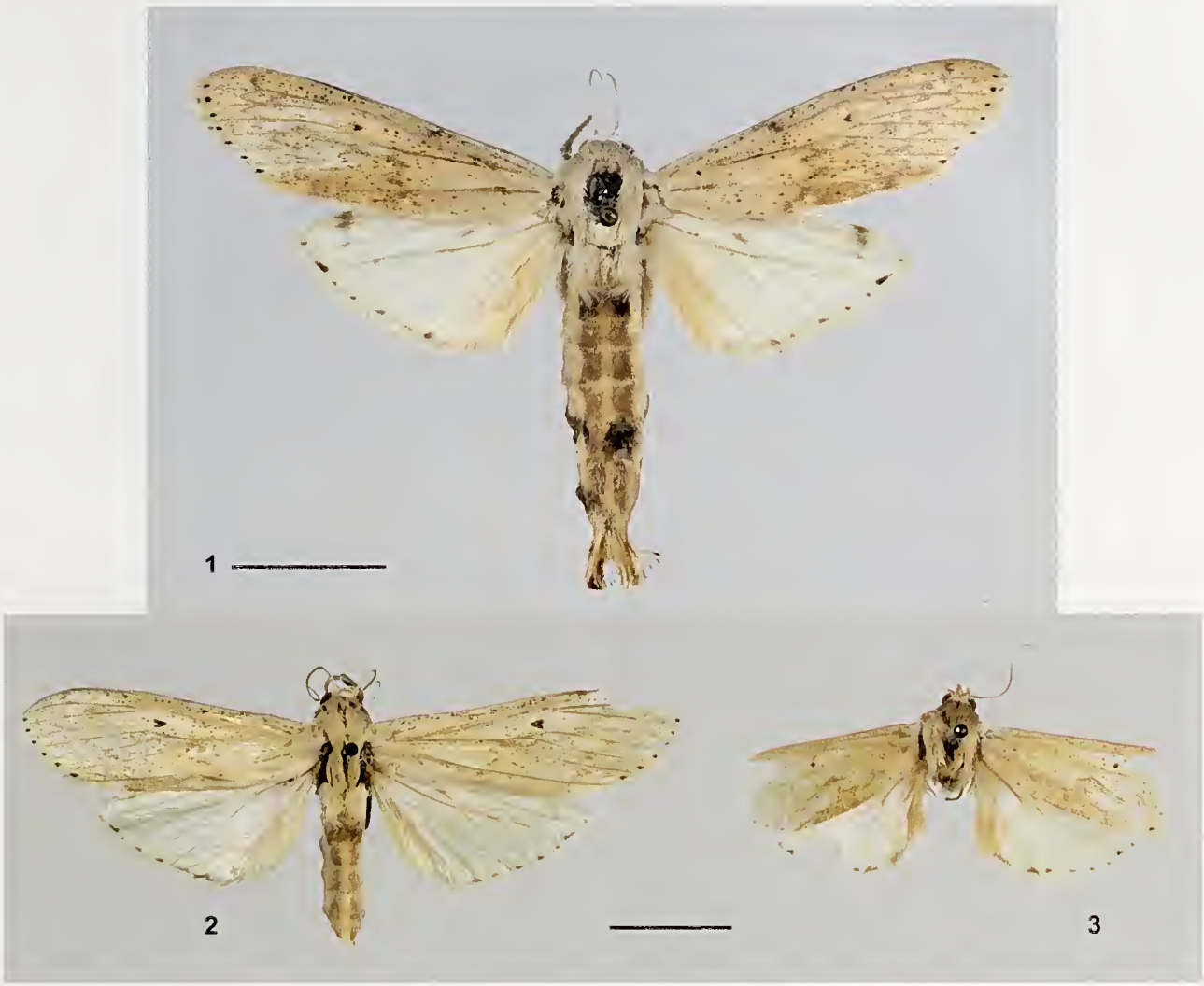
Photographs of the immature stages were taken with a Leica DFC 425 camera mounted on a Leica M205C dissecting scope. They were stacked using Zerene Stacker and enhanced with Adobe Photoshop Elements. Drawings were made with a camera lucida mounted on a Wild M10. One middle instar larva and a pair of adult abdomens were dissected after maceration in KOH at 60°C for one hour. They were then stained with chlorazol black and orange G, the latter mixed in lactic acid, and mounted in Euparal.

Our classification follows Landry (1995) who recognizes only one family (Pyralidae s.l.) instead of two (Crambidae and Pyralidae s. str.) in Pyraloidea (see also Regier *et al.*, 2012). Adult terminology follows Landry (1995) except for the use of 'phallus', instead of 'aedeagus', as recommended by Kristensen (2003). Color names are matched to Wikipedia (shades of brown, white, and yellow). Pupal terminology follows Mosher (1916). Larval morphology and setal nomenclature follows Stehr (1987), as applied to the Crambinae by Allyson (1986). Mandible terminology follows Passoa (1985) (see figures in Gilligan & Passoa, 2014: mandible morphology).

Only one mature larva of *M. nicaraguensis* was available for study. If a seta was broken on both sides of the body, the drawings record them by using of a pair of oblique crossbars to show the break. On the larval head, only the visible sockets of the MD setae of Stehr (1987) are shown. The broken antenna is shown with dotted lines. The larval mandible is illustrated from a probable 3rd instar (given its length) as we decided not to dissect the single mature larva of *M. nicaraguensis* associated with the type series.

The following acronyms are used: EAPZ for 'Escuela Agrícola Panamericana Zamorano', Francisco Morazán Department, Honduras; MELN for 'Museo Entomológico de León', León, Nicaragua; MHNG for 'Muséum d'histoire naturelle de Genève', Geneva, Switzerland; SMTD for 'Museum für Tierkunde, Senckenberg Naturhistorische Sammlungen Dresden,' Germany; SPIC for S. Passoa insect collection, Columbus, Ohio; USNM for National Museum of Natural History, Washington, D.C., U.S.A.

The molecular work was performed at the SMTD. DNA was extracted from a dried abdomen with the NucleoSpin Tissue kit of Macherey-Nagel according to the manufacturer's protocol and labelled as LEP2311. PCR amplifications were performed with the Bio-X-Act Short Taq DNA polymerase and the primers HybLCO (forward) and HybPat (reverse) following the methods of N. Wahlberg's lab (http://nymphalidae.utu.fi/Nymphalidae/Molecular.htm) with the following modifications in the initial mix: H20 (14.65 µl), MgCl2 (0.75 µl), both primers (0.5 µl), Bio-X-Taq (0.2 µl). The PCR program was that of Wahlberg's lab with the following changes in the cycles: denaturation temperature (94°), annealing time (40s). PCR products were analysed with a 1% agarose gel electrophoresis subsequently stained with GelRED and photodocumented under UV light. The clean-up of the PCR products was done with the ExoSAP-IT kit (USB Corporation) by mixing 0.1 µl ExoSap with 1µ H20 to 10µl of each PCR product. Samples for sequencing were prepared for each sequencing primers T3 and T7 as follows: 3 µl PCR product, 4.5 µl H20 and 2.5 µl primer (10 pmol/µl). The samples were sent to Macrogen Europe and further sequenced there. The forward and reverse sequences were aligned by eye and compiled under PhyDE 0.9971 (Müller et al., 2011). A comparison of the sequence with other COI barcode sequences was done on the BOLD Identification System (IDS)




**Figures 1–3.** Holotypes of *Myelobia* species. **1.** *Myelobia nicaraguensis* sp. n. **2.** *M. systrapega* (Dyar). **3.** *M. heinrichi* (Box). Scale = 1 cm.

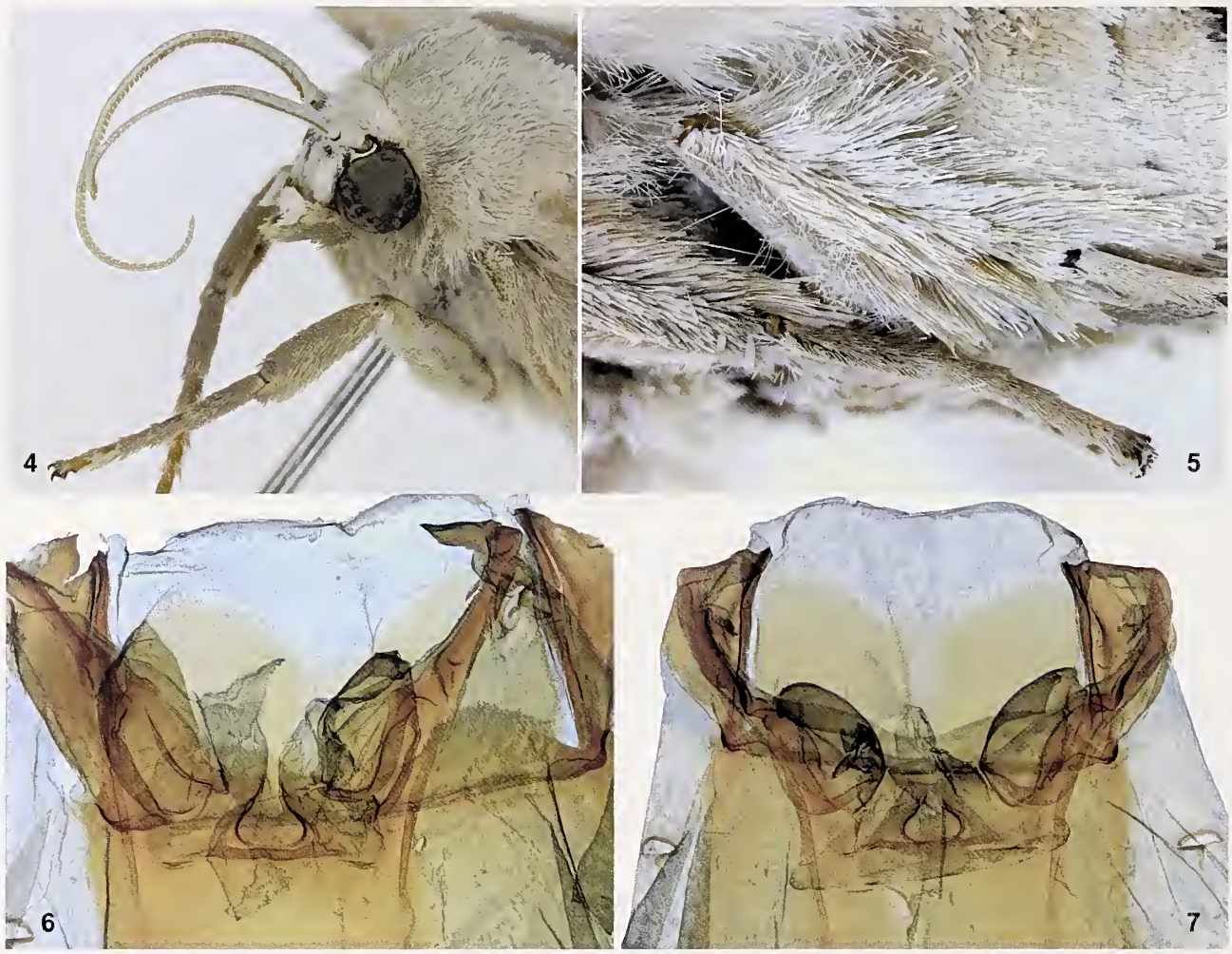
(Ratnasingham & Hebert, 2007). The sequence was aligned to the database with BLAST. The similarity of the barcode sequence with the sequences from BOLD was assessed using the Kimura 2-P parameter.

### *Myelobia nicaraguensis* Landry & Maes, sp. n.

**ZooBank LSID urn:lsid:zoobank.org:act:15ECC06B-83AF-455C-AD44-4C5F86176514**

**Diagnosis.** This species (Fig. 1) is most similar to *M. systrapega* (Dyar, 1913; Fig. 2), described from Veracruz, Mexico, and *M. heinrichi* (Box, 1931; Fig. 3), described from Peru. In size, color and wing pattern all three species are very much alike. One possible difference is the smaller dark spot at the end of the forewing cell in *M. nicaraguensis*. The forewing of *M. nicaraguensis* is also darker and the hind tibia is more densely set with longer scales than in *M. systrapega* or *M. heinrichi*. However, the primary types of *M. heinrichi* and *M. systrapega* are both somewhat damaged and the holotype of the former is missing about one fourth of both forewings. Differences in forewing color intensity may be due to the age and partial deterioration of the type specimens of both *M. systrapega* and *M. heinrichi*.

The uncus of the male genitalia provides the best diagnostic characters. Its dorsal margin forms a sharp (ca. 130°) angle between the helmet-like distal section and the basal shaft in *M. nicaraguensis* (Fig. 9) while this angle is less pronounced (105°) and broadly rounded in *M. systrapega* (Fig. 12). There is no bend between these two structures (180°) in *M. heinrichi* (Fig. 15). As best shown by the illustrations, the shape of the apical margin of the uncus also differs and a flange is present ventrally in *M. nicaraguensis* and *M. systrapega*, but not in *M. heinrichi*. Also, the lateral margins of the shaft of the uncus are produced into a triangle pointing ventrally just before the distal section in *M. nicaraguensis* and *M. systrapega* while this triangle is closer to the middle of the shaft in *M. heinrichi*. In addition, the apex of the gnathos is blunt in *M. nicaraguensis* while it is pointed and distinctly upturned in the other two species. The lack of material has prevented us from diagnosing female specimens.




**Figures 4–7.** Morphological features of *Myelobia nicaraguensis* sp. n. **4.** Head of male. **5.** Hindleg of male. **6.** Female tympanal organs. **7.** Male tympanal organs.

**Material examined**: Holotype ♂ (Fig. 1): 1- 'Nicaragua: Bluefields: | Finca Rio Kama | UV Light – X-2013 | Col. J.M. Maes'; 'HOLOTYPE | Myelobia | nicaraguensis | B. Landry & J.-M. Maes'; 'MHNG | ENTO | 00008724'. Undissected and deposited in MHNG.

**Paratypes**, all from Nicaragua: 3 ♂ (one dissected, genitalia slide MHNG ENTO 8723), 1 ♀, same data as holotype; 15 ♂, 2 ♀, same data as holotype except date, 24–30.ix.2013; 3 ♀, Bluefields, Finca Rio Kama, ex larva from *Guadua aculeata*, 6.x.2013 (J.M. Maes); 2 ♀, idem, 17.x.2013; 2 ♀ (one dissected, slide MHNG ENTO 8722), idem, 18.x.2013; 1 ♀, idem, 22.x.2013. Deposited in EAPZ, MELN, MHNG, SPIC, USNM. Specimens were collected at light at a farm house, 12°14'55" N, 84°00'53" W, elevation 20 m, while others were reared from larvae collected in plantations in an area up to 3 kilometers away from this farm house.

**Description. Male** (n=19). Figs. 1, 4, 5, 7–10. Head (Fig. 4) white to old lace towards occiput, with some light tan in middle of vertex, lighter still on fronto-clypeus. Labial palpus only slightly longer than widest diameter of compound eye, fallow to tan. Maxillary palpus tan on first two palpomeres, white on third, with apical scales directed medially, forming extended, flattened surface. Proboscis scales white. Antennal scales white; flagellomeres serrate, with sensilla trichodea about 0.5 X (near apex of flagellum) to 0.33 X (toward base) width of corresponding flagellomere. Thorax old lace at base, ivory towards apex; with large lateral bunch of thin scales extending posteromedially over first abdominal tergite from each side of metathorax. Foreleg tan, with taupe band along femur medially, light taupe at tip of distal tarsomere. Midleg tan, with thicker scaling on dorsal edge of tibia, light taupe at tip of distal tarsomere. Hindleg (Fig. 5) tan, femur dorsally set with longer and thin projecting white to light tan scales, ventrally lighter, tip of distal tarsomere light taupe. Forewing length: 21–25 mm (holotype, 25 mm); forewing color wheat, sprinkled with blackish brown scales; main markings a small blackish brown spot at end of cell, a slightly larger dark greyish brown diagonal spot submedially on costa, a zigzagged diffuse submedian line below cell connected to even fainter zigzagged line from below cell to before middle of R5, a faint and thin, subapical greyish brown zigzagged line, with external points between veins, slightly darker below costa, and seven apical blackish brown spots between veins; fringes wheat with small greyish brown spots at level of apical spots. Hindwing ivory with light wheat hair-like scales in anal sector and along veins; markings greyish brown as small, light dash submedially in cell, subapical line distinct only


**Figures 8–10.** Male genitalia of *Myelobia nicaraguensis* sp. n. **8.** Whole genitalia except phallus. **9.** Close-up of uncus. **10.** Close-up of phallus with vesica partly everted.

toward costa, and darker, more or less elongate spots on outer margin between veins; fringes ivory, spotless. Abdomen dorsally: first tergite with white to chocolate scales medially, laterally with bunch of light tan hair-like scales extending posteriorly over tergite II and curving medially; second tergite mostly coyote brown, apicomedially with wheat to earth yellow; remaining tergites with pair of longitudinal tan bands apically bordered light wheat on each tergites, with median longitudinal band wheat colored; apex of abdomen with bilobed tuft of elongate, thin scales wheat and tan; laterally and ventrally light wheat with narrow median, tan longitudinal band ventrally, reaching last sternite before genitalia. Tympanal organs (n=1). As described for the genus by Landry (1995), but praecinctorium short, triangular, and not distinctly bilobed (Fig. 7).

**Male genitalia** (n=1). Figs. 8–10. Uncus large; dorsal margin between laterally compressed, helmet-like distal section and basal shaft sharply angled at about 130°, apical margin more narrowly rounded at dorsal angle, almost straight towards ventral angle, ventrally with bilobed flange; lateral margins of shaft ventrally produced into triangles just before distal section. Gnathos short and stout, with lateral arms forming ca. 110° angle with ventral margin of distal section; apically with short projection at about 45° from ventral margin; dorsally towards apex with thickly sclerotized denticles. Tegumen with narrow arms forming right angle with dorsally closed, larger distal section. Valva simple, narrowing to 0.4 times basal width; dorsal margin with triangular, narrowly rounded projection at 1/4; ventral margin with short, broad bulge near middle, then slightly angled upward; medially at apex with group of thickened setae. Juxta with main, median part short, narrowing towards apex, apically truncated; with lateral projections symmetrical, narrow, slightly down-curved, apically pointed, about 1/3 longer than median part, reaching base of costal triangle of valva. Vinculum arms narrow, with short, apically rounded saccus about 3/4 length of lateral arms. Pseudosaccus a short, rounded, convex plate with apex rounded and base ending in short point. Phallus slightly down-curved, width about


**Figures 11–13.** Male genitalia of *Myelobia systrapega* (Dyar) lectotype (USNM). **11.** Whole genitalia. **12.** Close-up of uncus. **13.** Close-up of phallus.

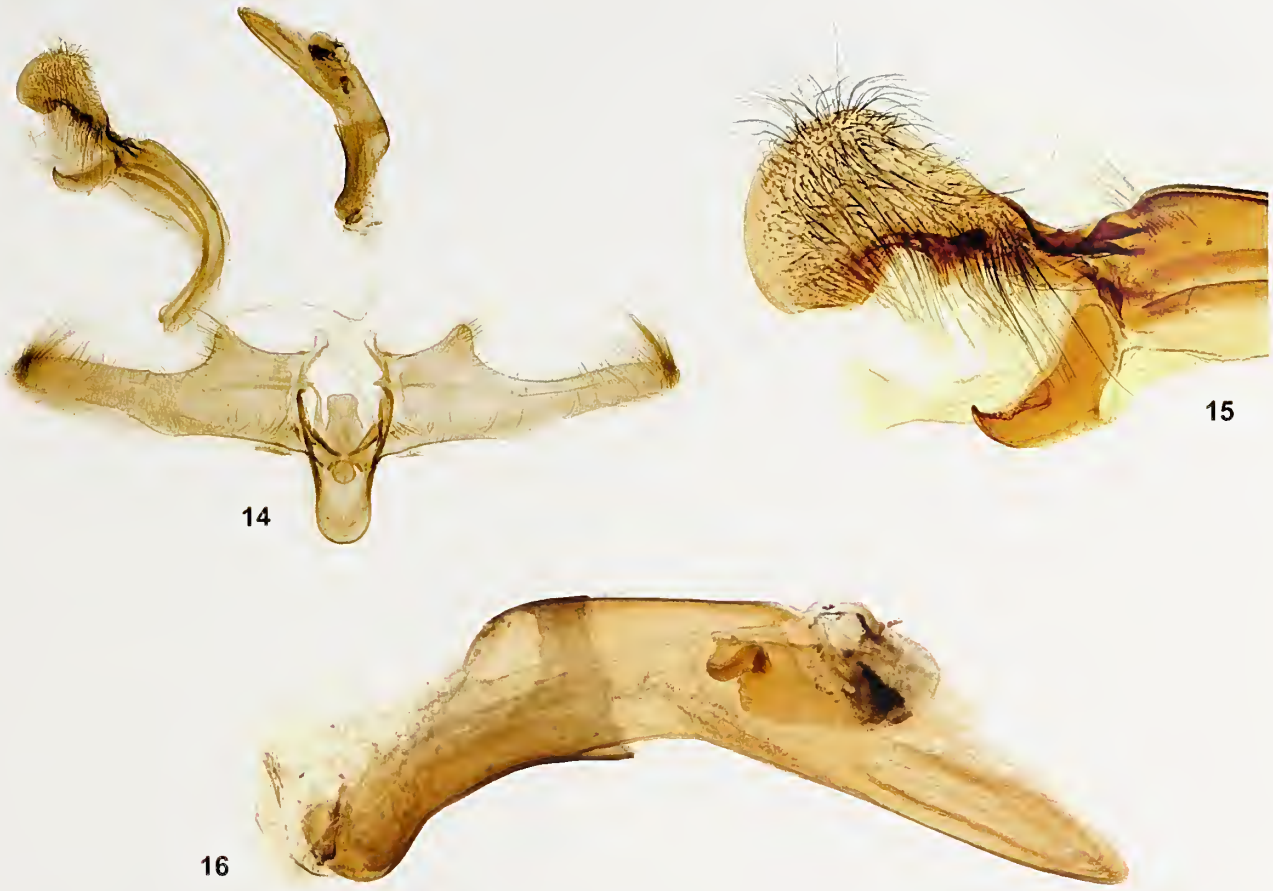
15% length, open dorsally on distal 1/3; without coecum penis; ductus and bulbus ejaculatorii almost four times as long as phallus shaft; vesica everting dorsally, with separate basal rounded flap proximally, covered with microscopic setae; single cornutus short, dagger like, with rounded base most thickly sclerotized.

**Female** (n=2). Forewing length: 25–31 mm; frenulum with four acanthae, the dorsal two shorter and thinner. Tympanal organs (n=1) as in male except praecinctorium more strongly developed and slightly bilobed (Fig. 6). Posterior margin of sternite VIII with rounded emargination covering more than half of width of sternite, with thicker cover of short, narrow scales along emarginated area.

**Female genitalia** (n=1) (Fig. 17). Papillae anales connected dorsally, narrow, with dense row of long setae all along apical edge, with dense cover of small, fine setae on rest of surface. Posterior apophyses narrow, straight, slightly tortuous, about 3/4 length of papillae anales. Anterior apophyses long, almost 3 times as long as posterior apophyses, very thin, slightly bent at middle. Segment VIII of medium length dorsally, narrowing ventrally to 1/4 dorsal length, not fused ventrally, with short, oval, desclerotized section along anterior edge ventrad from base of apophyses, with higher concentration of scale (or setal) sockets. Sterigma forming short depression walled ventrally and laterally with sclerotized wire-like incomplete rings. Ductus bursae wide, short, with thin sclerotized dorsal and ventral, convex plate-like walls abutting each other, with ventral wall distally curving upward, and both plates followed by short sclerotized ridges continuing into corpus bursae. Corpus bursae slightly wider than ductus bursae, very long, about 12x length of sclerotized part of ductus bursae, with parallel margins, densely scobinated all over, without defined signum but with rounded plate of thicker scobination near distal end laterally.

**Larva** (Figs. 18–31). Based on one mature larva and five specimens representing earlier instars (including one cleared and dissected). Mature larval length 42 mm. Body pale with tonofibrillary platelets especially prominent on subdorsal and lateral areas of A6-8. Dorsal pinacula on A1-8 paler than surrounding cuticle; subdorsal to ventral pinacula on A1-8 distinctly paler than surrounding cuticle and slightly bulged; pinacula of A9 almost concolorous with cuticle; anal shield with a few patches of tonofibrillary platelets on posterior portion, also almost concolorous with cuticle. Spiracles on prothorax and A8 comparable in size, distinctly larger than spiracles on A1-7.

**Head** (Figs. 18, 21–28) light yellowish brown, without markings, more darkly pigmented at stemmata 3-5, clypeus, and




**Figures 14–16.** Male genitalia of *Myelobia heinrichi* (Box) holotype (USNM). **14.** Whole genitalia. **15.** Close-up of uncus. **16.** Close-up of phallus.

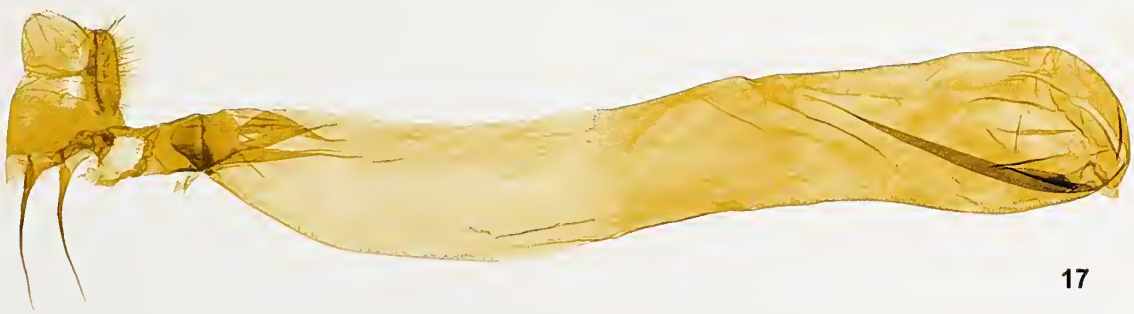



**Figure 17.** Female genitalia of *Myelobia nicaraguensis* sp. n.

around base of antenna. Front extending approximately one-half distance to epicranial notch. AF2 and AF1 widely spaced, AF2 well above apex of front, AF1 below it. F1 in line with the frontal pores. C1 and C2 equal in length. Labral setae in two groups of 3 setae, all more or less equal in length. P2 shorter and slightly posterodorsad of P1. A2 shorter than A1 or A3. L1 as long as A1. Six stemmata in semicircle, stemmata 1, 2 and 6 slightly larger than others, stemmata 3 and 4 closely spaced. S2 longer than S1, S3 between stemmata 5 and 6. SS2 longer than SS1 or SS3. Mandible with three obvious scissorial teeth and a small fourth one. Anterior mandibular seta longer and thicker than posterior one. Hypopharyngeal complex with thin spinneret rounded at apex longer than labial palpus, the dorsum covered with dense spines. Stipular setae present. Apical seta of antenna longer than basal segment. Maxillary palpus slightly longer than mesal lobe.

**Thorax** (Figs. 18, 28). Prothoracic shield nearly concolorous with head, without markings, and divided along midline. XD1, XD2 and SD1 in vertical line. SD2 much shorter than D1 or D2. Prespiracular pinaculum narrow, concolorous with shield, extending posteriorly below spiracle, with rosette of six tonofibrillary platelets present only on right side. L1 about four times as long as L2. SV group bisetose. V setae behind coxa and closely spaced, with length of each seta greater than distance between pinacula.

Mesothorax dorsally with wide triangular plate lacking setae on posterior edge of segment. D setae on oval pinaculum. SD2 short and slightly posterodorsad of SD1. L1 and L2 on small oval pinaculum. L3 short and on anterior edge of large circular pinaculum. SV group bisetose on large circular pinaculum. V setae closer to coxa than midline, on separate circular pinacula; distance between V1 on mesothorax approximately four to five times greater than separation of prothoracic V setae.

Metathorax dorsally with wide triangular plate similar to mesothorax but paler. Chaetotaxy as in mesothorax.

**Abdomen** (Figs. 18–20, 29–31). First abdominal segment. D1 on large circular pinaculum and about three times longer than shorter D1 seta. SD1 on large oval pinacula dorsad of spiracle. SD2 minute and anterodorsad to spiracle. L1 and L2 joined on same pinaculum, L3 longest of the group. SV group trisetose. V seta slightly closer to midline than to SV group.

Sixth abdominal segment. D setae on large oval pinacula, that of D1 slightly larger in diameter than D2. SD1 anterodorsal of spiracle. SD2 minute and anteroventrad of spiracle. L1 above L2, both joined on same pinaculum. L3 on elongate oval pinaculum. SV group trisetose, pinaculum indistinct. SV2 closer to SV3 than SV1. Crochets in irregular triordinal to weakly multiserial circle. V setae closer to coxa than midline.

Eighth abdominal segment. D1 pinacula and seta larger than D2. SD1 anterior to spiracle. SD2 minute, slightly anterior to SD1, below SD1 pinaculum, at level of ventral edge of spiracle. L1 and L2 joined on same pinaculum, both setae slightly posteroventrad of spiracle. L3 pinaculum about equal in size to L1-L2 pinaculum. SV group unisetose. V1 setae on A8 and A9 almost equally spaced.

Ninth abdominal segment. D1, D2 and SD1 nearly in vertical line. L1 unisetose and closer to SD1 than SV1. V1 on A9 twice as far apart as V1 setae on A10.

Tenth abdominal segment. Anal shield with D1 and SD2 aligned vertically. SD1 equidistant from D1 and SD2. Three L setae and one SV seta on outer side of anal proleg. Crochets irregular triordinal to weakly multiserial transverse arc.

**Pupa** (Figs. 32–39). Based on one exuvia (a female). Length 36 mm. Black brown on head, reddish brown on thorax dorsally, otherwise yellowish brown. Head heavily wrinkled, except for the smooth glazed eyepiece. Vertex narrow. Frons not produced. Invaginations for anterior arms of tentorium clearly indicated. Pilifers indistinct. Labrum an inverted truncated pyramid in shape. Antennae extending to slightly beyond half of forewing. Maxillary palpus small, triangular. Labial palpus concealed except for small triangular area. Maxillae extending to nearly middle of A2 and forewing. Prothoracic femur exposed, about half as long as rest of prothoracic leg; latter not reaching tip of maxillae and reaching slightly beyond middle of A1. Mesothoracic leg extending to about 3/4$^{th}$ length of forewing and about middle of A3. Metathoracic leg exposed, extending slightly beyond forewing and middle of A4, slightly separated apically. A4-6 ventrally with pair of proleg scars. Genital orifice on anterior portion of A8, without associated modifications of the cuticle. Anal orifice on A10 surrounded anteriorly by unmarked cuticle followed by 'crown' of short narrow grooves; laterally with 2-3 grooves along whole length of orifice and additional shorter grooves at apex; posteriorly with more grooves radiating from apex of orifice. Setae all very short: with one pair on prothorax just before mesothoracic spiracle; one pair on frontolateral lobes of metanotum; A1 bare; A2 and A3 with two pairs; A4 with four pairs; A5-7 with three pairs; A8 with four pairs; A9-10 with one pair. Mesothoracic spiracle long, slitlike. Cuticle of thorax and abdomen dorsally mostly smooth, with longitudinal creases, and with spinulose apical band of A4-6 all around these segments. Without lateral furrows on A10 or cremaster.

**DNA Cytochrome oxydase I barcode** (except first 66 base pairs) (GenBank accession number: BankIt1845402 MYELB001-15.COI-5P KT353043):

TGCAGAATTGGGTACCCCAAATTCTTTAATTGGTGATGATCAAATT
TATAATACAATTGTAACAGCTCATGCATTTATTATAATTTTTTT
TATAGTTATACCTATTATAATTGGTGGATTTGGAAATTGATT
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TAATTTCTAGAAGAATTGTTGAAAATGGAGCTGGAACTGGATGAA
CAGTTTATCCTCCTTTATCATCTAATATTGCCCATGGTGGAAGAT
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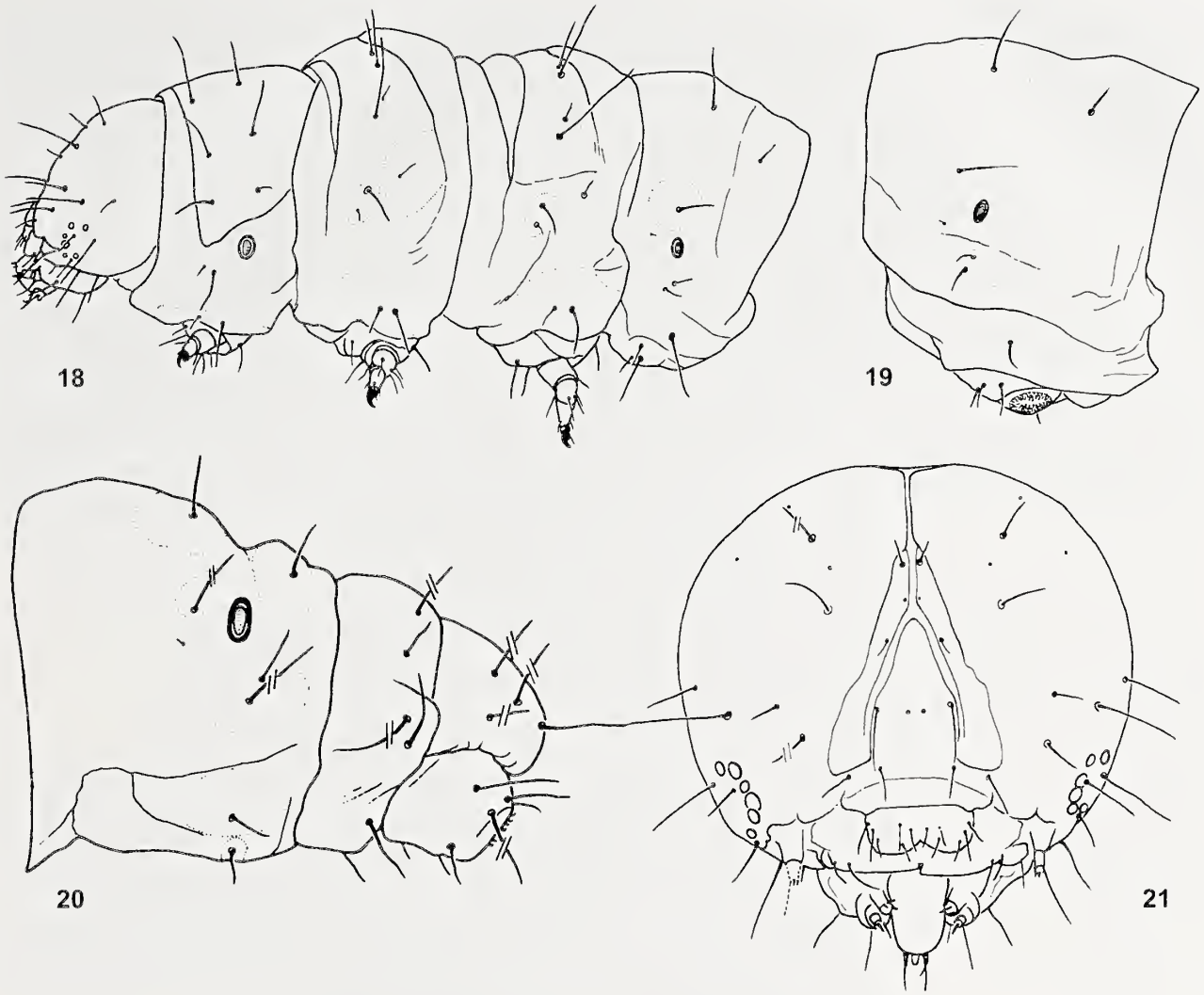
TAGCTGGAGCTATTACTATATTACTAACAGATCGAAATTTAAACAC
CTCTTTTTTTGACCCTGCTGGAGGGGGGGATCCAATTTTATATCAA
CATTTATTT

**Distribution.** So far known only from the Bluefields (= El Rama) region of Nicaragua, on the Atlantic side. The type locality is 25 km east of El Rama or 36 km northeast of Bluefields.

**Natural history.** This species was reared from bamboo, *Guadua aculeata* Rupr. ex E. Fourn. (Poaceae). Apparently larvae attack only branches, not emerging shoots, and may wander from branch to branch. A more detailed account of the natural history of this species will be published in a forthcoming paper.

## Discussion

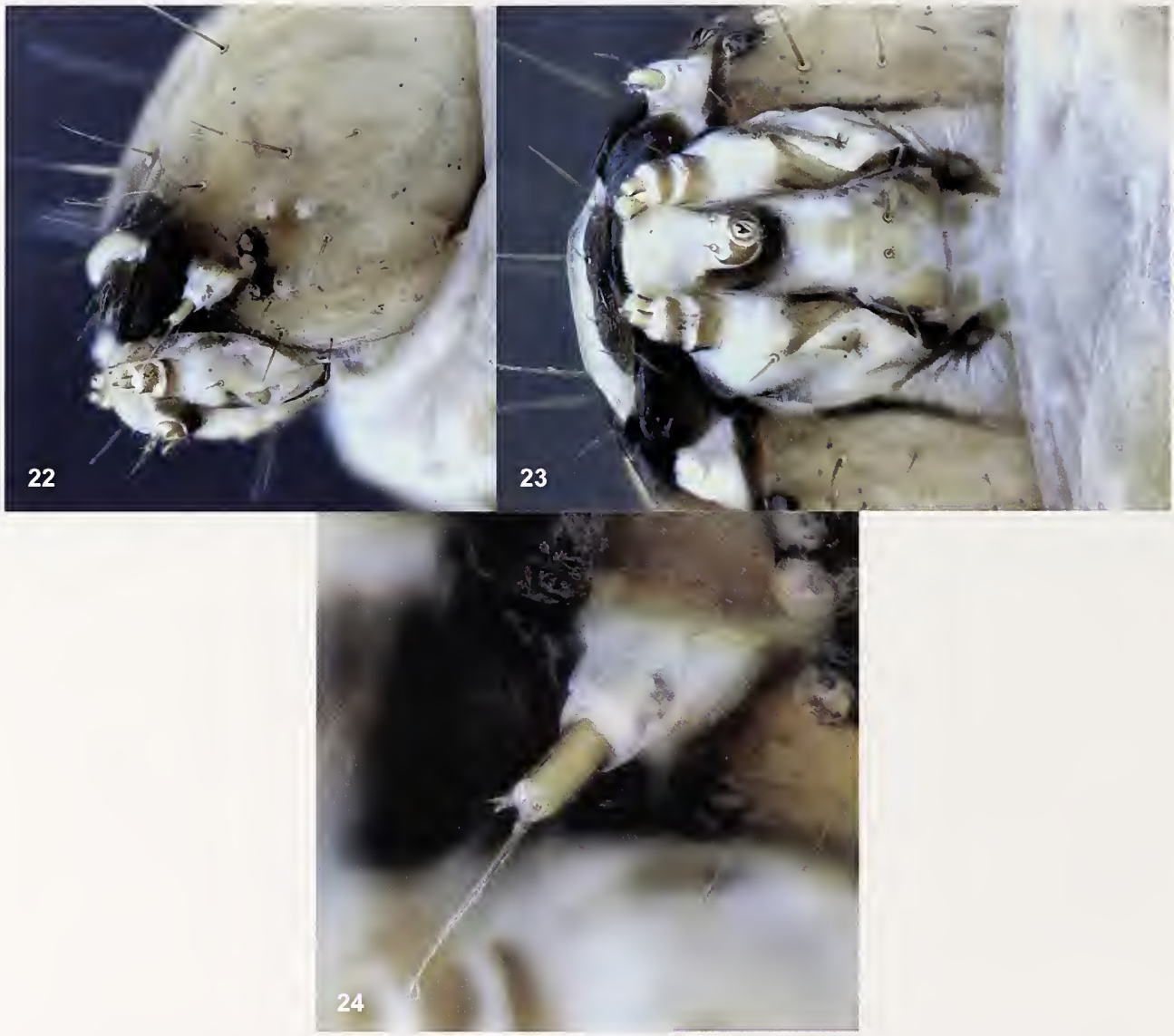
Landry (1995) based his characterization of *Myelobia* on only one species. The description of *M. nicaraguensis* differs from that summary in a few details, notably that the ductus bursae is differentiated in our new species. In addition to the holotype and the single paratype of *M. heinrichi* (Box) from Peru (Yahuarmayo, 365 m), BL examined an additional male specimen (BL slide 1805) of this species from Brazil (Rondonia, Cacaulândia, 140 m, xi.1991, V.O. Becker, coll. Becker 79589). The uncus of this specimen agrees with the holotype of *M. heinrichi*.




**Figures 18–21.** Mature larva of *Myelobia nicaraguensis* sp. n. **18–20.** Lateral view of head to A1 (**18**), A6 (**19**), A8–10 (**20**). **21.** Frontal view of head, slightly tilted to left.

Although information on *Myelobia* immatures is lacking for most species, there are several differences between the larva and pupa of *M. nicaraguensis* and *M. smerintha*, two species that may occur together on bamboo in Nicaragua. The larva of *M. smerintha* has the mesothoracic dorsum covered by a large plate that includes both the D and SD setae. This gives the impression of a larva with two "prothoracic shields" (Fig. 42). The mesothoracic plate of *M. nicaraguensis* is smaller, triangular and does not include either the D or SD setae (Figs. 18, 28). This is more typical of most crambine larvae that have a posterior shield on the mesothorax without setae (e.g. Allyson, 1986: 317). Another difference is in the height and shape of the SV pinaculum on A7 and A8. *Myelobia nicaraguensis* has a large elongate SV pinaculum that is approximately as high as the vertical diameter of the corresponding spiracle on that segment (Fig. 20). This contrasts with *M. smerintha* because the oval, not elongate, SV pinaculum on A7 and A8 is only half as high as the corresponding spiracle on that segment. Differences between the pupal stages are even more striking. The pupa of *M. smerintha* has the frons produced to a blunt point and the cremaster is a broad plate with two small points (Figs. 43, 44). In contrast, the frons is rounded in *M. nicaraguensis* and there is no trace of a cremaster (Figs. 32-34).

Landry (1995: 45, Fig. 4) included the genus *Myelobia* in his phylogenetic analysis of the Crambinae and suggested it was most closely related to *Diatraea*. Although no exclusive synapomorphy supported this relationship, the coecum penis was lost in the tribe Prionapterygini, the genus *Ancylolomia* and in both

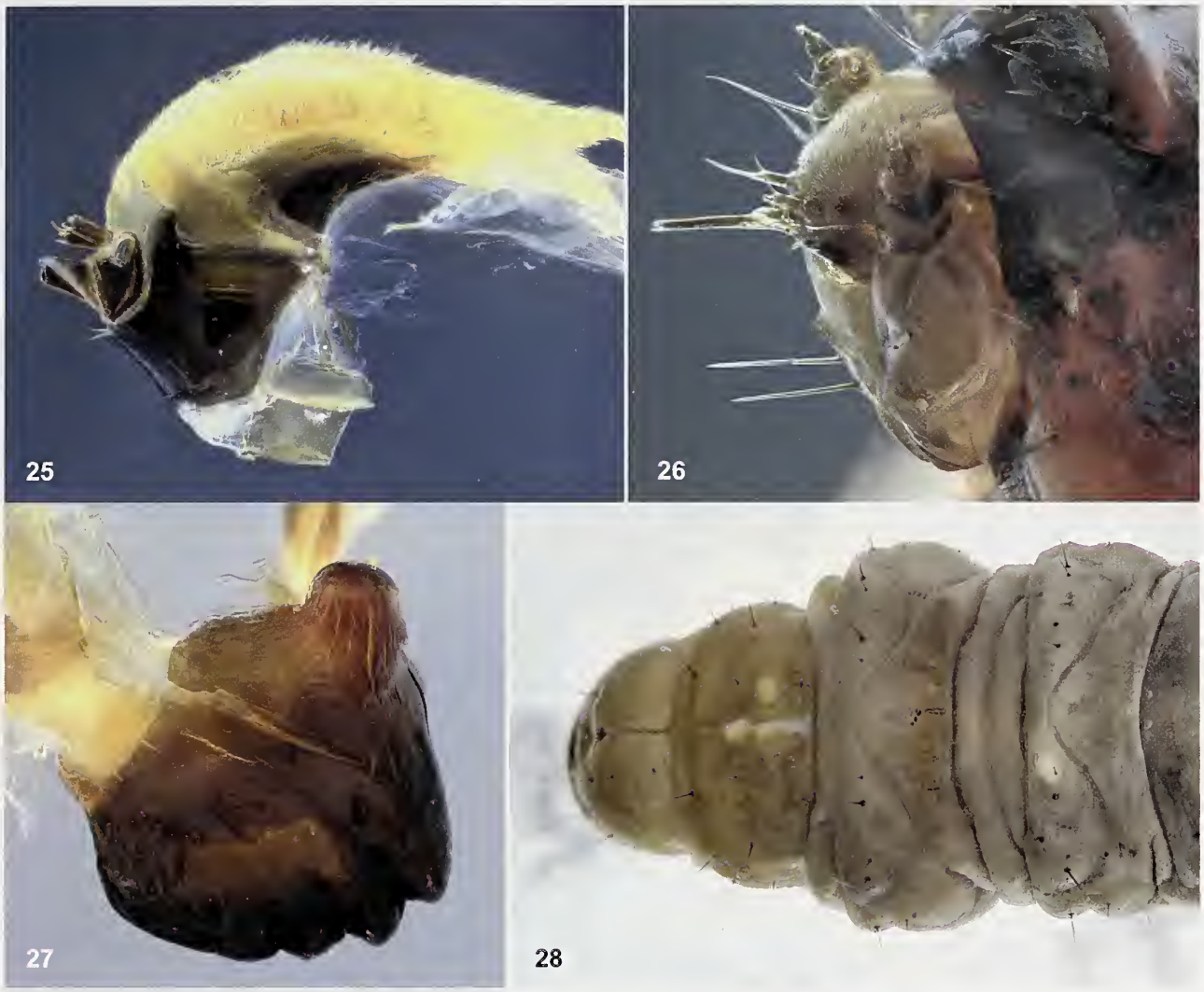



**Figures 22–24.** Head of mature larva of *Myelobia nicaraguensis* sp. n. **22.** Lateral view. **23.** Ventral view. **24.** Lateral view of left antenna.

*Myelobia* and *Diatraea*. We now have information on the genitalia of another *Myelobia* species (*M. nicaraguensis*) with preserved immatures for two members of this genus (*M. nicaraguensis* and *M. smerintha*). We can list several apparently overlooked characters of the immature stages that vary within the Crambinae and thus show promise for future morphological phylogenetic analyses. We have focused on Crambini, Argyriini (*Argyria* or *Urola*), Haimbachiini (*Eoreuma*) and economically important genera such as Chiloini (*Chilo*) and *Diatraea* for comparison because information on the immature stages is usually available in these taxa.

Egg morphology. There are two types of eggs in the Crambinae (Peterson, 1963). Crambini have eggs that are oval with obvious ridges (Matheny & Heinrich, 1972; Peterson, 1963). The eggs of *M. smerintha* (SPIC, Fig. 40), *Chilo* (e.g., Fletcher, 1914: Fig. 300; Peterson, 1963), *Eoreuma* (Johnson, 1981), *Urola* (Peterson, 1963) and *Diatraea* (Passoa, 1985; Peterson, 1963) are flattened and sometimes marked with a faint texture. The egg morphology of *Myelobia* is more similar to Argyriini (*Urola*), Haimbachiini (*Eoreuma*), Chiloini (*Chilo*) and *Diatraea* than Crambini.

Oviposition pattern. Eggs of the Crambini are laid singly without an adhesive (Peterson, 1963). The

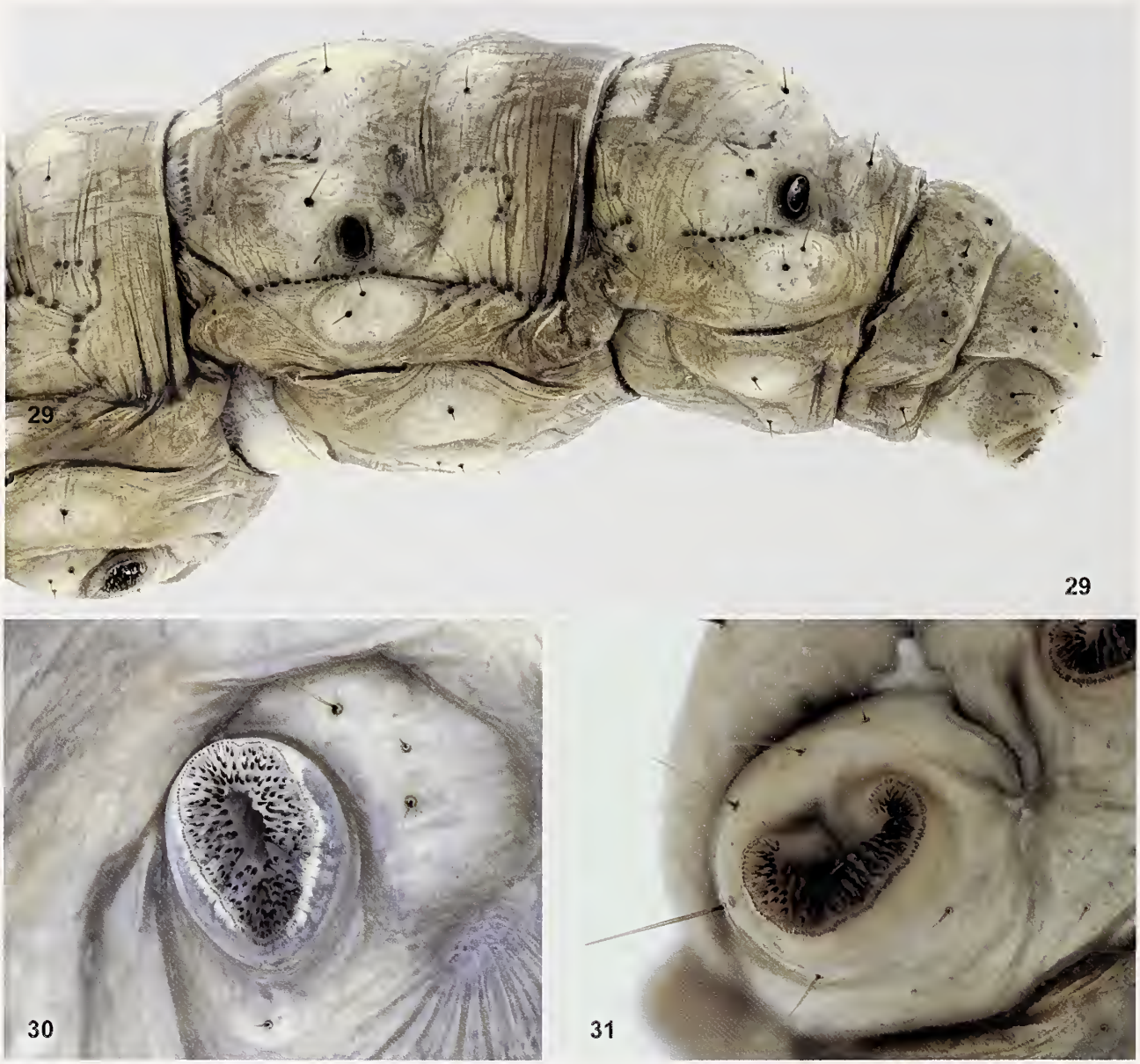



**Figures 25–28. 25–27.** Head of immature larvae of *Myelobia nicaraguensis* sp. n. **25.** Dissected hypopharynx, with spinneret and palpi broken (lateral view). **26.** Hypopharynx in situ of different larva, with spinneret entire (lateral view). **27.** Mandible (ventral view). **28.** Mature larva, head and thorax (dorsal view).

eggs of *M. smerintha* (SPIC), *Chilo* (e.g., Fletcher, 1914: fig. 300, Peterson, 1963), *Eoreuma* (Johnson, 1981), *Urola* (Peterson, 1963) and *Diatraea* (Passoa, 1985; Peterson, 1963) are laid in groups with an adhesive to prevent them from falling off the substrate. As with egg morphology, the oviposition behavior of *Myelobia* is more similar to that of Argyriini (*Urola*), Haimbachiini (*Eoreuma*), Chiloini (*Chilo*) and *Diatraea* than Crambini.

Mesothoracic and metathoracic SV setae. Crambini, *Argyria* (Allyson, 1986; Tan, 1984: 13), *Urola* (SPIC) and *Eoreuma* (Weisman, 1986) have a unisetose SV group on the mesothorax and metathorax. The SV group of *Myelobia* (*M. nicaraguensis* and *M. smerintha*) (SPIC, Fig. 19) and several *Chilo* and *Diatraea* (SPIC, Gilligan & Passoa, 2014) are bisetose in that position. This character groups *Myelobia* with Chiloini (*Chilo*) and *Diatraea* as opposed to the Argyriini or Crambini.

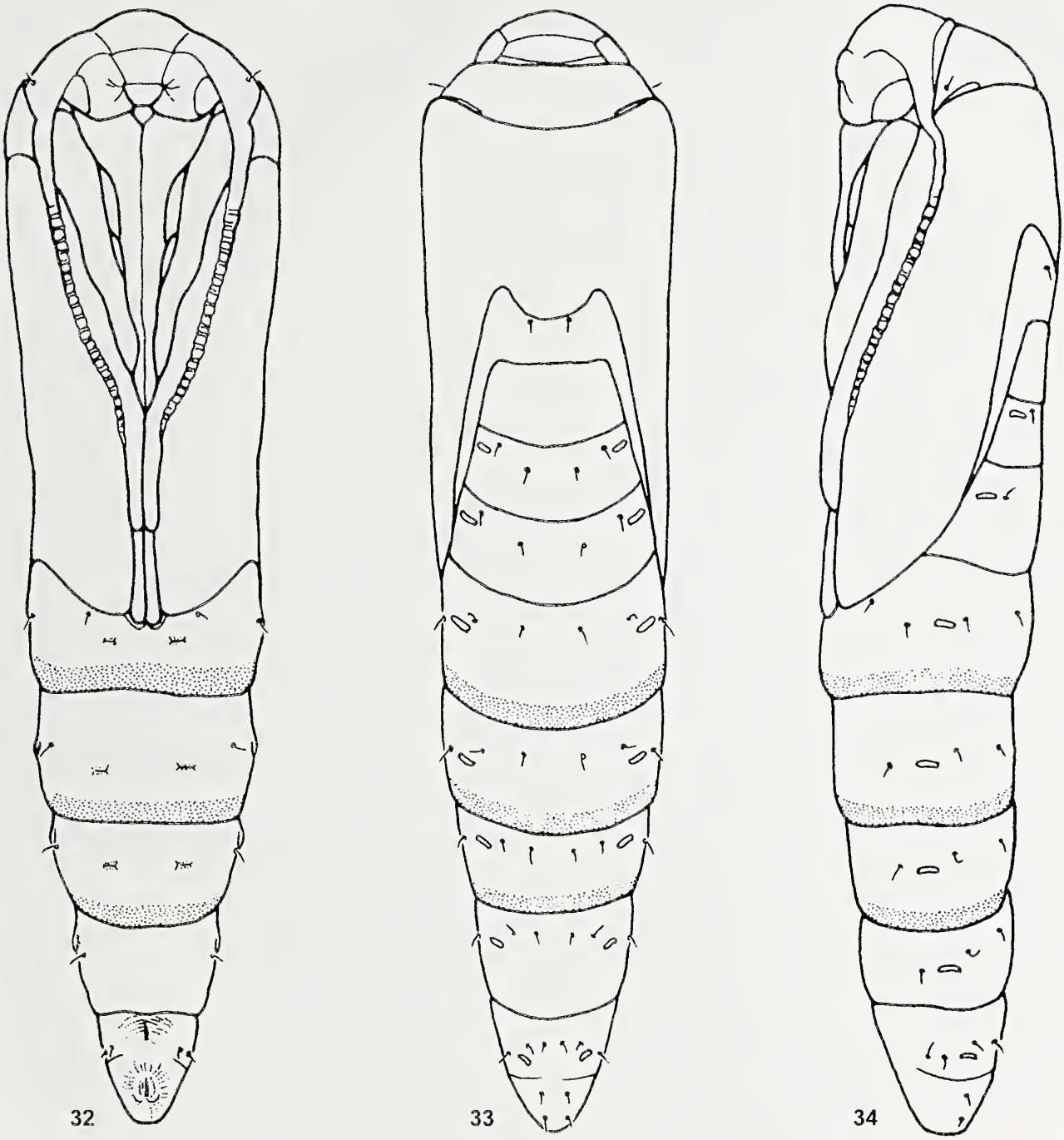
Pupal maxillae. Pupae of the Crambini have long maxillae that extend near the tip of the forewing (Passoa, 1985; SPIC). The maxillae of *Myelobia* (*M. nicaraguensis* and *M. smerintha*) (SPIC; Fig. 32), *Eoreuma* (Passoa, 1985) and several *Chilo* and *Diatraea* (SPIC) are short and only reach about half the forewing length. Both states exist in the Argyriini; *Argyria* has long maxillae (Passoa, 1985) whereas they are short in *Urola* (SPIC). This character groups *Myelobia* with the Haimbachiini (*Eoreuma*), Chiloini (*Chilo*) and *Diatraea*. Several other characters of




**Figures 29–31.** Mature larva of *Myelobia nicaraguensis* sp. n. **29.** Lateral view of A6-10. **30.** Left proleg of A3. **31.** Anal prolegs.

potential importance should be mentioned. The form of the cremaster is variable in the Crambinae (Passoa, 1985) but inconclusive with regard to the systematic position of *Myelobia*. A cremaster is absent in *M. nicaraguensis* but present in *M. smerintha*. The deep grooves found laterally on A10 in Crambini, *Chilo* (Passoa, 1985) and *M. smerintha* (SPIC) are absent in *M. nicaraguensis*, *Diatraea* and *Eoreuma*. The crochets of *Diatraea* and *Myelobia* are in a uniform circle (e.g. Fig. 29) as opposed to *Argyria* or *Eoreuma* that have the lateral crochets uniordinal and shorter in length than the longer triordinal mesal portion (Passoa, 1985). Although this character may well group *Myelobia* and *Diatraea*, not enough species have been studied to make a definitive statement.

In summary, there are some character states that associate *Myelobia* with *Diatraea*, the Chiloini and sometimes the Haimbachiini, but they should be put in a phylogenetic analysis together with molecular data to confirm any relationships. Characters of the immature stages strongly suggest *Myelobia* is not closely related to the Crambini. The description of




**Figures 32–34.** Pupa (based on exuvia) of *Myelobia nicaraguensis* sp. n. **32.** Ventral view. **33.** Dorsal view. **34.** Lateral view.


**Figures 35–39.** Exuvia of pupa of *Myelobia nicaraguensis* sp. n. **35.** Left mesothoracic spiracle. **36.** Apex of thorax ventrally. **37.** Left spiracle of A4. **38.** Spinulose apical band of A5. **39.** Ventral view of A8-10.


**Figures 40–44.** Immature stages of *Myelobia smerintha* (Hübner). **40.** Egg mass. **41.** Early instar, dorsal view. **42.** Mature larva, oblique dorsal view. **43.** Anterior half of pupal exuvia, lateral view. **44.** Cremaster, ventral view.

**Table 1.** Twenty best matches from the BLAST analysis of the barcode sequence of the *Myelobia nicaraguensis* sp. n. sample LEP2311 on all 3'742'723 barcode sequences available (>500bp). The name Crambidae in BOLD refers to a subgroup of Pyralidae s.l. as recognized here.

| Family | Genus | Species | Similarity (%) | Sequence ID (when available) |
|---|---|---|---|---|
| Crambidae | *Corynophora* | *torrentellus* | 91.67 | AANIC081-10.COI-5P |
| Crambidae | *Corynophora* | *argentifascia* | 91.67 | AANIC082-10.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 91.67 | ANICS202-11.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 91.5 | AANIC079-10.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 91.5 | ANICS203-11.COI-5P |
| Crambidae | *Corynophora* | *argentifascia* | 91.33 | ANICS206-11.COI-5P |
| Crambidae | *Myelobia* | *BioLep03* | 91.16 | BLPDM398-10.COI-5P |
| Pyralidae | - | - | 91.16 | LNOUF740-11.COI-5P |
| Crambidae | *Omiodes* | *continuatalis* | 91.16 | GBMIN22387-13.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 90.99 | AANIC084-10.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 90.99 | ANICS201-11.COI-5P |
| Crambidae | *Corynophora* | *torrentellus* | 90.99 | ANICS205-11.COI-5P |
| Crambidae | *Thliptoceras* | *manicalis* | 90.91 | - |
| Crambidae | *Massepha* | *grammalisDHJ01* | 90.89 | - |
| - | - | - | 90.83 | GMHGM157-13.COI-5P |
| Sphingidae | *Erinnyis* | *lassauxii* | 90.82 | LNOUE996-11.COI-5P |
| Crambidae | *Myelobia* | *BioLep03* | 90.82 | - |
| Crambidae | *Myelobia* | *BioLep03* | 90.82 | - |
| Crambidae | *Myelobia* | *BioLep03* | 90.82 | - |
| Crambidae | *Myelobia* | *BioLep03* | 90.82 | - |

the mature larva and pupa of *M. nicaraguensis* and *M. smerintha*, being based on a single individual in each case, remains to be verified with more material for some of the fine details, especially of the pupal head region. Our descriptions of the *Myelobia* immatures are the first published for the genus except for a brief and unillustrated description of the larva of *M. smerintha* by Dyar (1917).

Molecular results. We obtained a 1378 base pair COI sequence, including 592 base pairs of the barcode region (90% coverage). The three best-matching sequences returned from the BOLD analysis (http://www.boldsystems.org/) belong to *Corynophora torrentellus* (Meyrick, 1879) (Pyralidae, Crambinae; 2 sequence matches) and *Corynophora argentifascia* (Hampson, 1919) (Pyralidae, Crambinae) from Australia with 91.67% similarity (see Table 1). The nearest *Myelobia* match is sample BLPDM398-10 from the Area de Conservacion Guanacaste in Alajuela, Costa Rica with 91.16% similarity. This latter sample most probably represents *Myelobia smerintha* (Hübner, 1821).

## Acknowledgments

We are grateful to Alma M. Solis (USNM) for sending the primary types of *Myelobia systrapega* (Dyar) and *M. heinrichi* (Box) for comparison, and for her identification of the leaf-roller *Salbia*, to which Richard Mally and James Hayden also contributed. We also thank Christina Lehmann, Florence Marteau and Philippe Wagneur respectively for executing the pupal exuvia drawings and inking the larvae drawings, for producing the plates, and for the photos of the moths and for enhancing the genitalia photos. Mike Pogue (USNM) helped us by determining the specimen of *Scolecocampa* and Vitor O. Becker loaned us specimens. We are grateful also to Ecoplanet Bamboo S.A. for the initiative of planting bamboo, and especially to John Vogel, the pioneer of the realization of this idea. Our gratitude also goes to the personal in charge of the Finca Rio Kama, i.e. Sergio Sanchez, Henry Hernandez, and Felix Rocha, for their help in the field, and to Manuela Bartel (Senckenberg, Dresden), for amplifying the DNA sample and for sending it for sequencing. Specimens were sent with permit no. DGPN/DB-27-2013 of the MARENA (Ministerio del Ambiente y Recursos naturales) of Nicaragua, for which we thank Dr. Carlos Mejia, Director of the Biodiversity Department. Lastly, we thank the Charles A. Triplehorn Insect Collection at the Ohio State University, Columbus Ohio, for the use of the Peterson egg collection.

## Editor's note

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# Peripheral eye dimensions in Longwing (*Heliconius*) butterflies vary with body size and sex but not light environment nor mimicry ring

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**Abstract.** This study tests if tropical forest butterflies occupying similar light environments converge on eye morphology to meet shared demands of visual sensitivity. Total corneal surface area and facet diameters were measured and adjusted to body size for four species of *Heliconius* (Lepidoptera: Nymphalidae) butterflies that belong to two mimicry rings that frequent different light environments. Total corneal surface area and facet diameter differed among species, but not between mimicry rings and light environment. *Heliconius cydno* had the largest corneal surface areas, *H. erato* had the second largest, while *H. sapho* and *H. melpomene* did not differ from each other. *Heliconius cydno* and *H. erato* had larger facets than *H. cydno* and *H. melpomene*. Facet diameter was not linked to either mimicry ring or clade. Males had larger corneas relative to body size than females, but facet diameter did not differ by sex. As predicted, facet diameter differed by region of the eye. Lastly, we found that larger eyes had more facets. While the eyes of *Heliconius* generally seem to be larger than those of similarly sized butterflies, the hypothesis that light environment affects eye morphology was not supported and the finding that neither mimicry ring nor phylogeny explains facet diameter is perplexing, but suggests that adaptation to contrasting light environments might be instead found in the physiology of the visual system.

**Keywords:** Cornea, Eye size, Facet counts, Facet diameter, Mimicry.

## Introduction

Many animals use vision to gather information about their surroundings (Lythgoe, 1979; Land & Nilsson, 2012). Their success in doing this depends on the match between their eye structure and the light available for visual processing. Irradiance, a measure of light available, is nine orders of magnitude greater on sunny days than on starlit nights (Johnsen, 2011). As expected, terrestrial species that live at the extremes of this continuum display very different eye structures with nocturnal animals showing features that enhance photon capture at the photoreceptors (Warrant, 2006; Frederiksen & Warrant, 2008; Johnsen, 2011; Land & Nilsson, 2012). These features include larger eyes and facets than found in their diurnal relatives (Greiner *et al.*, 2004; Greiner, 2005; Warrant *et al.*, 2006; Somanathan *et al.*, 2008; Frederiksen & Warrant, 2008). Moreover, nocturnal and crepuscular species typically have superposition eyes in which a rhabdom (the microvilli component of the ommatidium's photoreceptors) is illuminated by light from several facet lenses enhancing sensitivity at the expense of resolution (Swihart, 1969; Horridge *et al.*, 1972; Warrant, 1999; Warrant *et al.*, 2004; Kelber, 2006). In contrast, diurnal insects (e.g. all non-skipper butterflies) often have apposition eyes in which the rhabdom in an ommatidium is illuminated only by light from the facet lens at the distal end of that ommatidium. Apposition eyes are much less sensitive than superposition eyes because photons from only one facet are caught by the individual photoreceptors.

Light environments that differ by several orders of magnitude in overall brightness can clearly lead to differences in eye morphology (i.e. night versus day), but how different are the eye features of diurnal animals that occupy habitats with smaller differences in available light (e.g. deep shaded forest vs. open field)? In this study, we test if eye morphology differs

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among four related species of diurnal *Heliconius* (Kluk) (Lepidoptera; Nymphalidae) butterflies that occur in light environments that can differ in brightness by one order of magnitude (Papageorgis, 1975; Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). This difference in brightness is relatively smaller than are differences in brightness encompassed by previous studies. For example, Frederiksen & Warrant (2008) compared the eyes of butterflies that fly at dusk to those that fly at midday when there is 100 times more light.

The four unpalatable species of *Heliconius* we studied include representatives of two different mimicry rings that occur in central Panama, the postman ring (*H. erato* and *H. melpomene*) and the blue-white ring (*H. cydno* and *H. sapho*: Brown, 1981; Chai, 1986). These two rings of Müllerian mimics occur in different microhabitats that present different light conditions (Gilbert, 1991; Mallet & Gilbert, 1995; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). *Heliconius erato* and *H. melpomene* occur in more disturbed and open habitats, while *H. sapho* and *H. cydno* occur in established forest with full canopy cover (DeVries, 1987; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). Endler (1993) quantified the differences in brightness (quantum flux) of forest understory and large open gaps in tropical forest in Panama. Large gaps, where *H. melpomene* and *H. erato* occur, are an order of magnitude brighter and are richer in long wavelengths than forest understory, where *H. cydno* and *H. sapho* occur (Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data).

Do co-mimics share eye morphology that is adapted to shared environment and similar behaviors? Here, the results presented test the predictions that mimetic *Heliconius* butterflies that occur in darker environments (*H. sapho* and *H. cydno*) will have larger eyes and larger facets to improve sensitivity, while postman butterflies which live in more open environments will have smaller eyes and facets (Warrant, 2006). Note that the mimicry rings do not reflect phylogenetic relationships among these species (Brown, 1981; Kozak *et al.*, 2015; Figure 1). *Heliconius cydno* and *H. melpomene* are more closely related than *H. sapho* and *H. erato*. Hence, if recent common ancestry is an important determinant of eye morphology, it is predicted that eye morphology will be more similar within these pairs than among mimetic pairs.

Several patterns of variation in eye size and facet diameter in butterflies are known from previous studies (Ziemba & Rutowski, 2000; Rutowski, 2000; Merry *et al.*, 2006; Rutowski *et al.*, 2009). Eye size and facet diameter increase with body size, males typically have larger eyes than females, and facets in the frontal region of the eye tend to be larger than in other eye regions (Land, 1997; Rutowski, 2009). Hence, our analysis took into consideration both size and sex of all sampled individuals and included measurements from several eye regions.

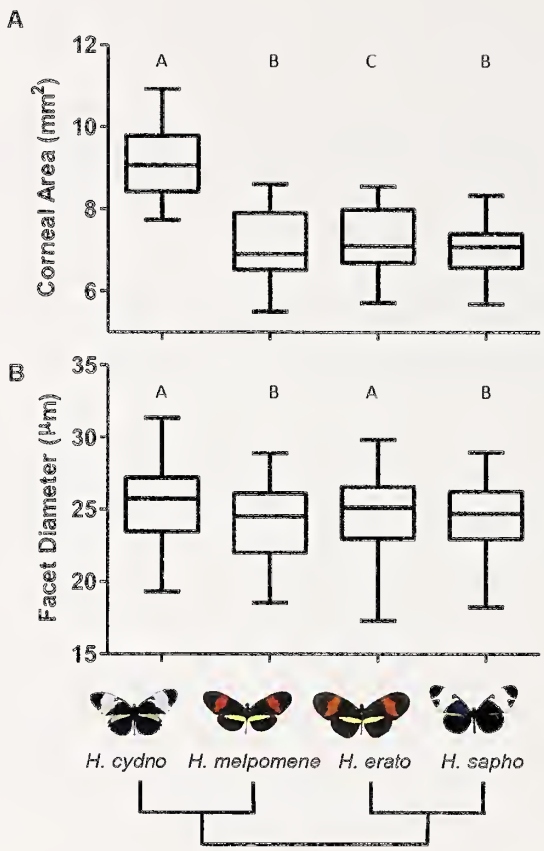


**Figure 1.** Interspecific differences in unadjusted eye morphology for the four *Heliconius* species studied. **A)** Absolute total corneal surface area. **B)** Mean absolute facet diameter. Letters (A, B, C) within each graph represent significantly different groups when controlling for body size. The data plotted here are not adjusted for body size unlike the statistical tests. Plots for each data set show the maximum and minimum values (upper and lower whisker, respectively), 1st and 3rd quartiles (top and bottom of box, respectively), and the mean (horizontal line within box). Phylogenetic relationships among these species are shown at the bottom (Brown 1981; Kozak *et al.* 2015). Note that *H. melpomene* and *H. erato* are found in brighter environments than *H. cydno* and *H. sapho*.

## Material and methods

### Specimen collection

Ninety-two adult *Heliconius* butterflies were collected for measurements in Parque de Nacional Soberanía in Panama from February to May 2013

**Table 1.** Sample sizes, body area measurements, and total corneal surface area for the *Heliconius* species studied. Means are given with standard deviations

| Species | Sex | N | Forewing (mm) | Femur (mm) | PC1 | Cornea ($mm^2$) |
|---|---|---|---|---|---|---|
| *H. cydno* | M | 12 | 39.6±1.76 | 4.57±0.34 | -1.17±0.87 | 9.45±0.90 |
| | F | 10 | 39.7±2.21 | 4.46±0.41 | -0.41±1.84 | 8.77±0.78 |
| *H. melpomene* | M | 12 | 34.8±3.19 | 4.26±0.37 | 0.32±1.07 | 7.32±1.03 |
| | F | 12 | 35.2±1.44 | 4.04±0.31 | 0.67±0.95 | 6.79±0.56 |
| *H. sapho* | M | 11 | 36.8±3.32 | 4.06±0.44 | -0.77±0.77 | 7.31±0.66 |
| | F | 12 | 38.8±1.51 | 4.34±0.27 | 0.67±.66 | 6.76±0.43 |
| *H. erato* | M | 12 | 32.5±2.26 | 3.61±0.30 | 1.37±1.09 | 7.27±0.93 |
| | F | 11 | 34.7±2.28 | 3.74±0.37 | 0.68±1.19 | 7.21±0.77 |

(Table 1). Adults with little wing wear were netted and then stored in glassine envelopes for transportation to lab facilities in Gamboa, Panama, where the butterflies were euthanized by freezing.

**Body size covariate**

As measures of body size we used hind femur length and forewing length of each individual measured with digital calipers to the nearest 0.01 mm (Rutowski, 2000; Rutowski *et al.*, 2009). Principal component analysis on these two measures revealed a first principal component that explained 90% of variation (hind femur length factor loading = -0.707; forewing length loading = -0.707). This component was used as a covariate representing body size in our analyses.

**Cornea preparation**

The head of each individual was severed from the thorax and the antennae, proboscis, and labial palps were removed. Following the methods of Ziemba and Rutowski (2000), the heads were soaked in 20% NaOH for 18 to 24 h to loosen the tissues behind the cuticular cornea. Once the soft tissues were removed, the cornea was cut along the dorsal-ventral axis and then laid flat on a microscope slide. A coverslip was placed over the cornea and then preserved and sealed with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI). These prepared slides were air dried for 24 h before being photographed.

**Total corneal surface area measurements**

Corneal squashes were photographed at approximately 20x magnification with a microscope (model MZM1, Askania Mikroskop Technik Rathenow, Germany) fitted with an OptixCam (Summit Series, The Microscope Store, Roanoke, VA) run with OCView Software (The Microscope Store, Roanoke, VA). A photograph taken of a micrometer scale was used to calibrate measurements made from other images. Total corneal surface area was measured by one observer in ImageJ with the lasso tool (Rasband, 2012); repeatability of these measurements was very high (intraclass correlation coefficient = 0.998).

**Facet diameter measurements**

Diameter of facets was measured in each of six regions of the eye: posterior, dorsal, anterior, anterioventral, ventral, and lateral (Figure 2). For these measurements, mounted corneas were photographed with the OptixCam attached to a compound microscope (Spencer Phase Star, American Optical, Hicksville, NY) at 100x magnification. The photographs were calibrated with a slide micrometer and all measurements were made within ImageJ. Within each region of each eye, distance was measured across ten facets in a row in two separate locations at least ten facets apart. The distance for each location was divided by ten to get an average facet diameter for each location. Then the two locations in each region were averaged to provide an average facet diameter for each region. As with total corneal

surface area measurements, one observer measured facet diameters and again repeatability was very high (intraclass correlation coefficient = 0.984).

### Facet counts

To further understand the eye morphology of *Heliconius* butterflies, the number of facets were counted for two individuals for each sex and species. Utilizing the total corneal surface area photographs, the cell counter plugin in ImageJ was used for counting the number of facets. We selected photos where all facets were easily countable.

### Statistical analyses

Body size principal components were calculated in R (R Development Core Team, 2008). All other tests were run in SPSS version 19 (IBM, Armonk, NY). Total corneal surface area was analyzed using a three-way nested analysis of covariance (ANCOVA). The covariate was PC1 of body size, the between factors were sex, mimicry ring, clade membership, and species. Species was nested both within mimicry ring and clade membership. Facet diameter was analyzed using repeated-measures ANCOVA. The facet diameters for each region of the eye were the within factor, and PC1 of body size served as the covariate. Sex, mimicry ring, and clade membership were the between factors, and again, species was nested within mimicry ring and clade membership. For both tests, post-hoc Helmert contrasts were implemented to determine differences among groups. All statistical inferences were made at the 0.05 level of significance.

## Results

### Total corneal surface area

As in other species of butterflies, total corneal surface area scaled positively with body size (ANCOVA, $F_{1,97}$=48.515, p<0.001; Figure 3) and males had larger eyes than females independent of body size ($F_{1,97}$=20.42, p<0.001; Figure 4). However, further Helmert analysis revealed that there was a significant difference between the sexes for total corneal surface area for *H. sapho* (p=0.004), and *H. cydno* (p=0.038), but corneal surface area did not differ by sex for *H. melpomene* (p=0.067) and for *H. erato* (p=0.332). Within each sex of each species there was a strong negative allometry in the relationship between eye size and body size, which means small individuals had relatively larger eyes compared to their larger counterparts (Figure 3).

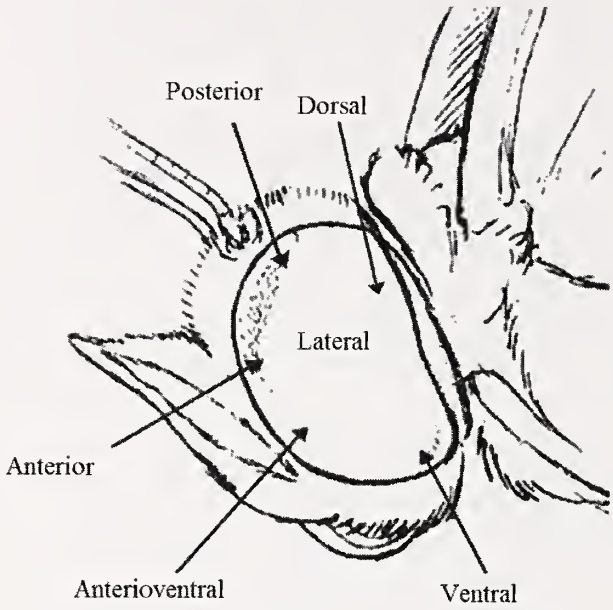


**Figure 2.** Eye regions in which facet diameter was measured. Figure modified from Rutowski (2000) and Merry *et al.* (2006).

Body-size-adjusted corneal surface area of *H. cydno* and *H. erato* were significantly different from each other and the other two species ($F_{3,47}$=46.365, p<0.001). Specifically, Helmert contrasts revealed that *H. cydno* had the largest eyes (p<0.001) while *H. erato* had the second largest (p<0.001; Figure 1). *H. sapho* and *H. melpomene* did not differ from one another and had the smallest eyes (p=0.064; Figure 1). Contrary to our prediction, there was no difference in total corneal surface area between the two mimicry rings ($F_{1,97}$=0.510, p=0.477) but the effect of clade was significant ($F_{1,97}$=40.394, p<0.001).

### Facet diameter

As expected from studies of other butterflies, facet diameters differed among eye regions (ANCOVA with Greenhouse-Geisser correction, $F_{3.95}$=210.39, p<0.001; Figure 5). Lateral facets were the largest, anterior and anterioventral facets were next largest in diameter; then facets became smaller from posterior to ventral to dorsal. Body size positively predicted facet diameter (ANCOVA, $F_{1,97}$=11.295, p=0.001; Figure 6), but facet size did not differ by sex ($F_{1,97}$=0.829, p=0.365), mimicry ring ($F_{1,97}$=0.001, p=0.970), or phylogeny ($F_{1,97}$=0.775, p=0.381). Facet size differed among species ($F_{3,47}$=7.438, p=0.001; Figure 1B). As with total corneal surface size, *H. sapho* and *H. melpomene* had similarly smaller facets (p=0.472) than *H. cydno* and

**Table 2.** Facet diameter by region of the eye as a function of species and sex. Means are given with standard deviations.

| | | | Facet Diameter (μm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Sex** | **N** | **Posterior** | **Ventral** | **Dorsal** | **Anterior** | **Lateral** | **Anterioventral** |
| *H. cydno* | M | 12 | 24.9±1.43 | 23.9±0.90 | 21.2±1.09 | 26.4±1.14 | 27.2±0.98 | 27.0±1.44 |
| | F | 10 | 24.6±1.48 | 25.3±1.51 | 21.1±1.18 | 26.9±0.78 | 27.5±2.05 | 27.7±0.64 |
| *H. melpomene* | M | 12 | 23.8±1.17 | 23.5±1.80 | 21.0±1.03 | 26.2±1.96 | 26.0±2.03 | 26.5±1.53 |
| | F | 12 | 23.9±1.29 | 22.3±1.50 | 20.9±1.75 | 24.9±1.79 | 25.9±1.23 | 24.3±2.34 |
| *H. sapho* | M | 11 | 24.6±1.04 | 23.9±1.25 | 20.8±1.69 | 25.8±0.93 | 26.2±1.23 | 25.5±1.93 |
| | F | 12 | 24.3±2.02 | 23.3±1.43 | 21.4±1.50 | 26.1±1.13 | 26.2±1.52 | 26.0±1.81 |
| *H. erato* | M | 12 | 23.4±1.50 | 22.8±2.15 | 21.5±1.79 | 26.7±1.18 | 27.1±1.42 | 26.3±1.98 |
| | F | 11 | 24.3±1.93 | 23.6±1.84 | 21.7±2.29 | 26.2±1.09 | 26.7±1.47 | 25.1±1.48 |

*H. erato,* which had the largest facet diameters and did not differ from one another (p=0.639). The data were suggestive of a three-way interaction of region by sex by species (ANCOVA, $F_{15,243}$=1.71, p=0.051). And as with total corneal surface area and body size, there was a strong negative allometry in the relationship between facet diameter and body size (Figure 6).

### Facet counts

Facet number was highly positively correlated with total corneal surface area ($R^2$=0.92 for males and $R^2$=0.73 for females; Figure 7). The largest corneas had the most facets and the smallest corneas had the fewest facets (Table 2). Males have absolutely larger eyes than females and therefore have more facets.

## Discussion

### Eye size varies with body size

Previous research has shown that eye size in Lepidoptera increases with body size (Yagi & Koyama, 1963; Rutowski, 2000; Rutowski *et al.*, 2009) and the *Heliconius* species examined here are no different. Here we found that larger *Heliconius* individuals have larger total corneal surface area and larger facets. However, we found the rate with which eye size changes with body size is much lower in *Heliconius* than reported for other butterflies (Rutowski, 2000; Figures 3 & 6). The very negatively allometric relationships between body size and eye size are unexpected and suggest selective pressures on *Heliconius* that favor development of large eyes regardless of body size. Regardless of the degree of allometry, eye performance is related to body size and depends on eye shape, facet number and facet size (Land, 1989; Land, 1997; Zollikofer *et al.*, 1995). Therefore, larger *Heliconius* butterflies should have increased sensitivity, acuity, larger visual fields or a combination of these characteristics (Rutowski, 2000; Frederikson & Warrant, 2008).

Interestingly, all of the *Heliconius* species we examined have a higher corneal surface area to body size ratio than that reported for other butterflies (Rutowski, 2000; Rutowski *et al.*, 2009). Rutowski (2000) found that the corneal surface area to body size ratio is close to 1:1 for 16 different species of butterflies with lower ratios of 1:2 and higher ratios of 11:10. Here we found corneal surface area to body size ratios greater than 2:1, indicating that *Heliconius* have the largest eyes relative to body size of butterflies studied thus far.

Larger total corneal surface areas could have several effects on vision including a larger visual field (ommatidia pointing in a larger number of directions), more acute and sensitive vision, or both. Visual field dimensions of butterflies are generally huge and do not change much with body size (Rutowski *et al.*, 2009). There is no reason to think this will not also be true for *Heliconius.* However, in *Heliconius* the number and diameter of facets do increase with body size. So, given no change in visual field dimensions, the increase in cornea size and in facet number should mean overall

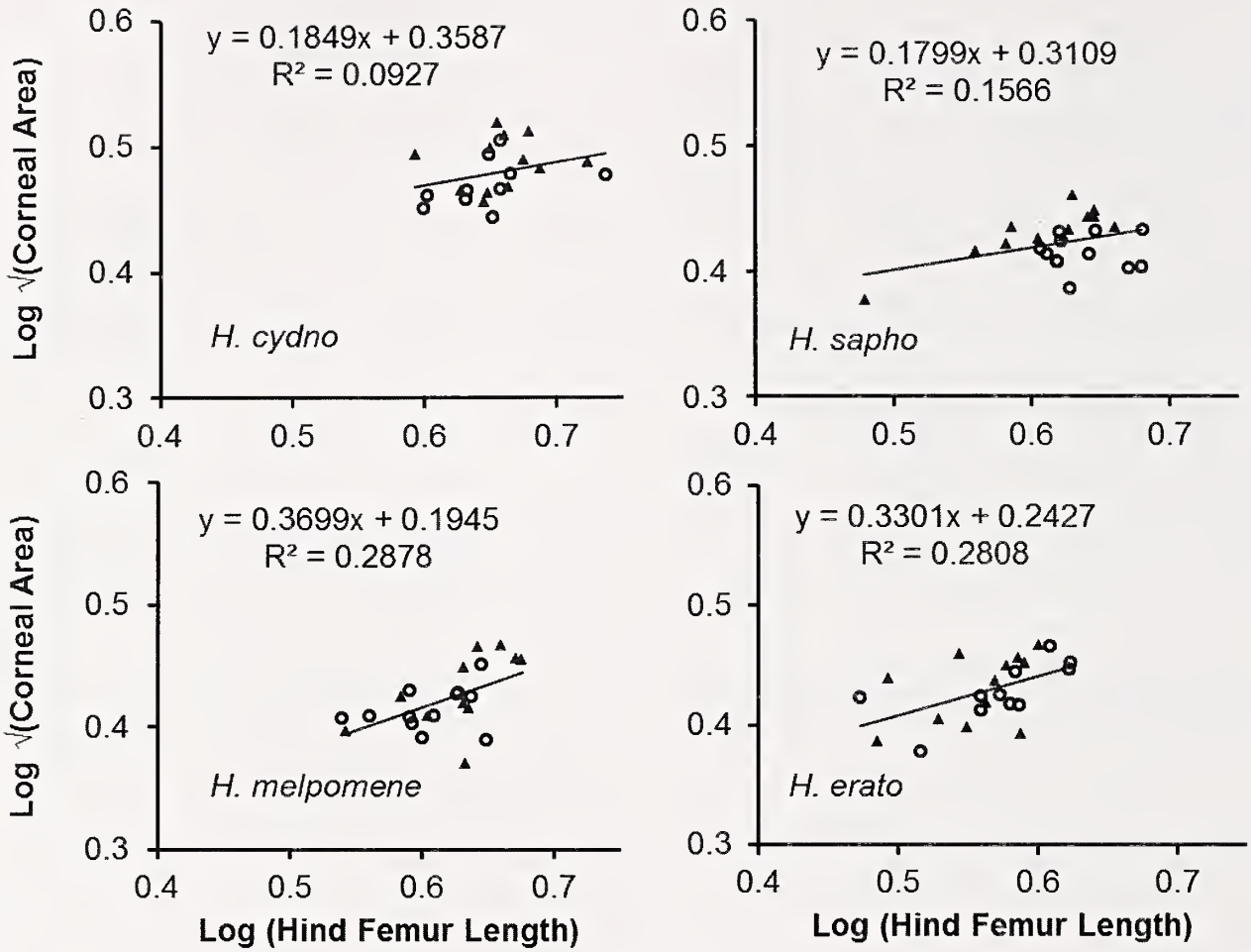


**Figure 3**. The relationship between eye size and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). The y-axis represents the log of the square root of the total corneal surface area and the x-axis represents the log of hind femur length. The double-logarithmic plot is used to determine if the relationship between total corneal surface area and hind femur length is allometric. A slope of 1 would indicate an isometric relationship between body size (hind femur length) and eye size (total corneal surface area). However, the slopes here indicate that eye size has a very negative allometric relationship with body size.

lower inter-ommatidial angles in larger eyes. Similarly, the increase in facet diameter with body size will mean a higher photon catch per ommatidium such that larger eyes should be more sensitive. Hence, *Heliconius* should have better low light vision than most other butterflies in the same body size range. What selective pressures might have driven this divergence is not clear. Perhaps it is that they frequent forest shade (i.e. low light) environments which makes visual detection and recognition tasks more demanding than those of butterflies in environments with higher light levels. Interestingly in Rutowski *et al.* (2009) the species examined in the *Heliconius* size range, *P. sylvia*, with its relatively smaller facets frequents open environments with high light levels.

**Blue-white males have larger eyes than females**

Previous studies showed that male Lepidoptera have larger corneas and facets than conspecific females (Yagi & Koyama, 1963; Ziemba & Rutowski ,2000; Rutowski, 2000; Lund *et al.*, 2001). Blue-white males had larger eyes than females when controlled for body size, but postman individuals did not differ in eye size between species. Why only blue-white individuals would have an intraspecific difference is intriguing because other studies hypothesize that male Lepidoptera have generally larger eyes as a result of the visual demands of finding mates (Yagi & Koyama, 1963; Rutowski, 2000).

### Facet diameter varies by region

The largest facets in butterflies are in the anterior regions of the eyes for maintaining flight and for locating and recognizing food resources, mates, and larval host plants (Land, 1997, Rutowski & Warrant, 2002; Rutowski, 2003; Rutowski *et al.*, 2009). We observed a similar pattern in the *Heliconius* species studied here but with large facets also in the anterioventral and lateral eye regions. Unlike in previous studies (Rutowski & Warrant, 2002; Rutowski *et al.*, 2009), there were no differences in facet diameters among the sexes or mimicry rings. This again supports the notion that vision may function similarly in males and females of *Heliconius* butterflies.

The lateral facets, located in the center of the cornea, are the largest for all four species, which contrasts with previous reports that largest facets in butterflies are found anteriorly and anterioventrally, most likely for locating and recognizing both host plants and mates (Merry *et al.*, 2006; Rutowski *et al.*, 2009). Large lateral facets may enhance processing of optic flow in flight, the pattern of apparent motion of elements in the visual scene as the observer moves (Srinivasan *et al.*, 2000). The greatest angular velocity of objects in the visual scene of a flying butterfly will be in the lateral regions and thus the lateral optical flow is most likely to suffer from visual blur which will be minimized when photon flux and signal to noise ratios are high. These conditions will happen when facets are large, such as they are in the lateral regions of the eye. Of course, this explanation warrants testing and further comparative research on compound eyes and optic flow is needed.

### Larger eyes have more but not larger facets

Very little is known about the relationship between eye size and facet number for the Lepidoptera. Ziemba & Rutowski (2000) found that although eye size differs between males and females in the butterfly *Asterocampa leilia*, the number of facets per eye was the same in males and females. Males of *A. leilia* have larger facets than females, which leads to a larger eye size without more facets. Unlike *A. leilia*, in *Heliconius* the sexes differ in the number of facets per eye. Furthermore, eye size correlates with facet number with similar negative allometry to body size as was found with corneal surface area and body size. Again, this negative allometry is likely due to selection for very large facets regardless of body size and because larger eyes have more facets instead of larger facets, a very negative allometric relationship would be predicted. This finding is comparable to what has been found in eusocial hymenoptera in which the larger the eye, the greater number of facets (Jander & Jander, 2002; Streinzer *et al.*, 2013).

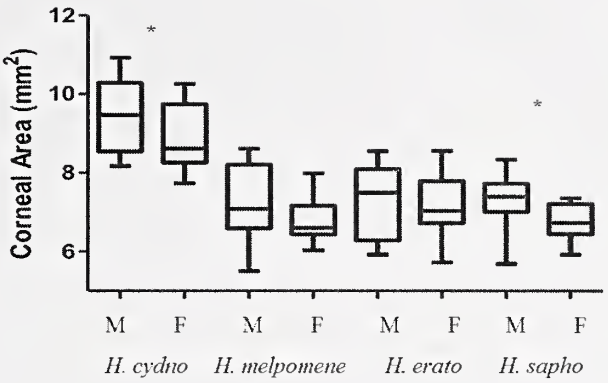


**Figure 4.** Absolute total corneal surface area for each sex of each species. See legend in Figure 1 for further details of the box-and-whisker plots. The asterisks mark intraspecific sexual differences that were significant at the 0.05 level.

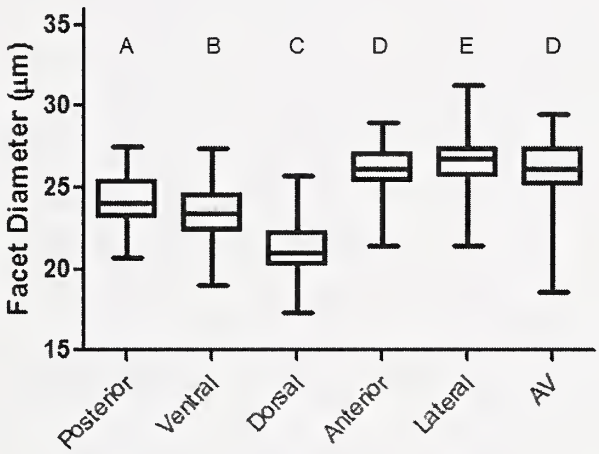


**Figure 5.** Mean facet diameter across different regions of the eye for all individuals of all species (n=92). Letters represent significantly different groups when body size is a covariate. Only anterior and anterioventral regions are not statistically different from one another.

### Eye morphology, mimicry ring and light environment

The predictions about the relationship between mimicry rings, which correspond to light environment, and eye features were not supported. One possible reason for this result is that differences in light

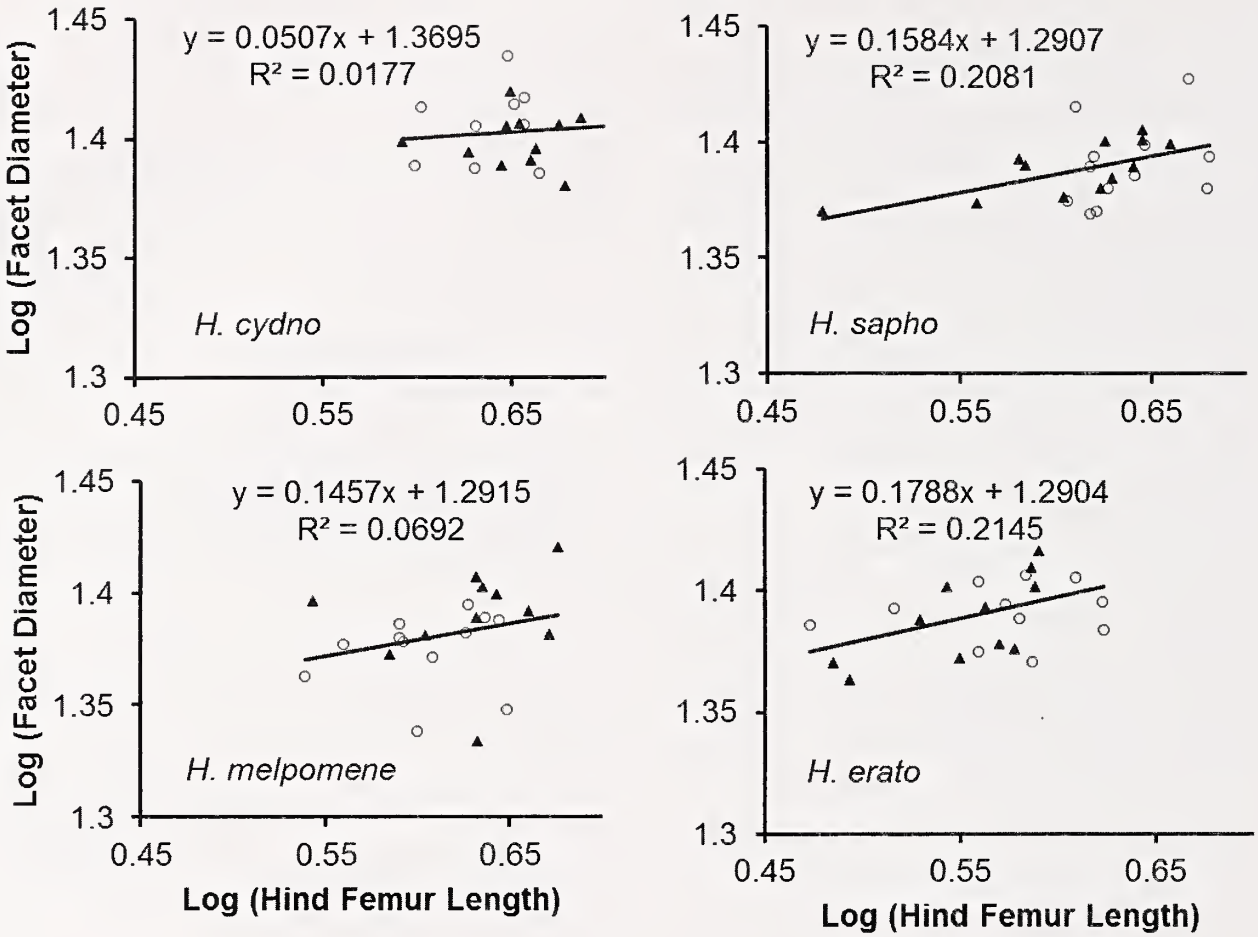


**Figure 6**. The relationship between facet diameter and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). See figure 3 for explanation of the double-logarithmic plots.

intensity where these species typically occur are too small to have shaped the peripheral features of eye morphology that we examined. Preliminary results from electroretinograms of these butterflies reveal that the blue-white butterflies that live in forest shade environments have greater absolute sensitivity (i.e. can see in darker environments) than the postman butterflies which live in very open environments (B. Seymoure *et al.*, unpublished). Because these two groups did not differ in the measures of eye structure reported here, physiological differences in eye performance between animals that live in different light environments are expected to be the result of differences in eye structure other than those measured here.

Apposition compound eyes can be rendered more sensitive through a pupil mechanism, by lengthening and/or widening the rhabdoms or through spatial and/or temporal summation of responses to dim light signals (Jonson *et al.*, 1998; Warrant *et al.*, 2004; Greiner *et al.*, 2005; Warrant, 2006; Land & Nilsson, 2012). In fact, Jonson *et al.* (1998) revealed that butterflies that occur in different light environments vary in pupil response with dim habitat species having a pupil mechanism that restricts photons entering the rhabdom in much dimmer environments than bright habitat species. Furthermore, Frederiksen & Warrant (2008) found that the crepuscular Owl butterfly (*Caligo memnon*) has four times the sensitivity of a similar sized diurnal butterfly that stems from not only increased facet diameters, but also wider rhabdoms and neural summation. Perhaps *Heliconius* individuals in darker environments have similar features that increase sensitivity. This is currently under investigation in our lab (B. Seymoure *et al.*, unpublished).

This work reveals several potentially fruitful research directions into the visual ecology and behavior of *Heliconius* butterflies. This study only investigated the eye morphology of four of the 44

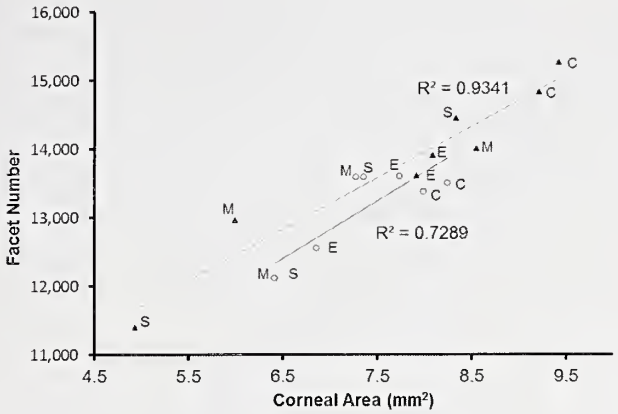


**Figure 7.** Relationship between facet number and total corneal surface area ($mm^2$) for selected *Heliconius* males and females. Letters near data points represent species: S = *H. sapho*, M = *H. melpomene*, E = *H. erato*, C = *H. cydno*. Lines represent least squares regression for males (closed triangles) and females (open circles).

*Heliconius* species and further *Heliconius* research is needed to understand why these species differ drastically from other butterflies and the role of ancestry in eye morphology. Furthermore, to understand how light environment has affected compound eye morphology, compelling studies could include phylogenetically-controlled comparisons of eye structure of diurnal species that differ in the light environments where they tend to occur. Such studies might also include a larger array of eye features including inter-ommatidial angles, visual field dimensions, pupillary responses, rhabdom lengths as well as physiological recordings such as electroretinograms or intracellular recordings. Such studies are currently underway in our lab and will shed light on the nature and tuning of visual adaptations in insects that occur in diverse light environments.

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## Disclosure

The authors have no conflicts of interest, including specific financial interests, relationships and affiliations relevant to the subject of this manuscript.

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