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The hilltopping mating system of the duskywing skipper *Erynnis tristis* (Lepidoptera: HesperIIDae)

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Abstract. Males of *Erynnis tristis* (Boisduval, 1852) perch at selected locations on a hilltop in central Arizona where they wait for the arrival of receptive females. They leave their perches to engage rival males in chases and, occasionally, in more elaborate spinning, descending flights in which one male appears to force his opponent down toward the ground. This latter behavior has not been previously recorded for *E. tristis* although it is convergent with that of a territorial hilltopping tachinid fly found at the same location. The nature of male-male interactions and the tendency of at least some individual butterflies to remain for some time at a particular perch, or small set of nearby perches, is consistent with male hilltopping territoriality, which has been reported for *E. tristis* at other locations. Although some males do return to the peaktop over periods of as much as three weeks, they do not exhibit fidelity to a particular territory unlike males of many other hilltopping insects at the same location. Moreover, they also differ from most hilltopping insects at this location in frequently choosing to perch in places other than on plants growing on the highest points of the ridge.

Key words: *Erynnis*, hilltopping, mating system, site fidelity, territoriality.

INTRODUCTION

Although the mating systems of a considerable number of butterflies are now known (Thornhill & Alcock, 1983; Dennis & Shreeve, 1988; Rutowski, 1991), skippers (HesperIIDae) remain relatively little studied, but see Shields (1967), Scott (1973), Shapiro (1977), Pivnick & McNeil (1985), Alcock (1988), and Ravenscroft (1994). One mating system that has been well documented for a few hesperIIDs is hilltopping in which males go to conspicuous landmarks where they wait for receptive females to arrive (e.g., Shields, 1967; Skevington, 2008). Among the hilltopping species is the duskywing skipper *Erynnis tristis* (Boisduval, 1852) (Shields, 1967; Bailowitz & Brock, 1991). Shields (1967) documented that males of this species defend

territories on hilltops in California and that females visit these sites to mate. This paper describes the natural history of *E. tristis* at an Arizonan hilltop. The data presented here are compared with those collected by Shields (1967). In addition, the mating system of *E. tristis* is contrasted with that of other hilltopping insect species with which the skipper co-exists in central Arizona.

METHODS

The main study of *Erynnis tristis* began on February 24 and ended a month later on March 24, 2009. This work was stimulated by casual observations and occasional records made over the preceding three years at Utery Mountain, north of Mesa, AZ. The study site was a peaktop (elevation about 900 m) in Upland Sonoran Desert habitat (33° 30' 08" N and 111° 38' 30.4" W) used by many species of insects as a mate rendezvous location (Alcock, 1987; Alcock & Dodson, 2008).

During the month long study in 2009, the peak was visited for between 1.5 and 3 hr on 16 days. On these days, *E. tristis* was found perching on plants along a portion of an undulating hilltop 275 m in length. This section of the ridge was censused several times by an observer during each visit to the peak. A record was kept of the plants occupied by the butterflies.

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In addition, during the month, 38 butterflies were captured in an insect net, marked distinctively on the wing or wings with DecoColor® paint pens, and released. (For 36 of these, I checked the underside of the hindwing for the small but conspicuous white marginal spots that are characteristic of *E. tristis* (Bailowitz & Brock, 1991); in all 36 cases, the spots were clearly present.) All marked individuals were captured on their perches and were therefore assumed to be males (Shields 1967). When marked individuals were resighted, their location was noted. These records can be compared with those made by Shields (1967) on a Californian population of *E. tristis*, especially with respect to the existence of favored perching sites, the extent of site fidelity at hilltops, and the period over which known individuals returned to a particular hilltop.

In addition to records made in the spring of 2009, small numbers of males were observed on four days from 10 to 19 September 2009 at the ridgetop during roughly hour-long periods in mid-afternoon.

All means are presented \pm 1 S.D.

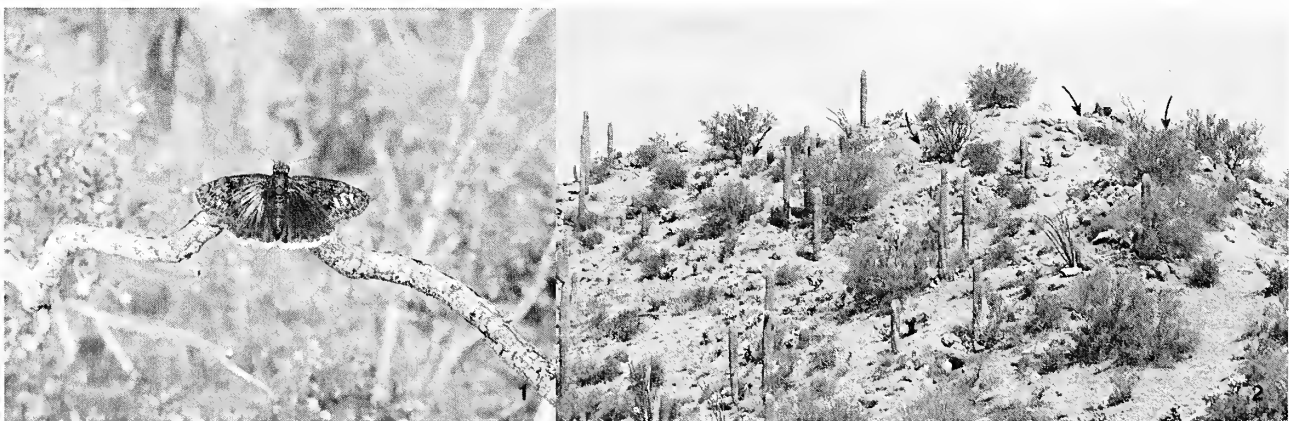
RESULTS

During the spring study in 2009, presumptive males were found perched (Fig. 1) on plants growing near the ridgeline from as early as 1055 A.M. (Mountain Standard Time) to as late as 450 P.M. In the preceding four years, *E. tristis* was recorded as present from as early as 1000 A.M. to as late as 430 P.M.

While at the peaktop, males perched on a wide range of shrubs, trees, and cacti. The species chosen included jojoba (*Simmondsia chinensis* (Link)

Schneider), creosote (*Larrea tridentata* (De Candolle) Coville), foothills paloverde (*Parkinsonia microphylla* (Torrey) Rose and I.M. Johnston), both living and dead buckhorn cactus (*Opuntia acanthocarpa* Engelmann & Bigelow), ocotillo (*Fouquieria splendens* Engelmann), and barrel cactus (*Ferocactus wislizenii* (Engelmann) Britton and Rose). One male also used a prominent boulder (about 1.5 m high) as his perch. Although the foodplants of *E. tristis* have been reported to be various species of oaks (Bailowitz & Brock, 1991), no species of *Quercus* has been recorded in the Utery Mountains to date (see <http://swbiodiversity.org/seinet>).

A total of twenty-three perch "sites" utilized by *E. tristis* were distributed along the 275 m census strip along the ridge that constitutes the top of Utery Mountain. A "site" was defined as an area of about 5 m in diameter containing from one to four nearby plants used by perching males. Seven of these sites were not located on the backbone of the ridge but were instead up to 8.5 m from the highest adjacent point on the ridge on the southfacing downslope (Fig. 2). Even those on the backbone of the ridge were often placed below nearby points of higher elevation. The mean distance between the generally well-spaced sites was 9.3 ± 8.8 m. Perching males were usually more than 10 m apart given that no more than 11 sites attracted a male on any one day during the study. The mean height of the perches themselves was 0.95 ± 0.33 m for a sample ($n=25$) of one or two perches per site. Some marked individual males moved from one plant to another within or between sites during the observation period; others restricted themselves to one or a very few neighboring plants where they remained for at least 55 min in five cases.



Figures 1 and 2. 1. A wing-marked male of *E. tristis* on a creosotebush in its territory in early March 2009. 2. A portion of the ridge that constitutes Utery Peak. Perches used by territorial males of *E. tristis* are indicated by arrows. Note that these plants were not located on the highest adjacent portion of Utery Peak.

Certain sites were far more likely to be occupied on any given day by a perching male than others. Over the 16 days of the study in 2009, three sites were occupied on at least 13 days; in contrast, seven sites were taken on just one or two days. The single most popular site in spring 2009 was also occupied in 2006, 2007 and 2008. During these three preceding years, plants in a total of 10 sites were recorded as being occupied by perching duskywings; eight of these were also utilized by perching males in the month long spring study in 2009.

When a male was present at his perch, he regularly launched flights of variable duration that took him out and around the shrub or tree before he returned (usually) to land on or near the spot where he had been prior to the flight. These flights occurred at a rate of 5.2 ± 1.8 per 5 min ($n = 17$ males observed on four days between March 15 and May 12, 2009). The vast majority (92%) of all recorded out and back flights ($n=88$) lasted less than 30 sec. In addition to apparently spontaneous flights, males also left their perches in pursuit of passing butterflies of their own and other species. When two male conspecifics interacted, the "resident" male usually chased after the "intruder" in a horizontal or gradually ascending flight that covered many meters. On other occasions ($n=7$), however, the two males quickly converted an ascending pursuit flight into a spinning descending flight in which the two individuals appeared to turn rapidly about one another at very close range. One male appeared to get above the other as the spinning pair descended until the lower butterfly was close to the ground, where it sometimes landed. At some point, the lower individual ended the interaction by flying rapidly away from the other male, which pursued the departing butterfly as it left.

Some of the males captured and marked on Usery Peak returned on one or more days subsequently. Of the 38 marked males, 15 (39%) were seen again on the peaktop on another day. The mean interval between first and last sighting for this sample was 8.5 ± 5.7 days. Site-specific fidelity was not strong; all 15 returning males perched in more than one site during the time they were observed on the peak with a mean number of 2.5 ± 0.6 sites taken. (The mean total number of days on which returning males were seen on the peaktop was only 2.9 ± 0.9 .) Thus, males were not strongly wedded to one perch or a few adjacent ones.

In 2009, hilltopping *E. tristis* were seen during five months from February through May, and again during September. In 2006, the species was seen on Usery Peak in August and October as well. Thus, the species appears to have two lengthy flight seasons at

this location separated by the very hot, dry months of June and July.

During the limited observations made in the mid-afternoon during September 2009, only a few males appeared. These individuals occupied a total of six sites, five of which had been occupied at least once during the spring study period. The most frequently taken territory in this brief fall study was the same one favored during the spring of the year. The site fidelity of males to their perches appeared to be very low, given that all attempts to watch perched individuals for 5 min each ended with the abandonment of the site by the observed male after a mean of 3.2 ± 1.0 min ($n=6$ males observed on three different days).

One mating pair was observed along the ridgetop in the afternoon of March 11, 2005.

The only congener of *E. tristis* seen on Usery Peak during the study was *Erynnis meridianus* Bell, 1927, which appeared on the hilltop on four days between 12 and 17 September 2009 between 1040 A.M. and 330 P.M. Three individuals were marked; one was seen on the day after marking at a site at the other end of the transect from where it had been captured and paint-marked on the wing. No more than three sites were held by this species during any one observation period of about an hour.

The behavior of *E. meridianus* was very similar to that of its congener. The males perched on shrubs growing along the ridge that constitutes Usery Peak. The plants chosen were a creosotebush, three different jojoba bushes, and dead staghorn cactus skeleton. The cactus skeleton had been frequently taken by *E. tristis* earlier in the year. Four individuals were watched for 5-min periods during which the presumptive males made no more than two flights out and back to their perch. Two of these flights were obviously triggered by a heterospecific butterfly flying near the perch. Two other cases of apparent male-male conspecific interactions took place, both involving ascending flights by the participants.

DISCUSSION

The mating system of *E. tristis* in central Arizona is very similar to that recorded for this same species in California (Shields 1967). Hilltopping males defend perches on plants from which vantage points they can presumably detect incoming females, although male-female encounters were much less often seen at Usery Peak than at the Californian hilltops where Shields (1967) observed 18 mating pairs of *E. tristis*. While waiting for females, males in both locations perched for variable periods from the middle of the day to the late afternoon on exposed branches and twigs of

shrubs. Shields (1967) recorded a maximum stay of 45 minutes; one male in the current study stayed at his site for at least 70 min. At both locations, some marked males returned to defend a perch or perches over a period of days. Returning males constituted only 11% of the sample in Shields (1967) study; at the Arizona site, resightings were considerably more common with returning males making up nearly 40% of the marked and released sample. Perhaps this difference derives from the fact that the Arizona hilltop was visited more often by an observer than the California hilltops. In keeping with this possibility, Shields (1967) recorded a maximum interval of four days between marking and resighting whereas three marked males at the Arizona site were seen from between 16 and 21 days after marking. Both the Arizona and California hilltops attracted perch-defending males year after year (for five consecutive years in Arizona and three years in the California study).

Shields (1967) noted that aggressive interactions with conspecifics were uncommon in the hilltopping *E. tristis* that he studied. He apparently did not observe the “spinning” descending male-male fights that occur on Utery Peak. These latter flights are highly convergent in form with the aerial interactions of the territorial tachinid fly *Leschenaultia adusta* (Loew 1872) (Alcock & Kemp, 2006). In both the butterfly and the fly, some males appear to be forced to the ground by their opponent. In contrast, males of many other hilltopping butterflies engage in elaborate ascending flights when challenged by a determined intruder. On these occasions, the two rivals spiral about one another as they fly more or less straight up into the sky. Sometimes, males of *E. tristis* also pursue intruders upwards but in a more gradual ascent that lacks the wing clashing element seen in species like the nymphalid *Chlosyne californica* (Wright, 1905) (Alcock, 1984) and various swallowtails (Rutowski *et al.*, 1989).

Another difference between the behavior of *E. tristis* and some other hilltopping butterflies lies in its selection of landmark perches. Species like the hairstreak butterflies *Atalides halesus* (Cramer, 1777) (Alcock, 1983) and *Strymon melinus* (Hübner, 1818) (Alcock & O’Neill, 1986) launch their territorial flights from elevated perches in prominent foothill paloverdes and creosotebushes growing right on the backbone of the mountain ridge at its higher points. These same perching plants are used by a host of other hilltopping insects (e.g., Alcock, 1981; Alcock & Dodson, 2008). Many other hilltopping insects, ranging from the butterfly *C. californica* (Alcock, 1984) to various flies and wasps perch on the bare ground just a few meters from these prominent plants. In

contrast, *E. tristis* rarely perched in any paloverde and never did so in the taller trees and other plants atop the ridgeline. Instead, the skipper favored low shrubs or small skeletal staghorn cacti, which often were several meters downhill rather than on the backbone of the ridge.

Yet another difference between males of *E. tristis* and the plant-defending hairstreaks has to do with the site fidelity of territorial males. When a marked male of *S. melinus* was resighted, there was a nearly 80% chance that it would be found in the tree where it had been seen previously (Alcock & O’Neill, 1986). Likewise, prolonged occupation of a particular tree was commonplace in *A. halesus* with some individuals holding the same tree for periods in excess of a week (Alcock, 1983). Although many males of *E. tristis* were seen on more than one day on the peak, they regularly shifted from one perch site to another, even on the same day.

The distinctive behavior of *E. tristis* provides support for the observation of Rutowski (1991) that the various components of butterfly mating systems, such as perch site selection, site fidelity and contest behavior, can be considered separately in terms of their adaptive significance. This approach leads to a number of questions about the mating system of *E. tristis* that will require additional research. For example, do males of *E. tristis* use perching plants ignored by other species as a means of reducing the number of mistaken pursuits of other species, chases that require the expenditure of time and energy? Do males shift perch sites relatively frequently because arriving females are more evenly distributed along the ridgetop than are the females of most other hilltopping butterflies, and therefore males gain less by staying on perches in a very few of the most visually prominent landmark plants? And finally, why have males of *E. tristis* (and a few other hilltopping species) evolved descending flight contests when so many other hilltoppers exclusively engage in ascending spiral flights when in territorial disputes?

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NOTE

Natural epizootic of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson infecting *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila México

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), is a common pest in corn and other crops (Lu & Adang, 1966). It is a pest of major economic importance capable of greatly reducing crop production rates (Carnevali & Florcovski, 1995). This insect has 5 to 6 larval instars which feed on tender leaves and stalks causing severe damage in every stage of plant development (Villa *et al.*, 2005; Wiseman *et al.*, 1966). FAW has been reported to be susceptible to more than 20 species of entomopathogenic fungi (Gardner & Fuxa, 1980; Sanchez-Peña, 2000). One of these is *Nomuraea rileyi* (Farlow) Samson, family Moniliaceae (Lezama-Gutierrez *et al.*, 2001) causing epizootics as observed in this study during August and September 2007 in non-irrigated corn plots in Buenavista, Coahuila. The experimental field is located 25°23'N, 101°00'W at an altitude of 1743 m above sea level with maximum average temperatures of 29°C and 86% relative humidity. The infected larvae were covered with a whitish fungus, later turning greenish-gray and identified as *N. rileyi* according to the morphological traits described by Ignoffo (1981). Temperature, relative humidity and rainfall measurements were obtained with the meteorological station of Universidad Autónoma Agraria Antonio Narro, located 100 m from the experimental site. FAW populations were observed within a 0.2-0.6 ha area. Samples were collected from corn leaves by finger tapping the plants recording the number of infected larvae. Putatively infested larvae were observed under the microscope to confirm *N. rileyi* infection.

Experimental corn plots had an average infestation of 1.2, 6.0, 4.1, 0.4 and 0.0 *N. rileyi*-infested larvae per meter in June, July, August, September and October

2007 respectively (Fig. 1). Populations of *S. frugiperda* decreased immediately after the samples taken in July; by September, the few larvae found on corn leaves infected with this entomopathogen were already dead. The larvae were fully covered with whitish hyphae (Fig. 2a and b) and a green mass of *N. rileyi* spores (Fig. 2c). Conidias as well as phialides were also observed (Fig. 2d). This research was focused on infected larvae during the growing stage of the corn plant, particularly at the corn boot formation. The largest number of dead larvae found was 6 larvae per linear meter in July. Larvae killed by *N. rileyi* were removed from the upper side of the leaves and examined at the laboratory and cultured in Petri dishes with Potato-Dextrose-Agar (PDA) and V8-Agar (V8A). Based upon the microscope observations of the characteristics of the infected larvae as well as slide observations, the fungus was identified as *N. rileyi* as reported by Barnett (1989).

Average maximum and minimum temperatures during the months of this study (June-October, 2007) was 28.2 and 13.2°C, averaging 20.65°C and a relative humidity average of 76.25% (Fig. 1). Sunlight hours

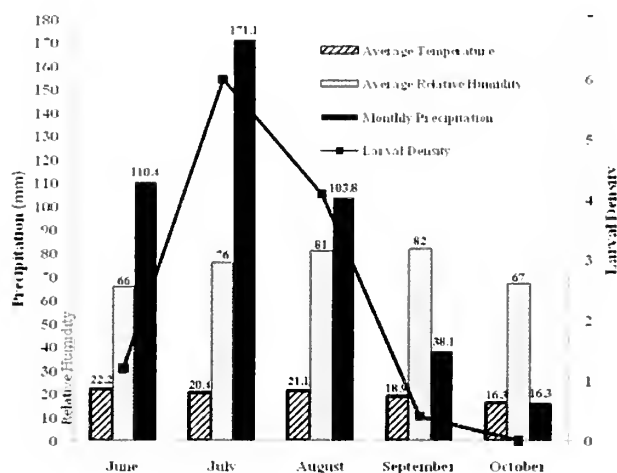


Figure 1. Natural occurrence of *Nomuraea rileyi* on *Spodoptera frugiperda* larvae in Coahuila, Mexico in 1997, correlated with precipitation, temperature and relative humidity.

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Figure 2. *Spodoptera frugiperda* larvae covered with a) and b) whitish mycelia of hyphae, c) mass of green spores *Nomuraea rileyi* attached on corn leaves and d) fungus conidia and phialides.

were between 2.1 to 8.4 indicating mostly cloudy days. The total number of rainy days and the amount of rainfall from June to October were 41 and 439.7 mm respectively (Fig. 1). These environmental conditions favored the growth and natural infective power of the fungus, as previously reported by Vimala-Devi (1966). Infectiveness and pathogenesis of *N. rileyi* is influenced by environmental conditions, mainly humidity which is the main requirement for conidia germination and fungus survival (Ignoffo & Garcia, 1985). The temperature range was also ideal for *N. rileyi*'s thriving and consequent unleashing of the infection (18 to 25°C). Temperatures over 35°C usually inhibit the development of this entomopathogenic fungus (Edelstein *et al.*, 2005).

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Constant eyespot display as a primary defense – survival of male and female emperor moths when attacked by blue tits

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Abstract. Large conspicuous eyespots, commonly found on the wings of butterflies and moths, have been shown to thwart attacks from predators. Previous experiments have focused on lepidopteran species that expose eyespots only when harassed by a predator. In contrast, we investigate the potential efficiency of the constantly exposed eyespots of emperor moths thus constituting a primary defense. We staged experiments between blue tits and moths having either intact or painted over eyespots. Moths with eyespots were killed as often as moths without eyespots and were, additionally, approached earlier by the birds suggesting that birds were not intimidated by their eyespots. Female moths weighed three times more than males and were less often eaten, suggesting that their large size intimidated the birds. We suggest that the constant eyespot display of the emperor moth may be associated with a cost, because potential predators seem to be attracted rather than intimidated by the display.

Key words: Eyespots, predation, anti-predator behavior, emperor moth.

INTRODUCTION

Through the pressure of predation, prey animals have evolved a variety of traits that permit them to avoid detection and subsequent attack by predators. Edmunds (1974) divided these adaptations into primary defenses, having the purpose of decreasing the risk of being attacked in the first place, and secondary defenses that operate during actual encounters with a predator. Examples of primary defenses include classical concepts like crypsis, aposematism and mimicry (e.g. Cott, 1940; Endler, 1981; Ruxton *et al.*, 2004), and secondary defenses include, for example,

different flight behaviors, various forms of retaliation such as stings and toxins, and also different forms of intimidating or deimatic behaviors (e.g. Humphries & Driver, 1971; Edmunds, 1974).

A trait often associated with intimidation is large and conspicuous eyespots that, to a human observer, resemble the vertebrate eye (Poulton, 1890; Cott, 1940; Blest, 1957; Ruxton, 2005). Eyespots can be conveniently defined as the presence of concentric rings of contrasting colour surrounding a central pupil (Kodandaramaiah *et al.*, 2009); it is a trait that is common on the wings of butterflies, moths and other insects, and also occurs in other animal groups, such as tropical fish, frogs, and birds (Cott, 1940; Blest, 1957; Edmunds, 1974). Large conspicuous eyespots on the wings of Lepidoptera have been hypothesized to function in two different ways (1) either by intimidating predators by creating the illusion that the predator's own enemy has suddenly appeared (the "intimidation hypothesis") or (2) or by being inherently intimidating due to their conspicuous and contrasting features (the "conspicuous signal hypothesis"; Poulton, 1890; Cott, 1940; Blest, 1957; Tinbergen, 1958; Ruxton *et al.*, 2004; Stevens, 2005, 2007; Stevens *et al.*, 2007). Although these hypotheses have been considered for over a century, support for these ideas are largely lacking (Ruxton *et al.*, 2004; Stevens, 2005), and only recently have the eyespots

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of the edible peacock butterfly (*Inachis io*) and the peacock pansy (*Junonia almana*) been shown to thwart predator attacks (Vallin *et al.*, 2005, 2006, 2007; Kodandaramaiah *et al.*, 2009). Recent field studies on spotted artificial prey have indicated that spot pattern features such as larger size and high internal pattern contrast are important for reducing predation, whereas spots occurring in pairs are no better than three spots or a single spot if they occupy the same total area (Stevens *et al.*, 2007, 2008a).

Large and conspicuous eyespots on the wings of butterflies and moths can either be hidden from view when the insect is resting, as in *I. io* and the eyed hawkmoth (*Smerinthus ocellatus*) or they can be displayed at all times, including when the insect is resting, as in the emperor moth (*Saturnia pavonia*). In the former case, the large conspicuous eyespots are exposed suddenly, only when a predator comes near, and conceivably can create a startling effect that effectively thwarts predator attacks, and hence represents a secondary defense (Blest, 1957; Edmunds, 1974; Ruxton *et al.*, 2004; Vallin *et al.*, 2005). It appears, however, that the antipredator efficiency of suddenly exposed large eyespots may be contingent on the behavior of the insect: when attacked by a bird the peacock butterfly suddenly opens and closes its wings in a repeated sequence and also tracks the movements of the attacking bird which creates the impression that the butterfly is actively defending itself. This antipredator behavior is very effective and recent experiments showed that 43 out of 44 peacocks with intact eyespots survived attacks by blue tits (*Cyanistes caeruleus*) (Vallin *et al.*, 2005, 2006). When the hawkmoth *S. ocellatus* is attacked it suddenly lifts its cryptic forewings thereby exposing the two large eyespots on the hind wings, and then moves rhythmically up and down continuously displaying its eyespots. This antipredator behavior seems to be less effective and experiments have shown that only 6 out of 27 eyed hawkmoths survived attacks from blue and great tits (*Parus major*) when the bird and lepidopteran interacted during 30-minute trials (Vallin *et al.*, 2007).

The conspicuous eyespots on the wings of a resting *S. pavonia* are constantly exposed and so cannot exert a startling effect, but when attacked the moth raises its forewings thereby exposing the hind wings which also bear eyespots that are identical in size and shape to those on the forewings (Fig. 1). Hence, the eyespots on the forewings represent a primary defense potentially deterring predators from attacking, whereas the sudden exposure of the eyespots on the hind wings represents a secondary defense that may make the predator abort its attack in progress

(Cott, 1940; Ford, 1955; Edmunds, 1974). Recently, the constant exposure of large conspicuous eyespots on the wings of *J. almana* were shown to elicit fear symptoms in attacking Great tits (*Parus major*) in an experiment, and when choosing between attacking a mounted butterfly with its eyespots intact or painted over, the birds attacked the eyespotless butterflies more often (Kodandaramaiah *et al.*, 2009). However, the combined effect of eyespots as a primary, and a secondary defense has never before been tested in a living lepidopteran.

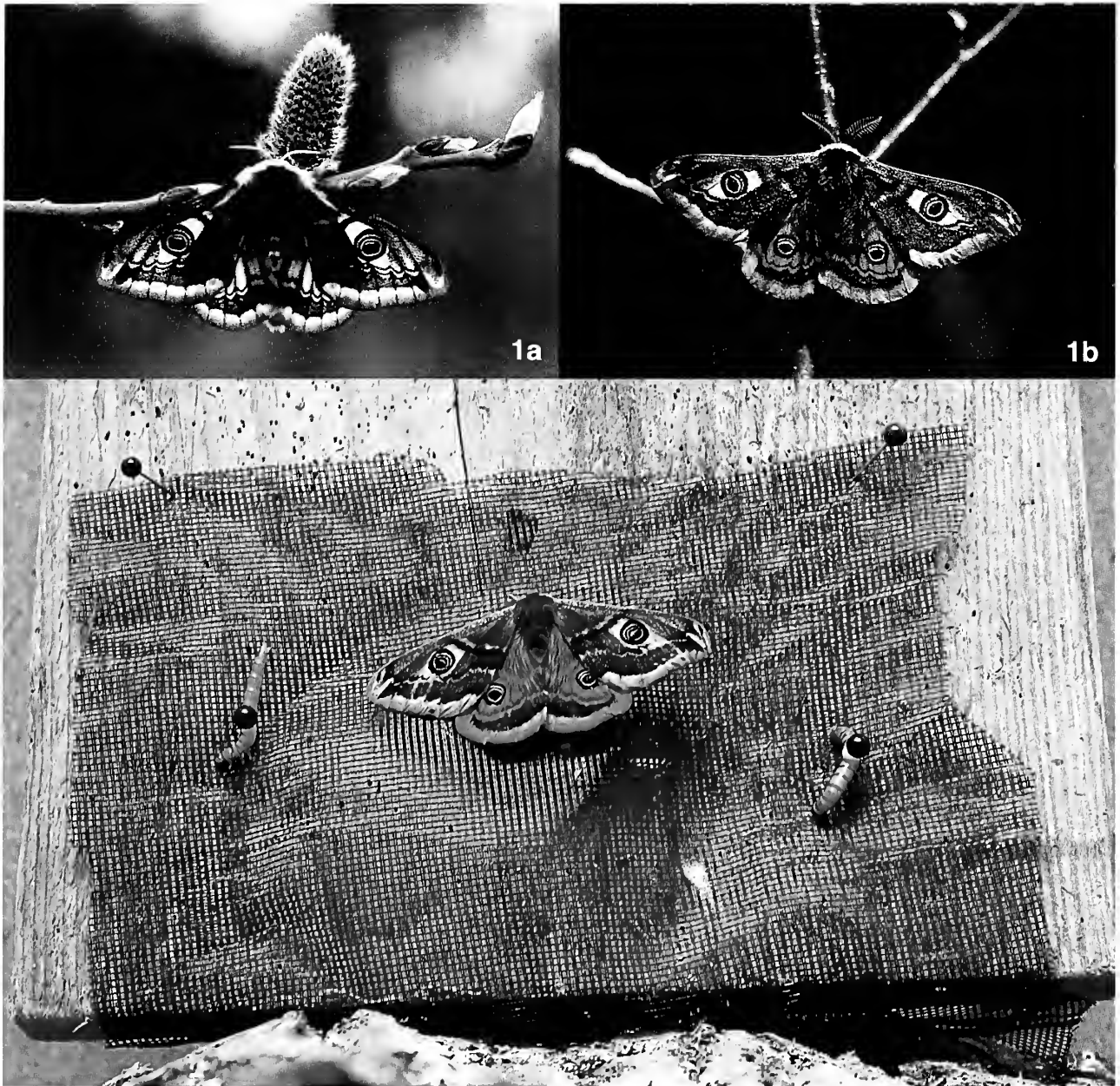
Another factor that is relevant when considering interactions between predators and potentially intimidating prey is the size of predator relative to prey. Indeed, Tinbergen (1958) pondered the possibility that an insect as big as *S. ocellata* might be too big a food item for a small passerine, and female *S. pavonia* are just as big as *S. ocellata*.

Hence, it is conceivable that large size of the insect prey itself can to some degree exert an intimidating function. In this context it is noteworthy that *S. pavonia* is sexually dimorphic both with respect to size, the males are approximately three times smaller than the females (see results), and whereas the hind wings of females are the same color as the forewings the male hind wings are bright orange red. This means that if size is an intimidating factor, female *S. pavonia* would be better defended against small passerines than males, whereas the opposite effect would be expected if red coloration exerts a strong warning effect, red coloration being typically associated with aposematic defense in insects (Ruxton *et al.*, 2004).

In this paper we address two issues: (1) whether the constantly displayed eyespots on the wings of *S. pavonia* thwart attacks by blue tits; we do this by staging experiments with living birds and moths that are either un-manipulated, have their eyespots painted over, or have been sham-painted on the basal parts of their wings leaving their eyespots intact, and (2) whether large size of the insect prey, or red hind wing coloration, can be effective as an anti-predation device; we do this by presenting the birds either male or female emperor moths.

METHODS

The two species used in these experiments, the prey, the emperor moth (*Saturnia pavonia*), and the passerine predator, the blue tit (*Cyanistes caeruleus*), both have a Palearctic distribution and so are largely sympatric (Rougeot & Viette, 1980; Perrins & Snow, 1998). Blue tits are opportunistic predators feeding on both seeds and insects; however, they feed their young exclusively insects which implies good insect



Figures 1-2 **1a**. A female emperor moth (*Saturnia pavonia*), in its resting posture constantly displaying the two conspicuous eyespots on the forewings. **1b**. A male emperor moth that after being disturbed has protracted its forewings and thereby displays also the two eyespots on the red-orange hind wings. **2**. A male small emperor moth sitting on a piece of gauze netting that was attached to a plank resting against the wall of the experimental room. To ensure consistent presentations, emperor moths were always placed over a small mark on the plank (not visible in the photo). To speed up trials, two mealworms were attached to the plank at a short distance from the moth.

catching skills (Gosler & Clement, 2007).

All trials were carried out at Tovetorp Zoological Research Station, located in the southeast of Sweden, approximately 90 km south of Stockholm. Blue tits were captured, outside their breeding-season, with mist-nets in the research station's surroundings (permit 619:M03 Swedish Bird Ringing Centre). They

were housed individually in indoor cages (80 x 60 x 40) cm equipped with perches for the birds to rest upon. In the cages, the blue tits had *ad libitum* access to water, sunflower seeds (*Helianthus annuus*), suet and mealworms (*Tenebrio molitor*). Experimental setup and procedures and housing of the birds were reviewed and approved by the regional ethical committee

(permit Linköpings djurförsöksetiska nämnd 49-01). After the completion of a trial, birds were banded, using rings from the Swedish Bird Ringing Centre, to enable future identification and to assure that a specific individual was never used in more than one trial. Birds were then released at the site of their capture. No bird was kept in captivity for more than a week. All birds maintained their condition during captivity and were healthy upon release.

Pupae of emperor moths, *Saturnia pavonia*, were obtained from Worldwide Butterflies Ltd. After eclosion, the emperor moths were kept in plastic cups, sitting on a piece of gauze netting stretched over the cup. The emperor moths were then transferred to a cool storage room (6°C) where they were kept until the time of the experiment.

The experimental protocol of this study consisted of five different treatments. One group of male emperor moths were left unmanipulated (male-unmanipulated), a second group (male-no eye) had their four eye-spots on the dorsal side of their wings covered with water-based grey acrylic-paint (Marabu Decormatt) and the third group (male-eye) was sham-painted on the basal part of their wings so as to leave their eyespots intact. In a similar manner, females were either left unmanipulated (female-unmanipulated) or had their eyespots covered with acrylic paint (female-no eye). Unfortunately, too few females eclosed to create a treatment with sham-painted individuals with their eyespots intact. The experiments in this study were performed on two occasions. Experiments with unmanipulated male and female emperor moths were conducted during February through early April 2005. Using the same experimental setup we performed the experiments on the painted emperor moths (male-eyes, male-no eyes, female-no eyes) in February and March 2007.

Trials were carried out inside a small room (2.3 x 2.4 x 1.9) m with one-way windows on two of the walls that allowed us to observe the interactions between a bird and an emperor moth without disturbing them. The room was lit by six daylight fluorescent tubes. A longitudinally cut log of sallow (*Salix caprea*) was placed on the floor so that one of its cut ends was in contact with one of the walls. At the other end of the log, a water bowl was placed on the floor to allow birds free access to water during a trial. On top of the log, a rough plank (80 x 20) cm was placed against the wall in a vertical position. Before a trial began, randomly a female or a male small emperor moth was transferred from its plastic cup, still sitting on the piece of gauze netting, to the plank. This was done by pinning the net to the plank using map pins. To enable consistent presentations, the emperor moth

was always placed over a small mark on the plank, situated 10 cm above the log. To mimic their natural resting position, the small emperor moth was placed head up in the experimental room. On both sides of the emperor moth, at a distance of approximately 4 cm, a mealworm was attached to the board using a map pin (Fig 2.). The function of the mealworms was to speed up the trials by encouraging the birds to approach the experimental set up.

A trial began with a bird being let into the experimental room by a small hatch in the door. We noted the time when the bird first visited the log and also the time to the first attack on either mealworm. Additionally, we measured the time elapsed until the bird executed its attack on the emperor moth. A trial lasted a maximum of 30 minutes but ended earlier if a bird killed and consumed an emperor moth. To get a crude indication of the palatability of the emperor moths, blue tits were always allowed to finish eating a seized insect before the trial was ended. All trials were observed directly and, additionally, recorded using a digital videocamera (Sony DCR-VX1000E). The video recordings allowed us to review trials and were also used to establish whether a bird made physical contact or merely performed an intention movement towards the emperor moth during a specific attack, something that would be difficult to differentiate between through direct observation.

To quantify the size difference between sexes, the left forewing of nine dead females and 14 dead males were measured using a plastic ruler with a millimeter scale. Furthermore, we weighed 13 females and 10 males within a few hours of eclosion on an electro balance to quantify the difference in mass between sexes.

Statistical analysis

All statistical tests are two-tailed and were conducted using Statistica for Windows 5.5 (StatSoft Inc.). All values given are mean \pm SE. Data on time to the first visit by birds on the log on the floor were log-transformed to achieve homogeneous variance in the different treatments. Non-parametric tests were used when analyzing data with more skewed distributions.

RESULTS

The five treatments did not differ in time to when the birds first visited the log with the emperor moth (ANOVA $F_{4,50} = 2.1$, $p = 0.10$, see Table 1 for sample sizes and mean values). Similarly, there was no difference in time to the first actual attack

on the emperor moths in the different treatments (ANOVA $F_{4,35} = 0.51$, $p = 0.73$). Finally, there was no difference between treatments in the number of attacks performed by the birds during the 30-minute trials (Kruskal-Wallis test: $H_4 = 5.47$, $N = 60$, $p = 0.24$). Hence, the birds attacked emperor moths with eyespots as soon and as frequently as moths without eyespots, which suggests that they were not intimidated by the conspicuous eyespots. Moreover, after 30 minutes of interacting with the blue tits 50% of the emperor moths in the treatments male-eyes (6 of 12) and male-no eyes (6 of 12) were still alive and thus there was no difference in survival. Furthermore, there was no difference in the number of times blue tits approached male-eyes (6.8 ± 1.8 , $N = 12$) compared with male-no eyes emperor moths (9.7 ± 2.5 , $N = 12$) (Mann-Whitney U-test: $U = 58$, $p = 0.44$). Accordingly, having eyespots did not confer any survival advantage to male emperor moths in our experiments.

Pooling the data on male and female emperor moths in treatments with or without eyespots, respectively, birds were found to visit the log on the floor after a shorter time in trials with emperor moths with eyespots (male-eyes, male-unmanipulated, female-unmanipulated) (171 ± 48 s, $n = 33$) compared with trials in which the emperor moths had their eyespots covered with paint (male-no eye, female-no eye) (317 ± 83 s, $n = 22$) (t-test: $t_{53} = -2.0$, $p = 0.05$; Fig. 3). Moreover, although 20 of 60 emperor moths were not attacked during the trials, there was no tendency for moths with eyespots intact not to be attacked; when pooling males and females only 50 % of moths (12/24) with eyespots painted over were attacked whereas almost 80 % (28/36) of moths with eyespots intact were attacked (Table 1).

Female emperor moths were approximately 3 times heavier (1.100 ± 0.076 g, $n = 13$) than males (0.373 ± 0.012 g, $n = 10$). Additionally, females had longer forewings (36.0 ± 0.9 mm, $n = 9$) than males (30.7 ± 0.7 mm, $n = 14$) (t-test: $t = 4.76$, $df = 21$, $p < 0.001$). Pooling the frequencies of surviving emperor moths of the three male treatments and the two female treatments, respectively, females (18 of 24) survived to a higher extent than males (14 of 36) (Fisher's exact test: $p = 0.008$; Table 2). The reason for the higher survival of females was not that they were attacked to a lesser extent, because a similar proportion both sexes were not attacked at all; out of 24 females 9 were not attacked (37.5 %), and out of 36 males 11 were not attacked (30.6 %) (Fisher's exact test: $p = 0.59$; Fig. 4a). However, among the emperor moths that were attacked 9 of 15 females survived, whereas only 3 out of 25 males survived (Fisher's exact test: $p = 0.003$) which shows that females were attacked less ferociously by the

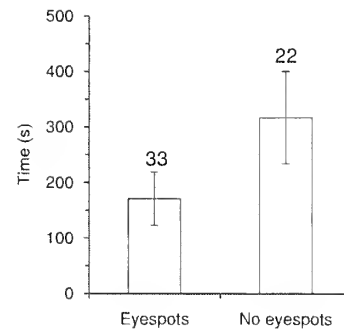


Figure 3. Time until a blue tit first visits an emperor moth with eyespots, or without eyespots. Data are mean \pm one standard error (whiskers). Numbers above whiskers are n-values.

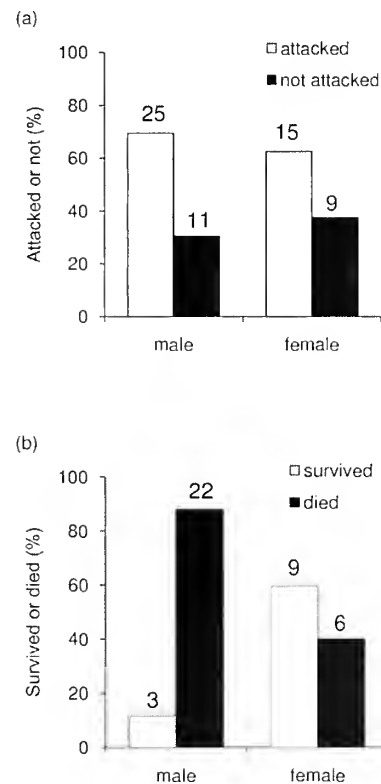


Figure 4. a. The proportion of male and female emperor moths that were attacked, or not attacked, during 30-minute trials with blue tits. **b.** The proportion of male and female emperor moths that survived, or were killed, when attacked by blue tits. Data are pooled for the three male, and the two female, treatments, respectively. Numbers above bars are n-values.

Table 1. Sample sizes and the mean time in seconds from the beginning of a trial to when a blue tit first visited the log on the floor (first visit), the time until the emperor moth was attacked for the first time (first attack), and the mean number of attacks. Sample sizes vary because individual blue tits did not necessarily perform all behaviors

| Treatment | first visit (s) | |
|----------------------|-----------------|-----------|
| | n | mean±SE |
| male-eye | 9 | 138 ± 55 |
| male-no eye | 12 | 322 ± 99 |
| male-unmanipulated | 12 | 264 ± 115 |
| female-no eye | 10 | 313 ± 144 |
| female-unmanipulated | 12 | 104 ± 44 |

blue tits (Fig. 4b). The birds consumed the thorax and the abdomen of the emperor moths that were killed, whereas the wings were left intact.

DISCUSSION

Our results suggest that the blue tits were not intimidated by the conspicuous eyespots on the wings of the emperor moths, as unmanipulated and sham-painted moths with their eyespots intact, were attacked as soon and often as moths with their eyespots painted over. On the contrary, the evidence suggests that the eyespots rather aroused the birds' curiosity, as the birds returned to inspect moths with eyespots sooner compared to moths without eyespots. The results also demonstrate that the larger females survived the attacks from the blue tits better than the males did.

Although the classic literature provides verbal arguments suggesting that conspicuous eyespots on the wings of butterflies and moths can be intimidating to birds (Cott, 1940; Ford, 1955; Edmunds, 1974),

this assertion rests on little experimental evidence (Ruxton *et al.*, 2004; Stevens, 2005). Hence, our finding that the eyespots on the wings of the emperor moth were rather ineffective in thwarting bird attacks may not be so surprising, especially given that previous experiments with the eyed hawkmoth produced similar results (Vallin *et al.*, 2007). In this context it is relevant to ask to what extent our experimental setup might have influenced the results, whether birds were less likely to be intimidated when interacting with the moths in a small experimental room? We think not for the simple reason that previous experiments with aposematic and/or with eyespotted insects and blue tits, or great tits (*Parus major*) as predators in the very same experimental room and set-up, have all shown that birds often exhibit obvious signs of fear (Wiklund & Tullberg, 1985; Vallin *et al.*, 2005; 2006, 2007; Kodandaramaiah *et al.*, 2009). Particularly strong evidence for this conclusion comes from earlier experiments in which blue tits were so intimidated by peacocks that only one out of 44 birds dared attack and kill a peacock when the insect had its eyespots intact (Vallin *et al.*, 2005, 2006).

It is more likely that the relatively low intimidating effect of the emperor moths is associated with their lack of efficient deimatic behavior. When comparing the intimidating capacity of the deimatic behaviour of the three eyespotted lepidopterans *I. io*, *S. ocellatus* and *S. pavonia*, it is obvious that that of *I. io* is considerably more effective. The effective defensive behavior of *I. io* may be due to their repeated sequence of opening and closing their wings which makes the eyespots appear and disappear in rapid succession together with their apparent "aggressive" behavior towards the potential predator. The defensive behavior of the emperor moth is more similar to the relatively ineffective one of the eyed hawkmoth, and once attacked emperor moths raise their forewings so that the eyespots on the hind wings are exposed and thereafter keep all of the four eyespots constantly visible performing slow

Table 2. Frequencies of surviving and killed emperor moths classified depending on whether they were attacked or not, after 30-minute trials with wild-caught blue tits.

| Treatment | total | attacked | not attacked | alive | dead |
|----------------------|-------|----------|--------------|-------|------|
| male-eye | 12 | 6 | 6 | 6 | 6 |
| male-no eye | 12 | 7 | 5 | 6 | 6 |
| male-unmanipulated | 12 | 12 | 0 | 2 | 10 |
| female-no eye | 12 | 5 | 7 | 10 | 2 |
| female-unmanipulated | 12 | 10 | 2 | 8 | 4 |

rocking movements as long as they are under attack. However, it is relevant to note that experiments with wings of the eyespotted peacock pansy (*J. abmana*) pasted on to a piece of cardboard so as to resemble a butterfly with its wings open, did indeed elicit signs of fear in great tits (Kodandaramaiah *et al.*, 2009). However, for obvious reasons survival of the prey could not be assessed in the *J. abmana* experiment, and while the study convincingly demonstrates that large eyespots can elicit fear in a small passerine bird it does not contradict the idea that an effective deimatic behaviour can increase the likelihood of prey survival when attacked.

Another factor that may have influenced the mortality of the emperor moth relates to the experimental setup, that we used two mealworms pinned in close proximity to the emperor moth which had the intended effect of encouraging the bird to approach the mealworms and hence also the moth. This could conceivably either increase, or decrease, the likelihood of bird attack, depending on the personality of the individual bird (Dingemanse *et al.*, 2004; van Oers *et al.*, 2004). Regardless of whether the birds that did not attack the emperor moths can be considered closer to the shyness end of the personality continuum between shyness or boldness, the presence or absence of eyespots on the wings of the moths was apparently irrelevant as the proportion of birds that did not attack the moths did not differ between moth treatments.

Yet another factor that might influence our results could be the extent to which the emperor moths can be considered to match their background. Stevens *et al.* (2008b) showed that the extent to which wing spots reduce predation can be context-dependent; in an experiment using artificial moth-like targets they showed that wing spots reduced predation when on conspicuous "prey" but increased predation on otherwise camouflaged "prey". In our experiment all birds devoured the mealworms and came in close proximity to the emperor moths none of which matched their background and so were rather conspicuous. Although it is difficult to assert objectively, we are convinced that both moths with their eyespots intact and those with their eyespots painted over were clearly discovered by the blue tits. Insofar as eyespots on conspicuous prey reduce bird attacks, this was not the case in our experiment; neither the number of attacks, nor the time to attack, was influenced by whether the emperor moths had their eyespots intact or painted over.

Why did female emperor moths survive significantly better than males? We contend that the most likely explanation is that the females' larger size per se

might be an advantage when encountering a small predatory bird such a blue tit which can conceivably be intimidated by a large insect. In a similar fashion, Gamberale and Tullberg (1996) showed that larger individuals (later instars) of the aposematic insect, *Trophidothorax leucopterus* (Heteroptera: Lygacidae), were less often attacked by chickens, *Gallus gallus*, compared with smaller individuals (earlier instars). Additionally, Exnerová *et al.* (2003), observed that equally large individuals of the aposematic insect, *Pyrochoris apterus* (Heteroptera: Pyrrhocoridae), were better protected against smaller, compared with larger passerines. Thus, to a predator, larger prey hold a stronger signal value compared with smaller prey and if the signal is associated with something negative, for example bad taste as described above, this will result in a better protected individual. Indeed, in our study the large females survived significantly better than the smaller males when attacked by the blue tits, 60% versus 12%, which suggests that the birds may have been somehow intimidated by the size of their potential prey. The fact that females survive better than males when attacked is also interesting in view of the fact that the hind wings of males are bright orange-red, a color usually conceived of as aposematic, whereas those of the females are grey and the same color as the front wings; however, this apparently did not confer higher survival upon males.

Conceivably the ultimate indicator of predator intimidation would be that the potential insect prey was not attacked at all. In our experiments about 30% of male and female emperor moths were not attacked, which may indicate that the blue tits were intimidated by their eyespots or their size or a combination of the two, in relation to the "personality" of the individual bird, as discussed above. Another possible explanation for why approximately one out of three blue tits did not attack emperor moths could be that these insects represent unfamiliar prey, and so may escape attack because of neophobia (cf. Marples *et al.*, 1998; Marples & Kelly, 2001).

In our experiment birds visited the log on the floor after a shorter period of time in trials with emperor moths with eyespots intact compared with emperor moths with eyespots painted over. Two hypotheses could explain this result. First, studies on humans (Attneave, 1954), pigeons (Delius & Nowak, 1982) and honeybees (Horridge, 1996) have shown that symmetrical patterns, such as eyes, are easier to detect, associate and remember compared with other patterns. Thus, it may be the case that emperor moths with exposed eyespots are simply detected earlier in trials, and consequently, the birds fly down to the log to make their initial assessment of the situation earlier.

The other possible explanation is that the eyespots of the emperor moth actually resemble those of a real predator, for example a small owl, which would present a real threat to the attacking bird (Cott, 1940; Edmunds, 1974; Ruxton, 2005, but see Stevens, 2005, 2007). Should this be the case, the result could also be explained in terms of predator inspection where a prey actively approaches a predator (Curio, 1978). The rationale behind this behavior is that a potential prey can gather information about the predator and at the same time convey the message that the predator has been spotted (e.g. Magurran, 1986; FitzGibbon, 1994).

In conclusion, this study of the efficiency of conspicuous eyespots as a primary defense in a living lepidopteran does not support the idea that eyespots on the wings of the emperor moth have an intimidating function and deter attacks from small birds. Rather, the evidence suggests that large eyespots, in this species, may be associated with a cost, because potential predators have their attention aroused rather than being intimidated by the prey's display. Finally, our results suggest that the size of the insect prey may have an intimidating function and can deter, or make less ferocious, bird attacks on larger moths and butterflies.

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NOTE

Collecting and eating *Liphyra brassolis* (Lepidoptera: Lycaenidae) in southern Thailand

The myrmecophagous lycaenid *Liphyra brassolis* Westwood, 1864, also known as the moth butterfly, has a broad distribution from northern India, South East Asia, Indonesia, the Philippines, New Guinea, and the Solomon Islands to northern Australia (Samson & Smart, 1980), and a second species, *L. grandis* Weymer, 1902, is endemic to mainland New Guinea (Parsons, 1999). The larvae of both butterfly species feed on the brood of weaver ants, *Oecophylla smaragdina* (Fabricius, 1775) (Johnson & Valentine, 1986; Parsons, 1999). These ants are ubiquitous in lowland tropical forests throughout the Indo-Australasian region where they construct large arboreal nests by stitching together foliage with larval ant silk (Hölldobler & Wilson, 1990; Azuma *et al.*, 2006). Adult butterflies are mostly crepuscular and, in contrast with their host ants, are rarely seen or collected, thus collection records are scant and there are many gaps in what is probably a more or less continuous distribution of *L. brassolis* coincident with the distribution of *O. smaragdina*. In Thailand, *L. brassolis* is known from single specimens collected at several locations (Fig. 1) and there are a few records from further south on the Malay Peninsular (e.g. Corbet & Pendlebury, 1992; Samson & Smart, 1980; Westwood, 1864). At the Center for Tropical Forest Science (CTFS) research site at Khao Chong in southern Thailand (7°33'N, 99°48'E), *O. smaragdina* ants are commonly seen, so it was thought that the moth butterfly may occur in the area.

Rather than opening *O. smaragdina* nests, we attempted to track down *L. brassolis* in and around Khao Chong by asking local people if they had seen the caterpillars. Throughout most of the range of *O. smaragdina*, their larvae and pupae are harvested

for human consumption, medicine, bird feed or fish bait (Bingham, 1903; Bequaert, 1921; Césard, 2004; Sribandit *et al.*, 2008) and this is particularly so in Thailand where the ants are known locally as 'red ants' and are a popular delicacy. Harvesting is done using a long pole with a bag or basket attached to the end. The bag is positioned under an *O. smaragdina* nest and shaken vigorously causing the nest to rupture and ant brood to drop into the bag (Fig. 2). We asked local people to look for large orange colored inquilines

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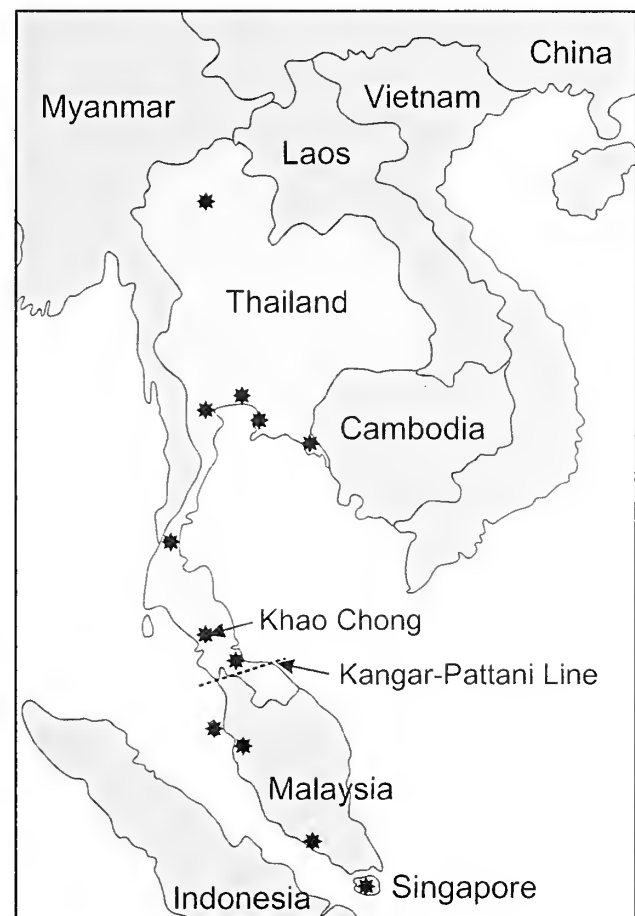


Figure 1. Collection locations for *L. brassolis* in Thailand and Peninsular Malaysia (Corbet & Pendlebury, 1992; Ek-Amnuay, 2006; Godfrey, 1930; Inayoshi, 2010; Pinratana, 1981; Samson & Smart, 1980; Westwood, 1864)

among the ant larvae, and offered a small reward to anyone who found one and brought it to us. As a result, during the ant-harvesting season at the end of February-early March 2010 we obtained two larvae and two pupae from within or near the Khao Chong Research and Conservation Promotion Station. These produced a male and a female *L. brassolis* (see Fig. 3); one larva was preserved in ethanol (Fig. 4) and the fourth one failed to eclose. The larva in Fig. 4 was collected by one of us (MR) on February 12, 2010 from an ant's nest in a Mangosteen tree, *Garcinia mangostana* Linnaeus, 1753, using local ant harvesting methods (Fig. 2). Fifteen ant's nests were harvested before the *Liphya* larva was found.

As part of the CTFS insect monitoring program at Khao Chong, butterflies are being DNA barcoded as a taxonomic tool and for future genetic studies. Thus, tissue samples comprising a single leg or small piece of larval tissue were placed in 95% ethanol and sent to the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert, 2007) where the 5' end of the mitochondrial gene, cytochrome *c* oxidase subunit 1 (barcode sequence) was characterized. Collection data and barcode sequences for the *L. brassolis* specimens are as follows. Male (Fig. 5) specimen number KHC6101; Khaobantot Mts, 236 m, Khao Chong, Trang Province, Thailand; 12 iii 2010; Kongnoo, Tongrod & Rienkaw; BOLD barcode accession number KHCBT180-10. Female specimen number KHC5981; same data as male except dated March 2010; BOLD barcode accession number KHCBT179-10. Larva (Fig. 4) specimen number KHC6141; same data as male; BOLD barcode accession number KHCBT482-10. Sequence variation among specimens from Khao Chong (0.152% - 0.456%) is consistent with the average within-species range for other tropical Lepidoptera (Hajibabaei *et al.*, 2006). All specimens are housed at the CTFS field station in Khao Chong but will be vouchered in the Forest Insect Collection of the Thailand Department of National Parks, Wildlife and Plant Conservation, Bangkok.

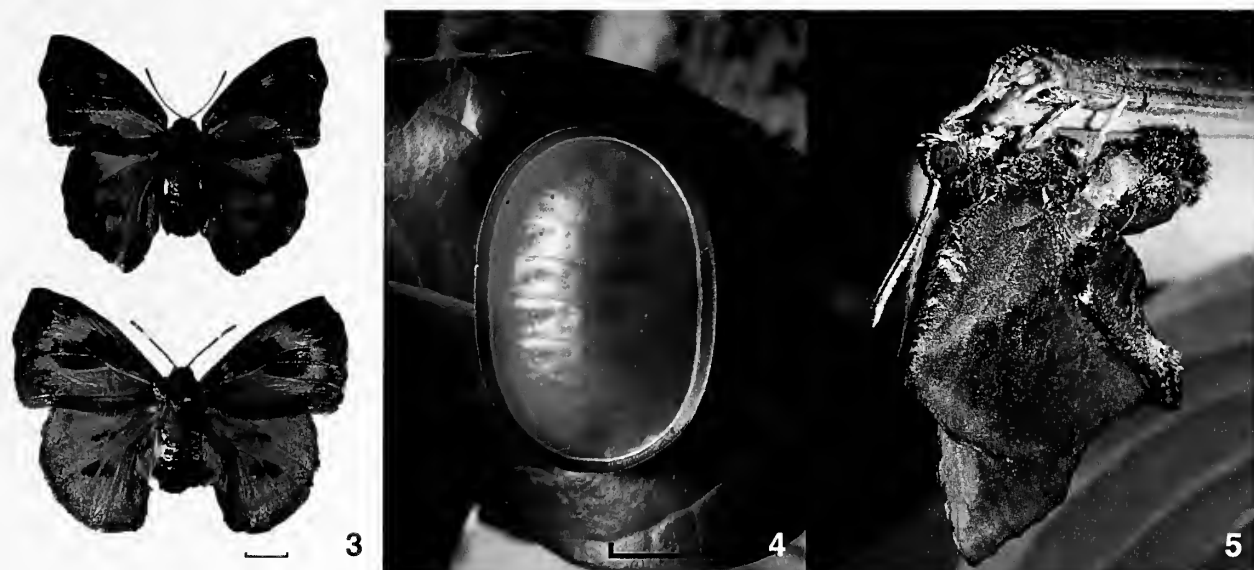
Adult *L. brassolis* at Khao Chong key to the nominate subspecies '*brassolis*' (Strand, 1911) and are remarkably consistent with photographs of specimens from Chang Mai, which are also recorded as subspecies '*brassolis*' (Ek-Amnuay, 2006). Specimens from peninsular Malaysia and Singapore, recorded as *L. brassolis abbreviata* (Corbet & Pendlebury, 1992), differ in having the black terminal band on the dorsum of the forewing in both sexes extending only part way to the base. It is uncertain where the subspecies' boundary lies, but based on available data, it is assumed to be the Kangar-Pattani Line. This line runs



Figure 2. Red ant harvesting in southern Thailand.

west-east from Kangar, Malaysia to Pattani, Thailand at about 7° N and is the most widely accepted position for the Indochinese-Sundaic boundary (Woodruff, 2003). Additional specimens and barcode sequences of *L. brassolis* from Peninsular Malaysia may help to resolve the subspecies' boundaries, and to test if DNA sequence variation parallels the differences in wing patterns.

When shown photos of *L. brassolis* larvae and told that they are found in 'red ant' nests, many local people stated they had eaten them. They thought the butterfly larvae were 'big' ants or 'queen' ants and were just as tasty as the other ant larvae, although the desirability of eating 'big ants' was variable among those people we asked. Entomophagy by humans is widespread in developing regions and some 240 species of Lepidoptera, mostly moth larvae, are known to be eaten (Ramos-Elorduy & Menzel, 1998); however, this may be the first documented record of lycaenid larvae being consumed intentionally as food. Furthermore, it is likely that 'big ants' (i.e. *L. brassolis* larvae) are encountered by ant harvesters in other parts of the *O. smaragdina* range; for



Figures 3-5. *Lyphyra brassolis*. 3. Male (upper) and female *Lyphyra brassolis* from Khao Chong, Thailand. Scale bar is 1 cm. 4. Dorsal view of *L. brassolis* mature larva from Khao Chong. Scale bar is 1 cm. 5. Adult male imago *L. brassolis* at Khao Chong.

example, ant harvesters in Cambodia recently located several *L. brassolis* caterpillars for a photo journalist (Mark Moffett, personal communication). Thus it is suggested that ant harvesters throughout the range of *O. smaragdina* may be a valuable source of distributional data and genetic material for the elusive moth butterfly.

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Two instances of inter-generic mating by Lycaenidae (Lepidoptera) in Maharashtra, India

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Abstract. Two instances of inter-generic pairing by Lycaenids are reported, the first instance between *Castalius rosimon* and *Talicada nyseus* and the second between *Chilades parthasius* and *Zizina otis*. Both instances were recorded from the same Indian village in the state of Maharashtra within a period of 2 months.

Key words: Inter-generic mating, Lycaenidae.

INTRODUCTION

Our concept of biological species is largely based on the concept of reproductive isolation. Therein, it is proposed that the pairing of opposite sexes of different species cannot produce fertile offspring, while members of the same species from distant locations, while differing superficially in response to climate and other local factors, can reproduce and produce fertile offspring.

Examples of interspecific, intergeneric or even interfamilial mating are rather rare in nature. Wright (1906) estimated this at roughly 1 in 140 pairings among North American butterflies, while Downey (1962) re-interpreted Wright's data and suggested a frequency of 1 in 70 pairings. At present, our understanding of the subject suggests that such behavior is aberrant and more or less constitutes the exception that proves the rule.

The only example of successful interspecific pairing in Asian Lepidoptera is between the closely related *Antheraea roylei* Moore 1859 and *A. pernyi* Guérin-Ménéville 1855 (Saturniidae) in China. The resulting hybrid, named *Antheraea x proylei* Jolly 1973 is used in commercial production of Tussah (Tasar) Silk. However, it has been proposed that *Antheraea roylei* is merely a sub-species of *Antheraea pernyi*, in which case the resultant fertile hybrid is perfectly logical.

We are unaware of any reports of such pairings observed in Indian butterflies. There is an example of an inter-generic courtship reported by Smetacek (2005) from the Kumaon Himalaya. Downey (1962) suggested that mating patterns may not be equally fixed throughout the range of a species so that some species may have fewer inter-specific mating barriers in some areas as compared to other areas.

MATERIALS AND METHODS

The present paper brings to notice two instances of inter-generic Lycaenid pairing observed near the town of Satara, Maharashtra. Both were photographed and the specimens involved were not taken.

The first (Figure 1) was between a male *Castalius rosimon* Fabricius 1775 (Common Pierrot) which mated with a freshly emerged female *Talicada nyseus* Guérin-Ménéville 1843 (Red Pierrot) before the female's wings had even dried. The event was observed by MB at 15.50 hrs Indian Standard Time on 30 July 2008 at Darre Budruk (= Dare Bk), a village near Satara (latitude 17° 68' 33" N longitude 73° 98' 33" E) in Maharashtra, India. The pairing was observed

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Figures 1-4. 1. A male *Castalius rosimon* mates with a *Talicada nyseus* female. 2. *Chilades parrhasius* mates with *Zizina otis*. 3. *Chilades parrhasius* and *Zizina otis* mating. 4. Mated *Chilades parrhasius* and *Zizina otis* after separation.

and photographed for about 2 minutes after which the pair flew away and were lost to observation.

The second instance of inter-generic mating was between a *Chilades parrhasius* Fabricius 1793 (Small Cupid) and *Zizina otis* Fabricius 1787 (Lesser Grass Blue) (Figures 2, 3 and 4). It was noted on 06 September 2008 at 15.15 IST at Darre Budruk, a village near Satara town in Maharashtra, India. The mating was observed for about 4 minutes and the pair separated later.

In both cases, courtship was not observed, since the first sighting was when the butterflies were already paired.

DISCUSSION

In the literature on North American Lepidoptera,

there are examples of hybridization between closely related Nymphalid species (Arbogast, 1976; Platt *et al.*, 1978; etc.); Papilionid species (Wagner, 1978) and Pierid species (Downey, 1962; Priestaf, 1974). In this context, Wynter-Blyth (1957) mentions a possible natural hybrid of *Papilio polymnestor* Cramer 1775 and *Papilio memnon* Linnaeus 1758, named *P. memnon* form *polymnestoroides* Moore 1902, from Bengal, where the two species occur together. In the Indian Lycaenidae, it has been suggested that *Heliophorus hybrida* Tytler 1912 is a hybrid between *Heliophorus brahma* Moore 1857 and *H. audrocles* Westwood [1851] (Evans, 1932). These are congeneric examples and inter-generic pairings appear not to have been reported so far.

While one of the authors (MB) observed two instances of inter-generic pairing within a period of 2 months, the other author (PS) has not observed such

inter-specific or inter-generic pairing in over 30 years of field observation mainly in the Western Himalaya, which lends support to Downey's (1962) suggestion that such pairings are commoner in some areas than in others.

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Feasibility of a combined sampling approach for studying caterpillar assemblages – a case study from shrubs in the Andean montane forest zone

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Abstract. We analyzed the suitability of a combined sampling approach – consisting of visual search and branch-beating – for quantifying tropical caterpillar communities. Surveys were conducted in the Ecuadorian montane forest zone, with two shrub species from the genus *Piper* serving as focal targets. We sampled 160 shrubs in the course of four experiments following a standardized sampling protocol. Subsequently each shrub was completely defoliated accompanied by an intensive leaf-by-leaf search, in an effort to extract as close to 100% of all present caterpillars as possible. We analyzed the resulting dataset with regard to completeness, taxonomical bias, and influences of daytime, complexity of shrub structure, or experience of the researcher. The standardized sampling protocol extracted between 50.6% and 71.6% of the caterpillars present on a shrub. A minor taxonomic bias of the sampling protocol was observed, but appears to be of a simple and predictable nature, and is therefore easy to account for. We did not find any significant influences of daytime. Structure and size of shrubs had a strong influence on sampling results with small and simply structured shrubs being sampled most completely, large and complex shrubs most incompletely in our dataset. Researcher experience did not appear to have an influence on the sampling efficiency or taxonomic composition of samples obtained when we compared caterpillars obtained by standardized sampling with those collected by exhaustive leaf-by-leaf search. Comparison of caterpillar sizes revealed however, that inexperienced field assistants tended to overlook large fractions of the smallest caterpillars entirely. We conclude that our standardized combined sampling approach is fairly suitable for studies concerning caterpillar communities, especially when resampling of the same shrub individuals is desired.

Keywords: Lepidoptera, *Piper*, beating tray, visual search, sampling efficiency.

INTRODUCTION

Herbivorous insects are a major fraction of all life on earth (e.g. Price, 2002). Their diversity and ecological roles have become a focus of many studies in the last decades. Lepidoptera are one of the largest taxa among this group, with currently approximately 155,000 species described (Pogue, 2009). While sampling of adults has been performed in largely identical ways for several decades, standardized

sampling of their larvae is less common. Especially in the tropics, where Lepidoptera are both especially diverse and particularly poorly studied, investigation of caterpillar communities and their ecology are still in a very early stage. Projects dealing with caterpillars employ a variety of collection methods such as canopy fogging (e.g. Floren & Linsenmair, 2001), complete destructive sampling (e.g. Rodríguez-Castañeda *et al.*, 2010), visual searching (e.g. Novotny *et al.*, 2002), or branch beating (e.g. Mody & Linsenmair, 2004). Canopy fogging (Adis *et al.*, 1998) has been widely used to study canopy arthropods, however caterpillars appear to be surprisingly rare in such samples (e.g. Basset, 1991; Floren & Linsenmair, 2001) and are probably highly underrepresented. Also, with many fogging protocols, only dead specimens are retrieved, making evaluation of their ecological roles impossible. Complete destructive sampling can be expected to yield highly complete samples and allows for feeding trials, but obviously renders resampling of the same plant individual impossible.

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Visual search and branch beating are both classic, low-tech, none-invasive sampling techniques for caterpillars. Visual search allows for the recovery of well attached or concealed feeding individuals, and additionally offers the possibility to record behavioral information. Branch beating can on the other hand be expected to be more effective in the recovery of small individuals. Both methods retrieve living caterpillars and therefore allow for successive feeding trials and rearing. However, both methods are only suitable for shrubs, treelets and lower tree branches due to their limitations based on the reach of the field researcher.

We here present a combination of visual search and branch beating as standardized sampling protocol with temporal replications for immature Lepidoptera on shrubs and address various questions concerning the suitability and applicability of this sampling approach for studying caterpillar communities.

In particular we aim at testing the following specific hypotheses:

- (1) Samples obtained by our combined standardized protocol retrieve the majority of individuals, but are nevertheless incomplete.
- (2) Sampling efficiency is higher on shrubs with simpler structure (i.e.: fewer, larger, hairless leaves).
- (3) Samples obtained by our protocol are unbiased with regard to higher taxa, feeding guilds, or size of caterpillars.
- (4) Sampling efficiency of the combined standardized protocol is independent of shrub size.
- (5) Sampling efficiency and composition of samples does not differ between collections taken during day and nighttime.
- (6) Samples collected by operators with and without sampling experience are comparable in terms of efficiency and composition.

METHODS

Study site

The study was conducted in southern Ecuador (province Zamora-Chinchipe), in the Reserva Biológica San Francisco (RBSF). This is a privately owned nature reserve adjacent to Podocarpus National Park which since 2007 forms part of the UNESCO biosphere reserve "Podocarpus-El Condor." The study area is located on the eastern slope of the Andes, where intensive ecological research has been conducted since 1997 (Beck *et al.*, 2008a). Caterpillars for the present study were sampled between 1800 and 2000m above sea level, in proximity to the Estación Científica San Francisco (3°58' S, 79°05' W). We

collected data in May 2008, December 2008, February to July 2009, and October 2009.

The RBSF area is covered by nearly pristine montane rain forest (Beck *et al.*, 2008b; Homeier *et al.*, 2008). Its moth fauna has been studied intensively since 1999 by light-trapping, offering insight into patterns of moth diversity and community structure at the level of adult stages (e.g. Brehm & Fiedler, 2003; Brehm *et al.*, 2003; Fiedler *et al.*, 2008; Hilt & Fiedler, 2008). In addition, life-histories and larval host plant affiliations of geometrid moths have been studied (Brehm, 2003; Bodner *et al.*, 2010).

Study organisms

We chose two species of *Piper* for experiments. Neither of them could be formally identified yet, and they are therefore referred to with their tentative names "*Piper* sp. I" and "*Piper* sp. III" (Fig. 1). Both species have been shown to harbor a substantial caterpillar community (F. Bodner, unpublished observations), dominated by species belonging to the geometrid genus *Eois*. While both *Piper* species exhibit a shrub-like growth form with maximum sizes usually around 2-3 m (sp. I) and 2-5 m (sp. III), they differ notably in structure and complexity. *Piper* sp. I has many small twigs with many rather small leaves (average leaf size \pm SD: 38.4 ± 5.5 cm²). The undersides of the leaves are covered with thin, short hairs, especially along the leaf venation. *Piper* sp. III has a more simple structure with fewer small twigs. The leaves are larger, tougher, smooth and hairless (average leaf size \pm SD: 128.4 ± 33.7 cm²). Our sampling considered all ectophagous and semi-endophagous caterpillars of any lepidopteran family. Only stem borers and leaf miners were not searched for. Eggs and pupae of Lepidoptera were also recorded, but not included in statistical analyses.

Sampling design

The standardized sampling protocol employed in this study usually consisted of two stages. First shrubs were visually searched for lepidopteran immatures. Afterwards shrubs were beaten over a beating tray, made of 1 m² of white cloth mounted on the frame of an umbrella drop net, to shake further caterpillars off the shrub. This two-staged procedure was chosen to extract a maximum of lepidopteran immatures present on the shrub. Beating usually only retrieves caterpillars, especially those species which more readily drop off the shrub when disturbed or attacked. Visual searching also yields at least a part of the eggs and pupae present as well as those caterpillars which



Figure 1. The two focal shrub species (a: *Piper* sp. I; b: *Piper* sp. III) and some of the caterpillar species (c: *Eois* sp. nr. *odatis*, d: species from the *Eois olivacea* complex, e: unidentified noctuid) from this study.

cling tightly to the branches or live as concealed feeders in leaf rolls, webs or alike. During both stages sampling effort was standardized by estimated shrub volume.

Four different experiments were carried out in the course of this study (Table 1) to analyze effects of researcher experience, sampling approach, plant species, and time of day when sampling was conducted.

Standardization

We used estimated shrub volume for standardization of sampling effort. From practical reasons sampling effort was not increased linearly with shrub volume, but in steps measured as sampling effort factor (SEF). When tailoring the SEF to shrub size classes, we allowed for a larger range of shrub volumes in the higher categories, whereas for smaller shrubs a more fine grained class division was accepted. This

aimed at avoiding excessive sampling effort at the upper end of the range of shrub sizes covered, or unacceptably low effort at the lower end of the size spectrum. This procedure was also implemented to balance against expected higher sampling efficiency for larger shrubs. We expected higher efficiency on larger shrubs because more leaf area can be visually searched simultaneously and more shrub volume can be accessed by individual beats. The SEF increased in the following fashion: 2 for a shrub volume of $1/6$ m³, 3 for $1/4$ m³ of shrub volume, 4 for $1/2$ m³ of shrub volume and +1 for every further $1/2$ m³ of shrub volume. For intermediate volumes SEF was adjusted to the nearest 0.5 for calculation of visual search effort only (see below).

Field work

We selected well accessible shrubs in the forest, mainly along paths, in various sizes from about 0.05

Table 1. List of experiments conducted to assess the feasibility of our sampling approach as a means of characterizing caterpillar assemblages of shrubs in the montane forest zone of southern Ecuador. Following the standardized sampling protocol, the number of remaining caterpillars present was evaluated by total defoliation of each shrub individual (Table 2).

| Experiment | carried out by | standardized sampling | shrub species | sampling time | number of shrubs |
|------------|--------------------------------|-----------------------|----------------------|---------------|------------------|
| P1 | experienced researcher | beating | <i>Piper</i> sp. I | day only | 37 |
| P2 | experienced researcher | searching and beating | <i>Piper</i> sp. I | day only | 29 |
| P3/I | inexperienced field assistants | searching and beating | <i>Piper</i> sp. I | day and night | 50 |
| P3/III | inexperienced field assistants | searching and beating | <i>Piper</i> sp. III | day and night | 44 |

Table 2. Caterpillars obtained as $cat_{(ss)}$ and $cat_{(es)}$ in the different experiments. Mean number of caterpillars per shrub \pm standard deviation are given for both $cat_{(ss)}$ and $cat_{(es)}$ for every experiment.

| Experiment | $cat_{(ss)}$ | Mean \pm SD | $cat_{(es)}$ | Mean \pm SD | Efficiency |
|------------|--------------|-----------------|--------------|-----------------|------------|
| P1 | 91 | 2.46 \pm 2.28 | 95 | 2.57 \pm 6.47 | 48.9% |
| P2 | 123 | 4.24 \pm 3.67 | 120 | 4.14 \pm 4.54 | 50.6% |
| P3/I | 118 | 2.36 \pm 1.72 | 92 | 1.84 \pm 1.71 | 56.2% |
| P3/III | 68 | 1.55 \pm 1.25 | 27 | 0.61 \pm 0.95 | 71.6% |
| Total | 400 | 2.50 \pm 2.39 | 334 | 2.09 \pm 3.96 | 54.5% |

m³ to about 2 m³ volume to assess possible size effects on the efficiency of the employed sampling methods. The field sampling consisted of the following stages:

1. Estimation of shrub volume by rough measurement.
2. Spreading of white sheets of cloth around and below the plant. If necessary surrounding undergrowth was cut down to allow for smoothing of sheets.
3. Visual search of the entire target plant for lepidopteran immatures for 1 \times SEF minutes (first stage of standardized sampling).
4. Beating on shrub 1 \times SEF times over beating tray which was held underneath the plant (second stage of standardized sampling).
5. Checking of sheets on the ground for caterpillars that had dropped off during search or beating, but had not been caught on the beating tray.
6. Complete leaf-by-leaf defoliation of the entire shrub during which every leaf was checked individually on both sides for lepidopteran immatures.

The first two experiments (P1 and P2) were performed by the first author who has years of experience in collecting and rearing lepidopteran caterpillars (Bodner *et al.*, 2010). The other two experiments were carried out by undergraduate students without previous experience with the sampling procedure of the experiments. We chose this setup to allow for analysis of effects of previous recorder experience or training on the completeness

and comparability of samples obtained.

Lab work and analysis

All caterpillars were photographed in the lab on scaled paper to allow for length measurement. All leaves of every sampled shrub were dried in an oven at 45°C for 72 hours and then weighed as a measure of available foliar biomass. For analysis, we coded caterpillars found during the two stages of the standardized protocol (stages 3 and 4) as $cat_{(ss)}$, those found outside of the standardized sampling protocol (stages 5 and 6) as $cat_{(es)}$ for exhaustive sampling. We calculated sampling efficiency as $\frac{cat_{(ss)}}{cat_{(ss)} + cat_{(es)}}$

for every experiment. Caterpillars were sorted by higher taxonomic levels (genus *Eois*, other Geometridae, other Macrolepidoptera, 'microlepidoptera') and feeding guild affiliation. True herbivores which feed on living *Piper* foliage were contrasted to non-herbivores (*viz.* feeding on epiphylls, lichens, mosses, or dead plant material). Data were analyzed by evaluation of contingency tables and ANOVAs calculated in Statistica 7.1 (StatSoft, 2005).

RESULTS

In total we collected 734 caterpillars from 160

shrubs (total volume: 87.9 m³, total dry leaf mass: 5.19 kg) in the course of the four experiments reported in this study. Of these, 400 were obtained by means of the standardized sampling approach (cat_(ss)), the remaining 334 were collected from sheets on the ground or during complete leaf-by-leaf defoliation of the shrubs (cat_(es)). The collected samples consisted mainly of members of the geometrid genus *Eois* (75.4% on *Piper* sp. I, 36.8% on *Piper* sp. III), other geometrid species (8.5% on *Piper* sp. I, 28.4% on *Piper* sp. III) and Noctuoidea (10.5% on *Piper* sp. I, 16.8% on *Piper* sp. III). While most caterpillars, especially the dominant genus *Eois*, were true herbivores (75.5%), a large fraction, notably consisting of other Geometridae and Noctuoidea, belonged to species feeding on dead leaves, lichens and other epiphylls (22.9%), as shown by extensive rearing trials (F. Bodner, unpublished observations). The remaining 1.6% of caterpillars could not be reliably assigned to either guild and were excluded from all analyses based on feeding guild affiliation. We additionally found 174 eggs and 16 pupae of Lepidoptera, but did not include them in statistical analysis as they were not the focus of the study and their samples can be expected to be far too incomplete even from exhaustive search to allow for any meaningful analysis.

The overall sampling efficiency was 54.5% for all four experiments and 56.4% for those three applying our combined sampling protocol. It ranged from 48.9% to 56.2% on *Piper* sp. I and was therefore similar for the three experiments dealing with this particular shrub species. The two experiments on this shrub species applying our combined sampling protocol (P2 and P3/I) retrieved the majority of caterpillars (50.6% and 56.2%), but only by a very narrow margin (Table 2). Sampling of shrub species *Piper* sp. III (experiment P3/III) was more effective with a yield of 71.6%. This was significantly higher (Chi²(DF=1) = 6.51, p<0.011) than in experiment P3/I, which was performed on *Piper* sp. I under otherwise identical conditions. Comparison of cat_(ss) and cat_(es) on higher taxonomical levels revealed a significant bias in two of the experiments (P1 and P2), but not so in the remaining two (Table 3). The same applies to analyses based on feeding guilds. Detailed inspection of the data shows that in both cases most of the effect was due to the genus *Eois* being underrepresented in cat_(ss). When comparing cat_(ss) and cat_(es) with regard to caterpillar lengths, an overall bias of the standardized sampling protocol towards larger caterpillars becomes evident (Table 4). Separate analyses of all experiments confirmed this effect only for P2 (Table 4, Fig. 2).

To analyze possible effects of shrub size on sampling efficiency, we combined all available data from the

Table 3. Comparison of cat_(ss) and cat_(es) on basis of higher taxa (genus *Eois*, other Geometridae, other Macrolepidoptera, 'microlepidoptera') and feeding guilds (herbivores, non-herbivores) by means of Pearson's Chi².

| Experiment | Taxa | | Guilds | |
|------------|-------------------------|----------|-------------------------|----------|
| | Chi ² (DF=3) | p | Chi ² (DF=1) | p |
| P1 | 24.20 | <0.00003 | 21.27 | <0.00001 |
| P2 | 8.88 | <0.031 | 8.01 | <0.005 |
| P3/I | 2.14 | >0.54 | 1.71 | >0.19 |
| P3/III | 6.01 | >0.11 | 1.04 | >0.30 |

Table 4. Results of ANOVAs comparing caterpillar lengths of cat_(ss) and cat_(es) for all experiments (Fig.2). DF: degrees of freedom.

| Experiment | DF Model | DF Residual | F | p |
|------------|----------|-------------|-------|---------|
| all | 1 | 729 | 15.66 | <0.0001 |
| P1 | 1 | 183 | 0.43 | >0.51 |
| P2 | 1 | 240 | 20.11 | <0.0001 |
| P3/I | 1 | 207 | 2.16 | >0.14 |
| P3/III | 1 | 93 | 0.97 | >0.32 |

Table 5. Correlations of sampling efficiency with mean foliar dry weight of shrubs per category for combined data from experiments with *Piper* sp. I (P1, P2 and P3/I), split into 6 (Q6; Fig. 3), 7 (Q7) and 8 (Q8) categories, respectively.

| | r | r ² | t | p |
|----|---------|----------------|-------|--------|
| Q6 | -0.9421 | 0.8875 | 5.617 | 0.0049 |
| Q7 | -0.8565 | 0.7336 | 3.710 | 0.0138 |
| Q8 | -0.7373 | 0.5436 | 2.673 | 0.0369 |

experiments on *Piper* sp. I (P1, P2 and P3/I) and assigned all shrubs to size categories by their dry leaf mass. We choose class borders in a fashion to distribute total dry leaf mass of shrubs over all categories as evenly as possible. Intermediate shrubs which did not clearly fall into one category were assigned to the one with lower total number of caterpillars. To rule out chance effects of category delimitations on the results, we performed this calculation three times, accepting 6, 7 and 8 categories, respectively. In all three cases correlation analyses of the overall sampling efficiency within each category versus the mean dry leaf mass of

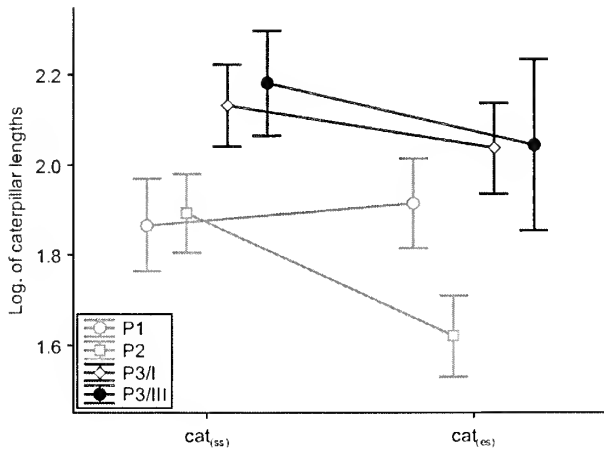


Figure 2. Average caterpillar lengths (log transformed) retrieved during the four experiments, segregated into those sampled by the standardized protocol ($cat_{(ss)}$) or during complete defoliation ($cat_{(es)}$). Whiskers are 95% confidence intervals. Grey: experiments by experienced researcher, black: experiments by field assistants. Empty symbols: *Piper* sp. I; filled symbols: *Piper* sp. III. Significance of experiment \times sampling group interaction (two-way ANOVA): $F(4, 723) = 5.5249$, $p = 0.00022$.

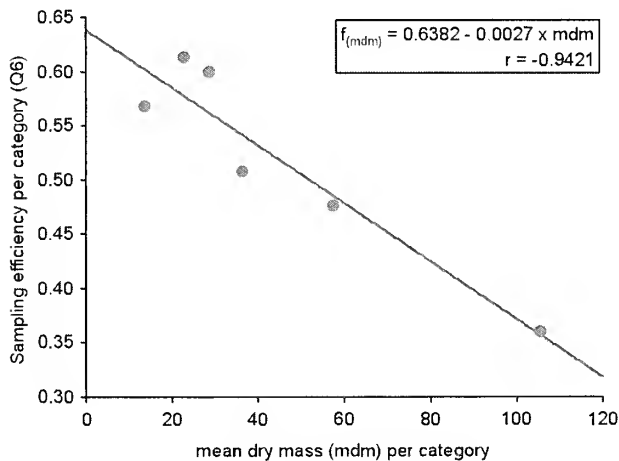


Figure 3. Relationship between sampling efficiency quotient and mean dry mass of shrubs for combined data of experiments P1, P2 and P3/I (*Piper* sp. I) split into six shrub size classes (statistical evaluation see Table 5). Regression line fitted by ordinary least squares regression.

all its shrubs showed a significantly negative effect of shrub size on sampling efficiency (Table 5, Fig. 3).

We set up contingency tables to address the question whether samples collected during day and night differ in efficiency and composition. No significant effects

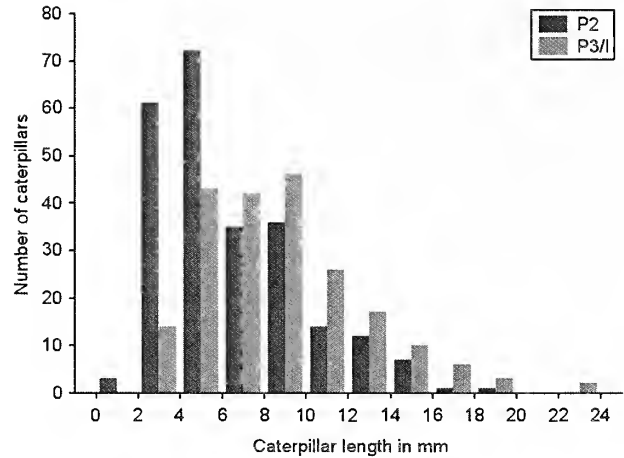


Figure 4. Frequency distributions of caterpillar lengths for experiments P2 (performed by an experienced observer, red bars) and P3/I (performed by inexperienced field assistants, blue bars) on *Piper* sp. I. Mean sizes of retrieved caterpillars differed significantly (see text). Note the difference between the experiments in the three lowest size classes.

of collection time were observed for either sampling efficiency or sample composition on levels of higher taxa or feeding guilds ($\chi^2(DF=1) < 6.68$, $p > 0.06$).

Comparison of experiments P2 and P3/I with regard to the effect of recorder experience on sampling results did not reveal significant differences in sampling efficiency, or taxon or guild composition. This was true both for $cat_{(ss)}$ and for the whole dataset ($cat_{(ss)} + cat_{(es)}$) ($p \geq 0.27$). However, average size of caterpillars obtained in total was significantly smaller ($F(1, 436) = 54.244$, $p < 0.0001$) when gathered by an experienced researcher (mean \pm S.D. = 6.55 ± 3.30 mm) as compared to data collected by inexperienced field assistants (8.87 ± 3.91 mm; Fig. 4).

DISCUSSION

Even though studies of caterpillar populations and assemblages frequently make use of both branch-beating (e.g.: Yela & Lawton, 1997; Mody & Linsenmair, 2004; Markó *et al.*, 2006) and visual searching/hand collecting (e.g. Novotny *et al.*, 2006; Dyer *et al.*, 2007) methods, studies that try to quantitatively assess their overall efficiency and possible biases are strikingly rare. Sampling of two species of *Piper* shrubs in the montane forest zone of southern Ecuador turned out to retrieve only slightly more than 50% of the caterpillars that were present on shrubs of *Piper* sp. I. Considering that especially some very small caterpillars will likely have been overlooked even during our high intensity

leaf-by-leaf search, the real efficiency can probably be expected to be a bit lower and may be below 50% even for the combined sampling protocol. In the light of these results our hypothesis (1) that sampling retrieves the majority of caterpillars on a shrub can at best be cautiously accepted. Efficiency of sampling on simpler structured *Piper* sp. III was significantly higher, as predicted by hypothesis (2). More detailed analysis revealed that the increase in overall efficiency on this shrub species was almost entirely due to visual search. The fraction of caterpillars recovered by beating was also slightly higher, even though the fraction of caterpillars still remaining on the shrub at this stage is smaller, revealing that beating efficiency has also increased notably. While higher search efficiency is probably due to lower availability of hiding places and less visual distraction of the researcher, higher beating efficiency is likely due to the smoother leaf surface of *Piper* sp. III offering a less strong foothold to caterpillars.

Comparison of $cat_{(ss)}$ and $cat_{(es)}$ on higher taxonomical levels revealed a significant bias in two of the experiments, especially in P1 where only sampling by beating was performed. In both cases the bias was almost entirely due to caterpillars from the genus *Eois* being underrepresented in $cat_{(ss)}$. *Eois* species are small-sized and usually very specialized herbivores. The limited data presently available indicates that many species may even be limited to a single host plant species (e.g. Dyer *et al.*, 2010; Strutzenberger *et al.*, 2010). This could explain their reluctance to drop off the plant, since they are unlikely to find a suitable host plant again. Such a behavior would render them underrepresented in samples acquired by beating. Identification of the individual caterpillar specimens did not indicate any entirely new *Eois* species that would have been acquired only by subsequent defoliation (even though species accumulation of *Eois* in the study area is far from being complete: Strutzenberger *et al.*, in press). Consequently, although hypothesis (3) has to be discarded, the sampling bias is of a predictable nature and in a small range that appears to be acceptable, since no herbivore species were overlooked.

Caterpillar assemblages on larger shrubs were sampled less completely as compared to small shrubs, falsifying hypothesis (4). We had not increased sampling effort in linear fashion with shrub size since we had expected higher per-effort-efficiency for larger shrubs, i.e. more shrub biomass can be sampled by a single beat or searched visually by turning one branch. Evidently this expected effect has either been overestimated or canceled out at least partly by other effects. One possible negative

size dependent influence is e.g. loss of recorder focus when visually searching larger numbers of leaves simultaneously. Beating efficiency on the other hand could be negatively influenced e.g. by tighter packing of branches and leaves, resulting in caterpillars shaken off from one leaf to land on another instead of the beating tray.

Samples taken under otherwise identical conditions during day and night did not significantly differ in any aspect, confirming hypothesis (5). This indicates that there is no reason to assume substantial day-to-night migrations of caterpillars on the sampled shrubs. Therefore caterpillar assemblages sampled during daytime should not be biased, e.g. due to missing nocturnal species. This confirms that the standardized sampling protocol is suitable for assessments irrespective of the time of day. However, we do not expect this to be necessarily true for other plant species, where diurnal migration of caterpillars might play a more important role (see e.g. Huogue, 1993).

Samples gathered by inexperienced field assistants did not significantly differ on a taxonomical basis from those taken by an experienced researcher and are therefore comparable and can be combined for analysis. Sampling efficiency was even calculated to be slightly higher for inexperienced assistants. This appears surprising at first glance, since one would suspect that experience in searching for caterpillars increases the number of caterpillars found during the same time during visual search at least. Closer examination of the size distribution of caterpillars collected during the experiments P2 and P3/I reveals, however, that the average size of all caterpillars ($cat_{(ss)}$ and $cat_{(es)}$ combined) was significantly larger on shrubs sampled by recorders without previous experience. This indicates that a larger fraction of small caterpillars was overlooked by inexperienced field assistants even during intensive leaf-by-leaf search. This also offers an explanation why the average number of caterpillars per shrub in both $cat_{(ss)}$ and even more so in $cat_{(es)}$ is lowest in experiment P3/I of the three experiments dealing with the plant species *Piper* sp. I. This leads to the conclusion that the efficiency of P3/I is particularly overestimated by the raw numbers and that the real efficiency is probably considerably lower for inexperienced field assistants as compared to experienced researchers.

CONCLUSIONS

We conclude that the two-staged sampling protocol presented in this study retrieved about half of the caterpillars which were in fact present on the sampled

Piper shrubs. While a taxonomic bias existed against well attached host-plant specialists, this bias was smaller than with beating alone and of a predictable nature. Overall sampling efficiency was only slightly increased by adding visual search to beating, possibly because the slight shaking of the shrub, which is unavoidable during search, caused caterpillars to hold on more tightly. However, besides the reduction in taxonomic bias, visual search also allows for the gathering of at least some part of the eggs, cocoons and pupae that would be completely overlooked by beating alone. Moreover, observations during visual research have the potential to yield information on behavior and functional connections between caterpillars and plants that are lost after beating. This includes the ability to distinguish between gregarious and solitary caterpillars. Sampling intensity has to be chosen in consideration of necessary sampling efficiency, but also with consideration of the size range of shrubs to be studied, lest sampling effort becomes unreasonably small or large at either end of the range. Linear increase of sampling effort might however lead to more homogenous sampling efficiency across shrub sizes.

Overall we consider the presented two-stage sampling protocol to be fairly suitable for studying caterpillar communities on shrubs, especially when resampling of the same shrub individuals in a time series is desired. At the same time the method is minimally invasive, since only the caterpillars present on the shrub at that time are affected and non-target animals can be freed again immediately.

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Evidence for the existence of three species in the genus *Archaeoattacus* (Lepidoptera: Saturniidae)

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Abstract. 27 specimens of the genus *Archaeoattacus* Watson [*in* Packard], 1914 were DNA barcoded. This resulted in three clearly different clusters: the first is a purely Himalayan group (i.e., nominotypical *Archaeoattacus edwardsii* (White, 1859)); the second is *Ar. staudingeri* (Rothschild, 1895) from Sundaland including the Malay Peninsula; the third comprises samples from all other parts of the continental range of the first species excluding the Himalaya, but also including the Malay Peninsula. For this population the name *Ar. malayanus* (Kurosawa & Kishida, 1984), **stat. n.** is available. The relationships between these three species as inferred from DNA barcode analysis were unexpected, with *Ar. staudingeri* being very close to *Ar. malayanus*, while *Ar. edwardsii* is more genetically distant in spite of the close similarity in morphology. Small, but evidently constant differences in male genitalia between the Himalayan *Ar. edwardsii* and the Indochinese *Ar. malayanus* support the distinction. The rooted mtDNA barcode tree, a distribution map, the types of the three species involved and several male genitalia are illustrated.

Key words: DNA barcoding, Oriental region, integrative taxonomy.

INTRODUCTION

The first two authors (W.A.N. & S.N.) were recently asked by Jeremy D. Holloway, London, about the status of several Sundanian species of Saturniidae for his

⁷⁷th contribution to the knowledge of the Saturniidae. (76th contribution: Nässig, W. A., Kitching, I. J., Peigler, R. S., & Treadaway, C. G. (2010): The group of *Cricula elaezia*: Comments on synonyms and priority questions, with illustrations of barcode similarity trees, distribution maps, a revised checklist and a formerly unknown female (Lepidoptera: Saturniidae). *Nachrichten des Entomologischen Vereins Apollo*, Frankfurt am Main, N.F. 31 (3): 145-165.)

⁷⁸th contribution to the Saturniidae fauna of China. (19th contribution: Naumann, S., & Nässig, W. A. (2010): Two species in *Saturnia (Rinaca) zuleika* Hope, 1843 (Lepidoptera: Saturniidae). *Nachrichten des Entomologischen Vereins Apollo*, Frankfurt am Main, 31 (3): 127-143.)

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new checklist of the Lepidoptera in this area. After preliminary analyses of the mtDNA COI barcode data we had gathered for the oriental genus *Archaeoattacus* Watson [*in* Packard], 1914, we observed genetic divergences suggesting the existence of three distinct species, thus contrasting with the current taxonomy of the genus recognizing only two species: *Ar. edwardsii* (White, 1859) and *Ar. staudingeri* (Rothschild, 1895) (see, e.g., Holloway, 1987: 112; Beck & Nässig, 2008: 160).

This result, which was not a total surprise to us since we had already seen similar patterns elsewhere (e.g., Naumann & Nässig, 2010), is further investigated in this work through an integrative approach combining molecular and morphological data.

HISTORY

The genus *Archaeoattacus* was described by J. H. Watson *in* Packard (1914: 265); its type species by original designation is *Attacus edwardsi* [*sic, recte edwardsii*] White (1859: 115) (Fig. 2), type locality “Dhargeeling” [= Darjiling, West Bengal, India].

A second species, *Attacus staudingeri* Rothschild, 1895 (: 36) (Fig. 4), was already included into *Archaeoattacus* by Watson *in* Packard (1914: 265) and

later also by, e.g., Schüssler (1933: 22) and Bouvier (1936: 321-322). Its type locality is “NW Java” [probably correct: W Java, see below]. Seitz (1926: 502-503) introduced a misspelling (“†*Archaeoattacus*”) without using it at the generic level and without making any clear distinction from species within the genus *Attacus*.

Watson *in* Packard (1914: 265) suggested to probably also include as third species in *Archaeoattacus* “a new smaller species, as yet undescribed, from Thibet, which M. Charles Oberthür has shown me in his collection.” This species was described by Oberthür (1914: 56) as *Desgodinsia watsoni* [gen. et sp. n.], but because of homonymy of the generic name (see Fletcher & Nye, 1982: 50, 145), it was then for a long time included in the genus *Samia* Hübner, 1819 (for a review, see, e.g., Lemaire & Peigler, 1982). Much later Brechlin (2007) described a new genus, *Archaeosamia* Brechlin, 2007, for the species *watsoni*.

About 50 years after Seitz, Schüssler and Bouvier, treating *Archaeoattacus* as a subgenus within *Attacus*, Kurosawa & Kishida (1984: 132) described *Attacus* (*Archaeoattacus*) *edwardsii malayanus* (Fig. 3), a subspecies from the “Cameron Highlands, Malay Peninsula.” Brosch *et al.* (1999: 39) formally synonymized this subspecies with *Ar. edwardsii*, because there did not appear to be constant and convincing differences from the broadly distributed non-Himalayan Asian mainland population in external and ♂ genitalia morphology. Peigler (1983 and especially 1989: 113) described some differences between the Malaysian and “Indian” specimens, but interpreted this as a clinal grade between “India” and Peninsular Malaysia along the Indochinese Peninsula.

This subspecies *malayanus* was also recorded from Borneo (Malaysia, Sarawak, near Miri) by Fukuda (2001: 90). However, this locality is doubtful (Peigler & Naumann, 2003: 32 stated categorically that this record is erroneous; U. & L. H. Paukstadt, 2006: 178-179 expressed doubt; but compare U. & L. H. Paukstadt, 2010: 163 where the authors mentioned “very few scattered records for Borneo”). There have been extensive collecting efforts in that area of Borneo, and to our knowledge this species was not found again. We think that it might have been either some mislabelled traders’ material that probably came from West Malaysia or even Thailand or further North, or, alternatively, it might have been a recently introduced population. A natural immigration into Sarawak from the Malay Peninsula, for a species preferring mountainous environments (U. & L. H. Paukstadt, 2006: 178-179 classified the taxon explicitly as a “highland taxon”), is, however, unlikely.

Lampe (1984 [20. x.], 1985) was the first author to report the sympatric occurrence of *Ar. edwardsii* and *Ar. staudingeri* on the Malay Peninsula; the paper by Kurosawa & Kishida (1984 [xii.]) was published shortly after in the same year, and they evidently did not know *Ar. staudingeri* from the Peninsula at that time.

RESULTS OF THE DNA BARCODE ANALYSIS: NEW EVIDENCE SUGGESTING THE EXISTENCE OF THREE DISTINCT SPECIES

A total of 27 DNA barcodes were generated (see Decaëns & Rougerie, 2008 for details and references relative to the laboratory protocols used) and analysed, including 16 samples of *Ar. edwardsii sensu lato* and 11 samples of *Ar. staudingeri*. The sample distribution for the former species ranges from West Bengal (Himalayan India) to the Cameron Highlands (Peninsular Malaysia), thus encompassing the range of the typical *Ar. edwardsii* (described from West Bengal) and of the synonymized subspecies *malayanus* (described from the Cameron Highlands). The generation of DNA barcodes for samples from other regions (e.g. India: Nagaland; Laos; Vietnam) is still pending, but these are not expected to affect the results presented here.

We also included in our analysis a few specimens of the closely related genera *Samia* and *Archaeosamia* as outgroups. [*Archaeosamia* is generally included within *Samia* (see Peigler & Naumann, 2003), and *Samia*, in addition to *Attacus* Linnaeus, 1767 and *Coscinocera* Butler, 1879, is often considered to be closely related to *Archaeoattacus*.]

The dataset for all the samples used in our analyses is publicly accessible on Bold (The Barcode of Life Data Systems, see Ratnasingham & Hebert, 2007) within the following projects: SNPUB, SKPUB, RRPUB, MPPUB and SWNPR. Sequences have also been deposited in GenBank (see Table 1).

The preliminary analysis of sequence similarity using the analytical component of Bold (Neighbor Joining analysis based on K2P distances) revealed the existence of three distinct genetic clusters, and this pattern was confirmed by analysis of the sequences using the maximum parsimony (MP) and maximum likelihood (ML) phylogenetic reconstruction methods (results not shown), all three methods yielding the same general topology. Here we present the results of the Neighbor Joining (NJ) analysis run in MEGA4 (Tamura *et al.*, 2007) using K2P distances, with bootstrap support values calculated after 1000 replications.

Table 1. GenBank Accession numbers for the specimens used here.

| Sample ID | Process ID | GenBank | Species | Deposition | Sequence Length | Sex | Country, Province/Island |
|--------------|-------------|----------|-----------------------------------|------------|-----------------|-----|------------------------------|
| BC SNB 1891 | SASNB796-10 | HQ579828 | <i>Archaeoattacus edwardsii</i> | CSNB | 658[0n] | ♂ | Bhutan |
| BC SNB 1892 | SASNB797-10 | HQ579829 | <i>Archaeoattacus edwardsii</i> | CSNB | 658[0n] | ♂ | Bhutan |
| BC SNB 1893 | SASNB798-10 | HQ579830 | <i>Archaeoattacus edwardsii</i> | CSNB | 658[0n] | ♂ | India, Arunachal Pradesh |
| B3220-wn-G02 | SAWNA260-09 | GU703545 | <i>Archaeoattacus edwardsii</i> | SMFL | 658[0n] | ♂ | India, West Bengal |
| BC-MNHN0032 | SPMNP025-07 | HQ599803 | <i>Archaeoattacus malayanus</i> | MNHN | 658[0n] | ♂ | China, Yunnan |
| BC-MNHN0033 | SPMNP026-07 | HQ599804 | <i>Archaeoattacus malayanus</i> | MNHN | 658[0n] | ♂ | China, Yunnan |
| BC-Roug1089 | SATWA995-07 | HQ599806 | <i>Archaeoattacus malayanus</i> | CRRR | 609[0n] | ♀ | China, Yunnan |
| BC-Roug1090 | SATWA996-07 | HQ599805 | <i>Archaeoattacus malayanus</i> | CRRR | 609[0n] | ♂ | China, Yunnan |
| BC-SK0202 | SASKA202-07 | HQ599810 | <i>Archaeoattacus malayanus</i> | CSKK | 474[0n] | ♂ | Malaysia, Pahang |
| BC-SK0203 | SASKA203-07 | HQ599809 | <i>Archaeoattacus malayanus</i> | CSKK | 658[0n] | ♂ | Malaysia, Pahang |
| BC-SK0207 | SASKA207-07 | HQ599808 | <i>Archaeoattacus malayanus</i> | CSKK | 658[0n] | ♂ | India, Meghalaya |
| BC SNB 1889 | SASNB794-10 | HQ599807 | <i>Archaeoattacus malayanus</i> | CSNB | 539[1n] | ♂ | Thailand, Ranong |
| BC SNB 1890 | SASNB795-10 | HQ579827 | <i>Archaeoattacus malayanus</i> | CSNB | 658[0n] | ♂ | Thailand, Chiangmai |
| B3220-wn-F12 | SAWNA258-09 | GU703355 | <i>Archaeoattacus malayanus</i> | SMFL | 658[0n] | ♂ | China, Yunnan |
| B3220-wn-G01 | SAWNA259-09 | GU703352 | <i>Archaeoattacus malayanus</i> | SMFL | 567[0n] | ♂ | Myanmar |
| BC-MNHN0031 | SPMNP024-07 | HQ599802 | <i>Archaeoattacus malayanus</i> | MNHN | 658[0n] | ♂ | China, Yunnan |
| BC-SK0204 | SASKA204-01 | HQ599814 | <i>Archaeoattacus staudingeri</i> | CSKK | 658[0n] | ♂ | Malaysia, Perak |
| BC-SK0205 | SASKA205-07 | HQ599813 | <i>Archaeoattacus staudingeri</i> | CSKK | 658[0n] | ♂ | Malaysia, Sabah (Borneo) |
| BC-SK0206 | SASKA206-07 | HQ599812 | <i>Archaeoattacus staudingeri</i> | CSKK | 658[0n] | ♀ | Malaysia, Perak |
| BC SNB 1884 | SASNB789-10 | HQ579822 | <i>Archaeoattacus staudingeri</i> | CSNB | 658[0n] | ♀ | Indonesia, Jawa Timur |
| BC SNB 1885 | SASNB790-10 | HQ579823 | <i>Archaeoattacus staudingeri</i> | CSNB | 564[0n] | ♂ | Indonesia, Jawa Timur |
| BC SNB 1886 | SASNB791-10 | HQ579824 | <i>Archaeoattacus staudingeri</i> | CSNB | 638[1n] | ♂ | Indonesia, Kalimantan Tengah |
| BC SNB 1887 | SASNB792-10 | HQ579825 | <i>Archaeoattacus staudingeri</i> | CSNB | 658[0n] | ♂ | Indonesia, Sumatera Barat |
| BC SNB 1888 | SASNB793-10 | HQ579826 | <i>Archaeoattacus staudingeri</i> | CSNB | 658[0n] | ♀ | Malaysia, Sabah (Borneo) |
| B3220-wn-F07 | SAWNA253-09 | HQ599811 | <i>Archaeoattacus staudingeri</i> | SMFL | 407[0n] | ♂ | Indonesia, Sumatra |
| B3220-wn-F09 | SAWNA255-09 | GU703357 | <i>Archaeoattacus staudingeri</i> | SMFL | 658[0n] | ♂ | Indonesia, Java |
| B3220-wn-F10 | SAWNA256-09 | GU703354 | <i>Archaeoattacus staudingeri</i> | SMFL | 658[0n] | ♂ | Malaysia, Sabah (Borneo) |
| B3220-wn-F04 | SAWNA250-09 | GU703543 | <i>Samia cynthia</i> | SMFL | 658[0n] | ♂ | Japan |
| B3220-wn-F05 | SAWNA251-09 | GU703544 | <i>Samia cynthia</i> | SMFL | 658[0n] | ♂ | Japan |
| B3220-wn-F06 | SAWNA252-09 | GU703356 | <i>Samia cynthia</i> | SMFL | 658[0n] | ♀ | Japan |
| BC-Roug1035 | SATWB068-07 | HQ599815 | <i>Archaeosamia watsoni</i> | CRRR | 634[0n] | ♂ | China, Fujian |
| BC SNB 1872 | SASNB777-10 | HQ599816 | <i>Archaeosamia watsoni</i> | CSNB | 658[0n] | ♂ | China, Guangdong |
| BC SNB 1876 | SASNB781-10 | HQ579820 | <i>Archaeosamia watsoni</i> | CSNB | 658[0n] | ♂ | China, Jiangxi |

The three clusters within the genus are:

- (1) a Himalayan group of *Archaeoattacus edwardsii* specimens;
- (2) *Ar. staudingeri* from Sundaland including the Malay Peninsula (Lampe, 1984, 1985);
- (3) a group of *Ar. edwardsii* samples from non-Himalayan continental Asia (China, Thailand), including the Malay Peninsula.

These three clades, as displayed in Fig. 1, are all well

supported (bootstrap values of 100, 82 and 76 for the clusters (1), (2) and (3) described above, respectively). Clearly, *Ar. edwardsii* as currently defined (including all populations from the Himalaya, mainland Asia and the Malay Peninsula) does not form a cohesive genetic group with respect to DNA barcode data, and the genetic structure rather suggests the existence of three distinct lineages, with the lineage distributed in non-Himalayan mainland Asia and the Malay Peninsula

slightly larger, the valve apex is slightly more elongate and rounded; the phallus is slightly longer, with a slightly different shape, and its apical sclerotisation before the vesica is longer. These differences cannot easily be seen from photographs (scales are often slightly different among pictures), but when the genitalia are compared directly, they are easily visible. There is some variability in the ♂ genitalia of *Ar. malayanus* studied by us (GP WAN/SMFL no. 2130/10-2132/10; GP SNB 374/99-382/99, 394/99-395/99; Figs. 6-10), but all are smaller than *Ar. edwardsii*, and there is evidently no overlap with that species. There is almost no difference in ♂ genitalia between *Ar. malayanus* and *Ar. staudingeri* (see Fig. 11), an observation corroborating the relationships resulting from the analysis of barcode data.

The preimaginal instars of *Ar. edwardsii* from N. India (Himalaya) and of *Ar. staudingeri* from N. Sumatra were illustrated in colour by Lampe (2010: 260-261, pls. 254-255, legend p. 357) and, *Ar. staudingeri* from Borneo, by Häuser *et al.* (1996) and also (but with respect to Himalayan *Ar. edwardsii* or Assamese *Ar. malayanus*?) in several other publications. The differences in larval morphology between the Himalayan *Ar. edwardsii* and the Sundanese *Ar. staudingeri* are only minor but clear (compare the illustrations in Lampe, 2010). The preimaginal stages of the Indochinese population of *Ar. malayanus* have evidently not yet been described and illustrated in colour. Pinratana and Lampe (1990: pl. 1, fig. 1), show a larva of *Ar. edwardsii* from "N. India" (probably from the same source like in Lampe 2010?), but as the authors often do not provide exact locality data, this is not fully conclusive ("N. India" may be either the Himalayan *Ar. edwardsii* or the Assamese *Ar. malayanus*!) and requires further studies on basis of the updated taxonomy of the genus.

As a consequence of the congruent results of our molecular and morphological analyses, we propose to reinstate the previously synonymized taxon *malayanus* as a valid species within the genus *Archaeoattacus*. An updated checklist for the genus is provided below, including the geographical distribution of the three species as currently known to us.

It is interesting to note that in their later instars, the larvae of *Archaeoattacus*, as far as is known, present a unique characteristic that we consider a possible behavioural synapomorphy for the genus: they could all be considered "lumberjacks," chewing the twigs of their foodplants and letting them fall to the ground (e.g., Kuyten, 1962; Nässig, 1983; U. & L. H. Paukstadt, 1989; Häuser *et al.*, 1996). Also, the mature larvae produce a lot of silk and use it to fix many leaves, after cutting the petioles, to the twig near their cocoon, possibly to enhance its camouflage. Both traits may occasionally occur in species of the genus *Attacus*, but they have

never been reported as regular behaviour there.

Abbreviations

Abbreviations of collections:

BMNH: The Natural History Museum, London (formerly British Museum (Natural History)), U.K.
CRRR: Collection Rodolphe Rougerie, Rouen, France.
CSKK: Collection Steve Kohll, Kayl, Luxembourg.
CSLL: Collection Swen Löffler, Lichtenstein/Sachsen, Germany.
CSNB: Collection Stefan Naumann, Berlin, Germany.
CWAN: Collection Wolfgang A. Nässig, now in SMFL.
MNHN: Muséum National d'Histoire Naturelle, Paris, France.
MZB: Museum Zoologicum Bogoriense, Cibinong, Bogor, West Java, Indonesia.
NSMT: National Science Museum, Natural History, Tokyo, Japan.
SMFL: Senckenberg-Museum, Frankfurt am Main, Lepidoptera collection, Germany.
ZMHU: Zoologisches Museum der Humboldt-Universität, Berlin, Germany.

Other abbreviations and conventions:

‡Invalid and unavailable name.
 BC [no.] Barcode [with number].
 GP [no.] Genitalia dissection [with number].

ANNOTATED CHECKLIST OF THE GENUS

ARCHAEOATTACUS

Distribution data are also illustrated on a map (see Map). The doubtful records for SW India from Cotes (1891) are not included.

Archaeoattacus edwardsii (White, 1859)

Attacus edwardsii White (1859: 115, pl. Annulosa lvii); type locality [India, West Bengal], [Darjiling]. Type (syntype) in BMNH [photo examined, Fig. 2].

Distribution: Himalaya (N. India, Nepal, Bhutan, China: Tibet).

Nepal: Allen (1993: 54, central & eastern hills up to 2500 m, Pokhara valley 350 m); Haruta (1992: 93, Godavari; 1994: 159, Khosi, Pheksinda).

India, West Bengal: Darjiling, Mangpu road, 1900 m, leg. W. Thomas, BC B3220-wn-G02, GP WAN/SMFL 2127-2129/10 (CWAN in SMFL). – Uttarakhand: Masuri (Mussoorie): Cotes (1891: 73). – Arunachal Pradesh (north of the Brahmaputra river, Himalaya): Along District, near Rapum, 2000-2100 m, 28.31589° N, 94.15221° E, leg. Bretschneider, BC 1893 SNB (CSNB). Near Rapum, 2000 m, 28.53176° N, 94.24941° E, leg. Bretschneider (CSNB, CSLL). Rapum, 1800 m, 28.31995° N, 94.15325° E, leg. Bretschneider (CSNB). [For Arunachal Pradesh, see also below under *Ar. malayanus*.]

China: South Central Tibet, Linzhi (29°38'44.84" N, 94°22'22.85" E); Zhang *et al.* (1986: col. pl. 6, fig. 55 ♂).

Bhutan: Mongar Dzongkhag, 5.5 km NNW Limithang, Yonkola, 1600 m, 27°18'31" N, 91°9'48" E, leg. P. Kautt & S. Naumann (CSNB). Thimphu Dzongkhag, Mo Chu valley, 16 km NW Punakha, 1500 m, 27°41'54" N, 89°46'8" E, leg. P. Kautt & S. Naumann, BC 1891 & 1892 SNB (CSNB).

Ar. edwardsii ranges in the Himalaya from about 350 m to at least 2500 m (both extremes reported in

Nepal, Allen, 1993: 54).

♂♂ arrived in Bhutan at light between 20:00 and 23:30 h; specimens were observed flying in Nepal between vi. and viii., in West Bengal in late vi., in Bhutan in mid-vi., in Arunachal Pradesh in mid-vii.

Cotes (1891: 73) reported *Ar. edwardsii* also from Mysore (Karnataka) and the Western Ghats (cited by, e.g., Peigler, 1989: 113, but not listed by Arora & Gupta, 1979). However, we have never seen specimens from there and believe that this might be based on a misidentification. In case that there really is a population of an *Archaeoattacus* living in western and southwestern India, specimens should be barcoded to assess their identity.

***Archaeoattacus malayanus* (Kurosawa & Kishida, 1984), stat. n.**

Attacus (Archaeoattacus) edwardsii malayanus Kurosawa & Kishida (1984: 132); type locality Peninsular Malaysia, Cameron Highlands. Holotype (by original designation) in NSMT, Japan [photo examined, Fig. 3].

Formally synonymized with *Ar. edwardsii* by Brosch *et al.* (1999: 39).

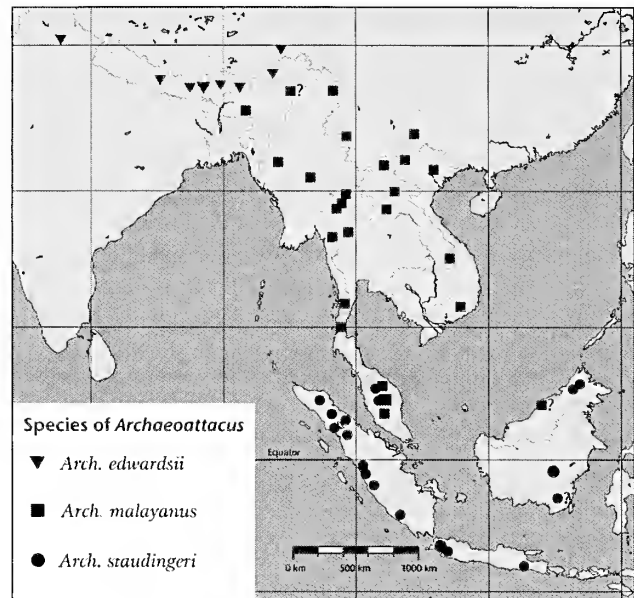
Distribution: India (Meghalaya; southern Arunachal Pradesh?, Nagaland?, no barcode results yet from these latter two); Myanmar, China, Thailand, Laos, Vietnam, Peninsular Malaysia; ?Borneo?.

India, Meghalaya: Khasi Hills, vic. Shillong, traders' material, GP WAN/SMFL no. 409/86, 2131/10-2132/10 (CWAN in SMFL). Khasi Hills, Shillong env., Mawphlang, 600-1000 m, GP 374, 375, 382/99 SNB, BC 1894 SNB (CSNB). Meghalaya, [Khasi Hills], BC SK 0207. — Arunachal Pradesh (south of the Brahmaputra river), Jairampur, [Changlang District, 20°21'5" N, 96°0'59" E; the city is at an elevation of around 880-900 m], from a photo by A. Vaidya (*in litt.* to I. J. Kitching, pers. comm.). [This specimen comes from a locality south of the Brahmaputra river valley and is most likely *Ar. malayanus*, but we have no specimens and barcode results from there.]

Myanmar, Kachin State: Nan Sa Bon, 25 km N Putao, 800 m, 27°23' N, 97°40' E, leg. S. Murzin & V. Siniaev (CSNB). Zi Yar Dam, 65 km NW Putao, 1250 m, 27°50' N, 97°1' E, GP 376/99 SNB, leg. S. Murzin & V. Siniaev (CSNB). Wa Sa Dam, 50 km NW Putao, 950 m, 27°39' N, 97°2' E, leg. S. Murzin & V. Siniaev (CSNB). Nan Thi, 50 km E Putao, 950 m, 27°31' N, 97°55' E, GP 377/99 SNB, leg. S. Murzin & V. Siniaev (CSNB). — Shan State: Shoe Pin (Shopping) Village, Utut Ni Pass, 1773 m (CSLL). Sekya Inn Village, Utut Ni Pass, ca. 10 km W Pindaya, 1712 m (CSLL). — Chin State: Mt. Victoria (Nat Ma Toung) Nationalpark, Chin Hills, Mindat City, Kall Nue, 1457 m (CSLL). — Kayin State: Dawna, NE Moulmein [Mawlamyine], ca. 1300 m, GP 378/99 SNB (CSNB, CSLL). — Tanintharyi Prov.: Tenasserim (CSLL) [no barcode yet available].

China, Yunnan: Zhu & Wang (1996). (NW), Dexin [Deying], Baimaxueshan, 4000 m [more reliable: 2500 m], leg. Ying *et al.*, BC B3220-wn-F12 (CSNB, CWAN in SMFL, CSLL). SW, Daxueshan, Yongde, 3500 m (CSLL). S, Xishuangbanna, 27 km NW Jinghong, vic. Beng Gang Ha Ni, 1800-2000 m (CSLL). — Guangxi: W. Guangxi, near E Yunnan borderline, Laogongshan, Xiling, 1800 m, leg. Li (CSNB).

Note: The altitudes given in the locality lists, especially for Chinese localities, refer mainly to the records of dealers, and we strongly suspect that altitudes given as, e.g. 4000 m or higher, for localities in Yunnan or Tibet, refer to the height of the mountain summit rather than to the real collecting locality somewhere on its slopes; serious data confirmed by European collectors range over



Map. Distribution data of the 3 species of the genus *Archaeoattacus* in SE Asia. Symbols may represent more than one locality in close proximity; not all places from the labels were located on maps. Symbols of type localities slightly enlarged. Localities with “?” mean either the locality does not appear to be part of natural distribution (*Ar. malayanus* in Borneo, Sarawak; Fukuda, 2001), or it has only been located approximately (Mt. Bayutawar, Kalimantan Selatan, CSNB), or the locality is based on a photograph only (*Ar. malayanus* in southern Arunachal Pradesh). Data compiled from literature, CWAN, SMFL, CSBN, CSLL and other sources. Map base from OMC, Martin Weinelt (www.aquarius.geomar.de/omc/, downloaded on 23. v. 2006; this address is no longer in existence), modified and localities added.

lower altitudes (see also Naumann & Nässig, 2010: 56).

Thailand, Chiang Rai Prov.: Huai Khom (CSLL). — Chiangmai Prov.: Doi Phahompok, vic. Mae Ai, 2000 m, leg. T. Ihle, BC 1890 SNB (CSNB, CSLL). Vic. Chiangmai/Doi Inthanon, no details, trader's material (Pinratana & Lampe, 1990: 4, as “*Ar. edwardsii*”; CWAN [GP WAN/SMFL 2130/10] in SMFL, CSLL [1500 m]). Doi Inthanon, road km 37.4 ranger station, 1681 m (CSNB). Doi Suthep, 1400 m (CSLL). Doi Angkhang, 1400 m (CSLL). Maetaeng, GP 379/99 SNB (CSNB). — Tak Prov.: Doi Mussoe, 812 m (CSLL). — Ranong Prov.: Isthmus of Kra, Ranong TV Station, 500 m, BC 1889 SNB (CSNB, CSLL).

Laos: Brosch *et al.* (1999: 38); Central Laos, ca. 100 km E Louangphrabang (= Luangprabang), ca. 1400-1600 m (CWAN in SMFL). Phongsaly, Gnoi-ou (CSNB). — Louang Prabang Prov.: Phou Khoun, 1500 m (CSLL). — Vientiane Prov.: Ban Viang Kham, ca. 15 km S Phou Khoun, 950 m (CSLL). — Attapu Prov.: Dak Pok (CSLL).

Vietnam, [Hoang Lien Son/Lao Cai Prov.]: Mt. Fansipan area, Chapa vic., several localities, 1600-2400 m, GP 381 & 395/99 SNB (CSNB, CWAN in SMFL). Ta Phin, 1900 m (CSLL). — [Prov.] Plato Thai Nguyen: Mt. Ngoc Linh, 15°2' N, 107°59' E, 900-1400 m (CWAN in SMFL). — Lam Dong Prov.: Bhu Son, Lam Ha, 1320 m (CSLL).

Peninsular Malaysia: Genting Highlands, 1500 m (Lampe, 1984,

1985); Cameron Highlands (Kurosawa & Kishida, 1984); U. & L. H. Paukstadt (2004: 138-140; *Ar. edwardsii* is a "highland taxon"). Cameron Highlands, leg. H. F. Wong, GP 394/99 SNB (CSNB). Cameron Highlands, rd. Ringlet-Tanah Rata, Bharat tea estate, ca. 1800 m, GP 380/99 SNB, leg. S. Naumann (CSNB). Cameron Highlands, Tanah Rata, 1400-1500 m (CSLL). Cameron Highlands, Gunung Berinchang, 1700 m (CSLL). West, Fraser Hill (CSLL). – Perak Prov.: 83 km E Gerik, 920 m (CSLL).

‡Borneo: Malaysia, Sarawak, Miri? Doubtful locality data (mislabelled traders' material or introduced population?) from Fukuda (2001: 90), see Peigler & Naumann (2003: 32), U. & L. H. Paukstadt (2006: 178-179; but also 2010: 163).

Ar. malayanus is ranging from ca. 500-2400 m.

Pinratana and Lampe (1990: 4) report specimens collected in Chiang Mai province, Thailand, in v-viii. Specimens were found in Myanmar in iii. (Tenasserim), viii./ix. (other provinces), in Yunnan in v. and other months, in S Laos in iii., in Peninsular Malaysia in xii-v. and ix. The tropical populations will probably be found at any time of the year, except during extended dry periods.

As there was never any distinction made between the Himalayan and extra-Himalayan populations of "*edwardsii*" prior to this work, and because both species are found in "North India" (i.e., *Ar. edwardsii* in the Himalaya only, *Ar. malayanus* in Meghalaya, probably southern Arunachal Pradesh and Nagaland), the literature concerning distribution, morphology and preimaginal stages (and other papers) is rather ambiguous with respect to the actual taxon treated therein. For example, the specimen from Myanmar figured by Peigler and Naumann (2003: fig. 89) as "*Archaeoattacus edwardsii*" surely is *Ar. malayanus*.

Archaeoattacus staudingeri (Rothschild, 1895)

Attacus staudingeri Rothschild (1895: 36); type locality "NW Java;" from label of lectotype: "Tjisolak, [18]92-93, G[e]relak" [6°57'0" S, 106°26'0" E, which in fact is W, but not really "NW" Java (close to the southern coast of W Java!)]. – Lectotype in ZMHU (designated by Nässig *et al.*, 1996: 25) [examined, Fig. 4].

Distribution: Sundaland (Sumatra, Peninsular Malaysia, Borneo, Java). – Peigler and Wang (1996: 59) suspected *Archaeoattacus staudingeri* to also live on the island of Bali, based on a picture on a T-shirt. However, this has not been subsequently confirmed (see Peigler & Naumann, 2003: 47), and we believe that the species does not inhabit Bali. From the eyespots shown on the T-shirt image, we suppose that a specimen or, more likely, a photograph of *Archaeoattacus edwardsii* or *Ar. malayanus* was the model for that (not quite naturalistic) depiction.

Indonesia, Java: West Java, Tjisolak, G[e]relak, Java occ. mer. [= NW Java], type locality (of the lectotype); Gn. Halimun National Park, 900-1000 m; Gn. Gedeh, 1000 m; Goalpara, 1500 m (all in MZB, see U. Paukstadt *et al.*, 2008: 229, 231). East Java, Malang, 1200 m, leg. U. & L. H. Paukstadt, BC 1884 & 1885 SNB (CSNB, CWAN in SMFL).

Indonesia, Sumatra: Aceh (= Nanggroe Aceh Darussalam), several localities (Nässig *et al.* 1996: 25-26; U. & L. H. Paukstadt 2009: 339). – Sumatera Utara, Sibolangit Plateau (van den Bergh 1915). Several localities not far from Lake Toba (e.g., Sindar Raya, 300 m; Naga Raja, 350 m; "Holzweg 3" near Prapat, 1150 m; "Sipirok III", 1300 m, leg. E. W. Diehl (Nässig *et al.*, 1996: 25-26). – Sumatera Barat, Bukittinggi [formerly Fort de Kock] (van Eecke

1930). Padangpanjang, 775 m (Nässig *et al.*, 1996: 25-26). Padang, 800 m (SMFL). Mt. Singgalang, 1000 m, leg. S. Jakl (CSNB). Mt. Intan, Solok region, 900 m, (CSNB). Mt. Sanggul, Landai village, 1300 m, leg. S. Jakl (CSNB). 3 km NE Landai, 1200 m, leg. S. Jakl, BC 1887 SNB (CSNB). Mine Tambang, 6 km N Padangaro, 460 m (CSLL). Mt. Korinji (Kerinci), 3 km S Padangaro, 1000 m (CSLL). – Lampung, Pagar Alam, in MZB (Nässig *et al.*, 1996: 25-26, U. Paukstadt *et al.*, 2008: 231). Mt. Pesagi (CSLL).

Indonesia, Borneo, Kalimantan: Kalimantan Tengah, Gn. Payang, 400[-800] m, ca. 0.87742° S, 115.07975° E, BC 1886 (CSNB, CSLL). Kalimantan Selatan, Mt. Bayutawar, 400-800 m (CSNB). Mt. Meratus, 500-700 m (CSLL).

Malaysia, Borneo, Sabah: Mt. Kinabalu area: Taman Kinabalu Park Headquarters (1200-1500 m), Poring Hot Springs (600 m), Sayap (1000-1100 m) (Häuser *et al.*, 1996: 171) (CWAN in SMFL, CSLL). Mt. Trus Madi, 1100[-1600] m, GP 383/99 SNB, BC 1888 SNB (CSNB, CWAN in SMFL, CSLL). Mt. Marapok (CSNB).

Malaysia, Peninsula: Cameron Highlands, 900 m (Lampe, 1984, 1985; CSNB); U. and L. H. Paukstadt (2004: 140-141; *Ar. staudingeri* is a "typical lowland taxon"). – Perak: 30 km E Gerik, 420 m (CSLL).

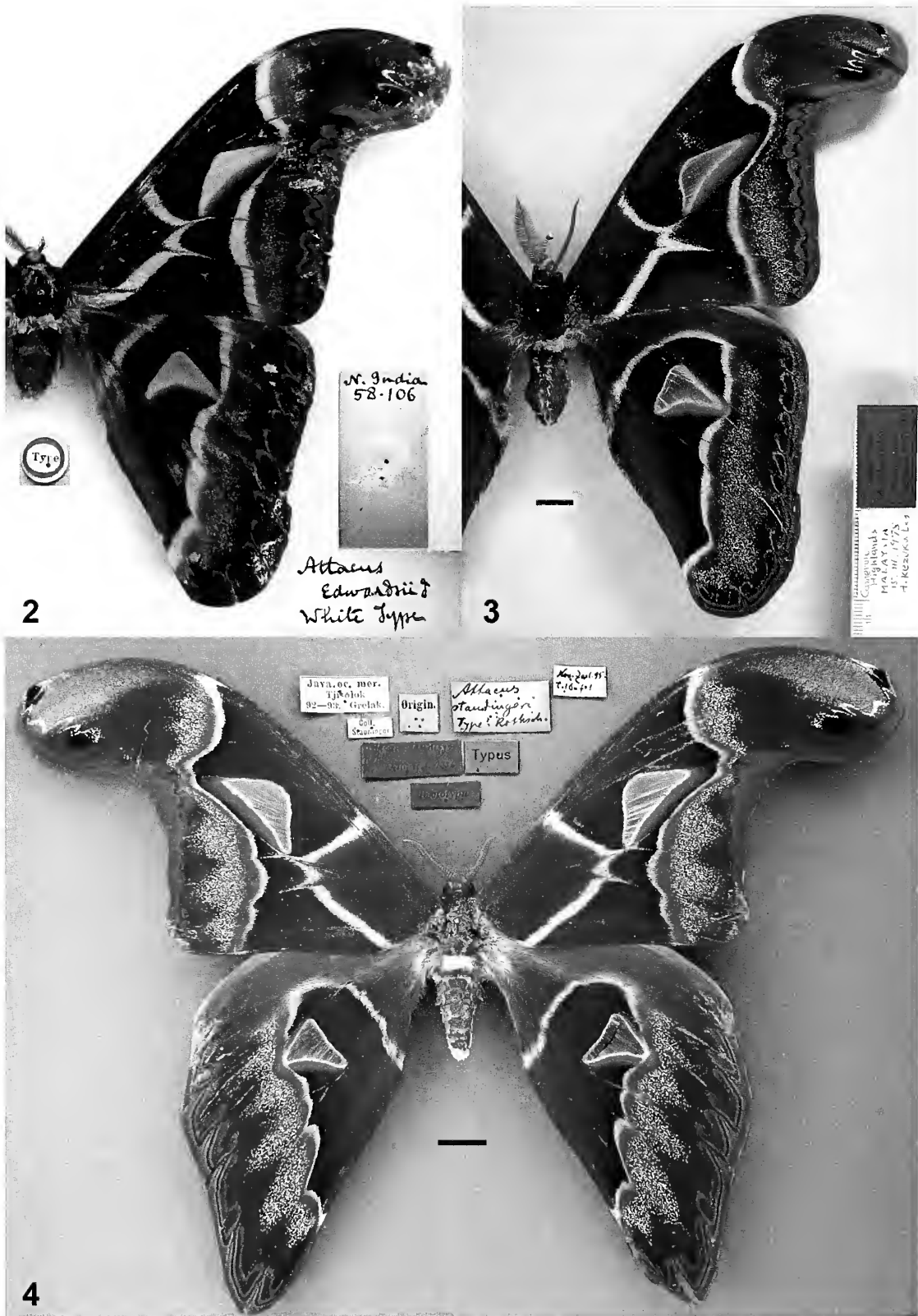
Ar. staudingeri ranges from about 300 to 1800 m elevation (both extremes observed on Sumatra: Nässig *et al.*, 1996: 25-26; U. & L. H. Paukstadt 2009: 339). Observations took place in iii. in Borneo, vi-x. in Sumatra, ix. in Perak. The tropical populations will probably be found at any time of the year, except during extended dry periods.

Ar. staudingeri was never seriously doubted as being a distinct species; the differences in external imaginal (and larval) morphology are quite clear. Our results based on DNA barcodes, reporting that the species is genetically closer to *Ar. malayanus*, were then rather unexpected.

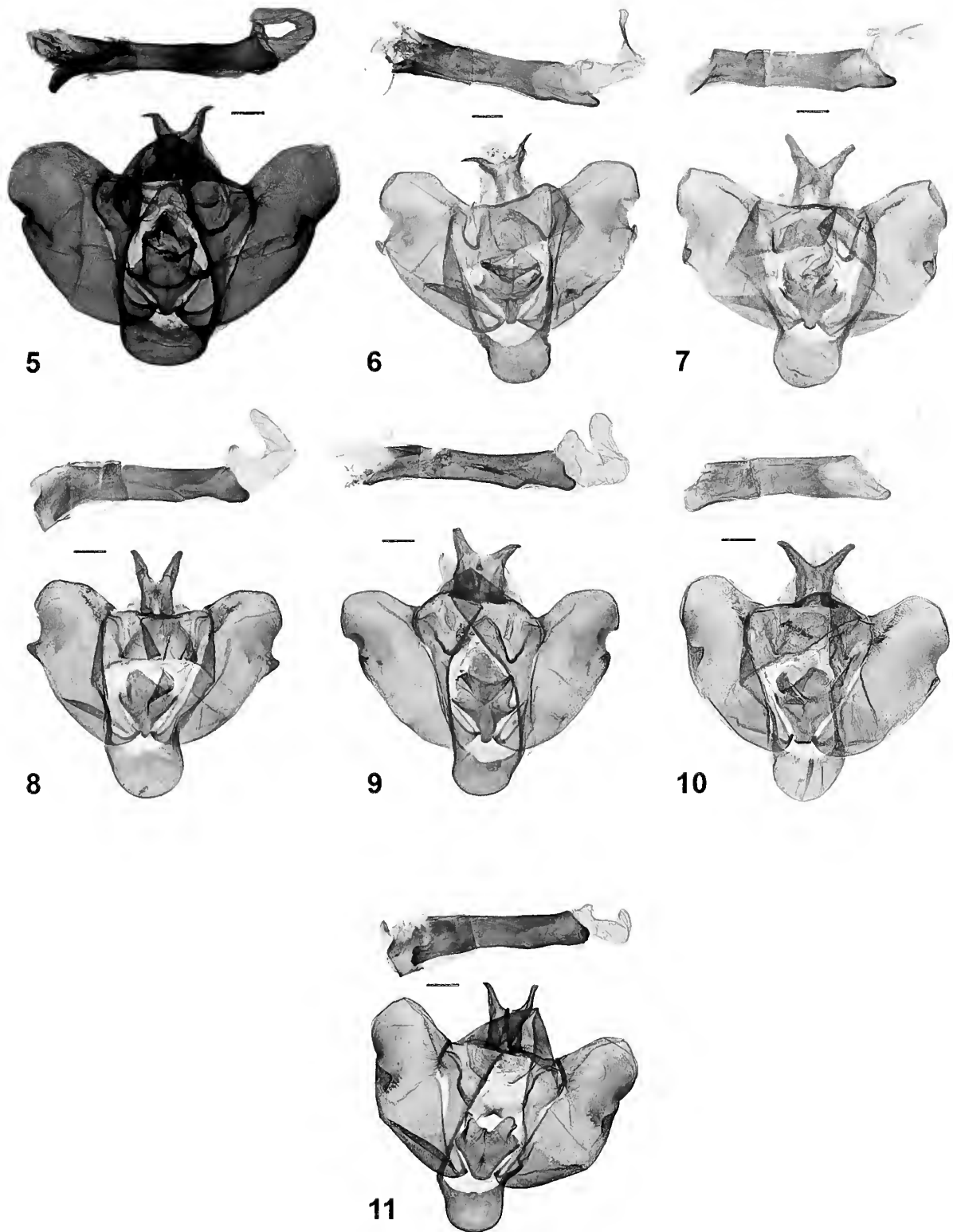
DISCUSSION

Although not supported by the collecting data at the lowest altitudes (which are very similar in all three taxa), the general trait attributed to *Archaeoattacus edwardsii* (including *Ar. malayanus*) of preferring higher elevations and *Ar. staudingeri* preferring lower elevations (U. & L. H. Paukstadt, 2004: 140-141) is probably correct when considering all the data for the many specimens observed, and when considering the highest elevation records at which these taxa have been recorded.

Considering the variation of DNA barcode sequences within each of the three species now recognized within the genus *Archaeoattacus*, it is now interesting to observe a peculiar geographic structure shared by *Ar. malayanus* and *Ar. staudingeri*. The few samples sequenced from the Malay Peninsula (in the case of *Ar. malayanus*, including also the Isthmus of Kra, Thailand, Ranong prov.; we do not yet have barcode data for the specimen from Myanmar, Tenasserim) diverge significantly from those of other conspecific populations (up to 2% and 1.7% within *Ar. malayanus*



Figures 2-4. 2. Syntype ♂ of *Attacus edwardsii* White, 1859, BMNH (photo © The Natural History Museum, London). 3. Holotype ♂ of *Attacus (Archaeoattacus) edwardsii malayanus* Korosawa & Kishida, 1984, NSMT (photo V. Zolotuhin). 4. Lectotype ♂ of *Attacus staudingeri* Rothschild, 1895, ZMHU (photo S. Naumann). Specimens not to the same scale; scale bars (where present) = 1 cm.



Figures 5-11. ♂ genitalia of *Archaeoattacus* species. 5. *Ar. edwardsii*, India, West Bengal, Darjiling, GP 2127/10 WAN. 6. *Ar. malayanus*, Peninsular Malaysia, GP 394/99 SNB. 7. *Ar. malayanus*, Myanmar, Dawna, GP 378/99 SNB. 8. *Ar. malayanus*, N. Thailand, GP 379/99 SNB. 9. *Ar. malayanus*, N. Vietnam, GP 381/99 SNB. 10. *Ar. malayanus*, India, Meghalaya, Khasi Hills, GP 375/99 SNB. 11. *Ar. staudingeri*, Borneo, Malaysia, Sabah, GP 383/99 SNB. Genitalia not exactly to the same scale; scale bars = 1 mm. Scans ex CSNB: U. Brosch. Phot. 5: W.A.N.

and *Ar. staudingeri*, respectively). There is no evidence that such divergence reflects an actual separation of lineages deserving a particular taxonomic status, but it is very interesting to observe that in both cases, the divergence occurs in the region where the two species live in sympatry. Further sampling would be necessary to confirm this pattern. The observed divergence may be caused by some degree of geographical isolation of populations inhabiting the Malay Peninsula, or alternatively we may be observing an interesting case of incipient speciation driven by reinforcing natural selection in the contact zone between the two species (Hoskin *et al.*, 2005).

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Atkins, A. F. 1975. The life history of *Anisynta tillyardi* Waterhouse and Lyell (Lepidoptera:Hesperidae: Trapezitinae). Australian Entomological Magazine 2: 72-75.

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PAPERS

The hilltopping mating system of the duskywing skipper *Erynnis tristis* (Lepidoptera: Hesperidae)

John Alcock

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1

Constant eyespot display as a primary defense – survival of male and female emperor moths when attacked by blue tits

Adrian Vallin, Sven Jakobsson and Christer Wiklund

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9

Two instances of inter-generic mating by Lycaenidae (Lepidoptera) in Maharashtra, India

Milind Bhakare and Peter Smetacek

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23

Feasibility of a combined sampling approach for studying caterpillar assemblages – a case study from shrubs in the Andean montane forest zone

Florian Bodner, Stefanie Mahal, Maren Reuter and Konrad Fiedler

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27

Evidence for the existence of three species in the genus *Archaeoattacus* (Lepidoptera: Saturniidae)

Wolfgang A. Nässig, Stefan Naumann and Rodolphe Rougerie

Published online 21.12.2010

37

NOTES

Natural epizootic of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson infecting *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila México

Claudio Ríos-Velasco, Ernesto Cerna-Chávez, Sergio Sánchez Peña and Gabriel Gallegos-Morales

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7

Collecting and eating *Liphyra brassolis* (Lepidoptera: Lycaenidae) in southern Thailand

Rod Eastwood, Pitoon Kongnoo and Manus Reinkaw

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19