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Cover illustration: Male Gulf Fritillary, *Agraulis vanillae* (Linnaeus), nectaring on its primary foodplant in Florida, passion-vine, *Passiflora incarnata*, Gainesville, FL Oct. 17, 2014. Photo by Charles V. Covell Jr.

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COLORATION AND STRUCTURE OF THE WINGS OF *CHORINEA SYLPHINA* BATES

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ABSTRACT. The structure and the origin of transparency of the wings of *Chorinea sylphina*, a species of glasswinged butterflies, were explored using optical microscopy, scanning electron microscopy, UV photography, spectrophotometry, and optical polarimetry. We found that for normally incident light, the clear transparent areas of the forewings and hindwings exhibit significant transmission as well as minuscule reflection throughout the visible regime in the electromagnetic spectrum. We found that the transparency results from the sparsity, the semitransparency, and the upright orientation of single scales on the wing membrane. The red and dark brown colors of the nontransparent areas of the wings have a pigmentary origin. Coherent scattering from the slanted and overlapping lamellae in the scale ridges and diffraction from every scale's longitudinal network of parallel ridges are responsible for blue iridescence and shimmer at large viewing angles. The transparent areas of the wings function as absorbing linear polarizers, due to both the parallel ridges and the almost unidirectional orientation of individual scales on those areas.

Additional key words: butterflies, transmittance, reflectance, anti-reflection, polarization

While biologists associate the colors of living creatures with mate finding and/or camouflage against predators (Hill 2010), engineers and physicists are increasingly attracted to the intense and often iridescent colors displayed by some tropical butterflies, such as of the genus *Morpho*, because of the potential to reproduce those colors with engineered microstructures rather than via pigments (Kinoshita 2008, Saito 2011, Dushkina & Lakhtakia 2013). The production and use of pigments must be reduced, if not eliminated altogether, because these activities release vast amounts of volatile organic compounds into the biosphere (Brown et al. 1990, Yu & Crump 1998, Leung et al. 2005), causing both chronic and acute distress to humans and other life forms (Mølhave 1990, Bailey et al. 2010, Casals-Casas & Desvergne 2011, Nurmatov et al. 2015).

The diversity and origin of the butterflies' bright iridescent coloration have been extensively studied over the last few decades (Vukusic 2006, Berthier 2007, Kinoshita 2008), and applications have been sought for cosmetics, textiles, security paper, and automotive paints (Saito 2011, Dushkina & Lakhtakia 2013, Poncelet et al. 2015). Comprehensive research focused on the detailed structure of single scales and their contribution to the overall colors in a wing (Kinoshita & Yoshioka 2005, Vukusic 2006, Giraldo 2007, Kinoshita 2008), and led to sophisticated bioinspired nanostructures and bioreplication techniques (Huang et al. 2006, Saito et al. 2006, Martin-Palma et al. 2008, Saito et al. 2012, Gupta et al. 2015, Zobl et al. 2016).

Some researchers are now showing interest in the reasons for and the physical mechanisms underlying clear (i.e., colorless) transparency, which is rare among lepidopterans. Among clear-winged butterfly species, examples are *Greta oto* (Hall 1996) and *Pteronymia zerlina* (Bolaños Martinez et al. 2011), both from the family Nymphalidae, tribe Ithomini, as well as *Chorinea sylphina* Bates of the Riodinidae (Bates 1867-68). Studies of their habitat and life style in captivity (i.e., in butterfly houses) as well as in the wild show that the clear transparent wings easily camouflage the butterfly at rest and also protect it in flight from predatory birds (Johnsen 2001).

The clear transparent wings of *G. oto* were the first to be investigated for their structure, due to the remarkably low haze and reflectance over the portion of the electromagnetic spectrum that is visible to humans (Binetti et al. 2009, Siddique et al. 2015). Scanning electron microscopy (SEM) revealed that the

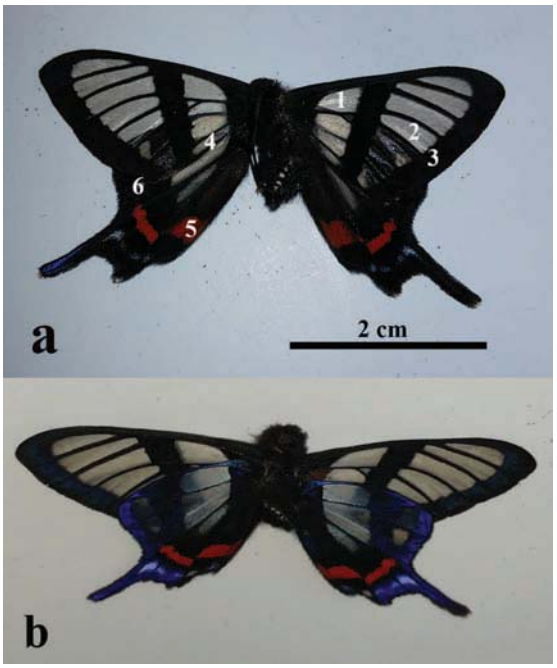

FIG. 1. *Chorinea sylphina*. (**a**) Dorsal view at a small viewing angle. (**b**) Ventral view at a large viewing angle. The investigated areas of the wings are numbered in Fig. 1a as follows: (**1**) inner transparent, (**2**) outer transparent, and (**3**) dark areas of the forewing; (**4**) inner transparent, (**5**) red, and (**6**) outer transparent areas of the hindwing.

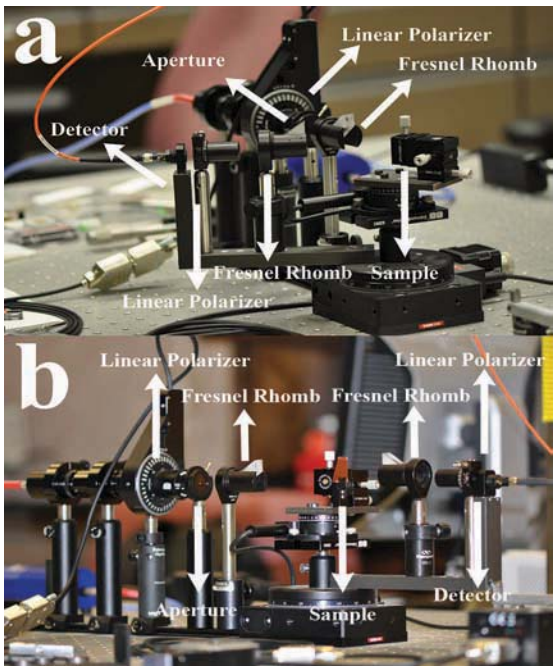

FIG. 2. Variable-angle spectroscopic system configured to measure the (**a**) circular reflectances and (**b**) circular transmittances of a sample. The same configurations but with both Fresnel rhombs removed were used to measure the linear reflectances and linear transmittances.

membrane of any transparent part of a wing is covered by miniature bristles, also called microtrichia, of height and thickness about 2 μm and 40 μm, respectively, and spaced 40–50 μm apart from each other. Similar microtrichia were observed also in other butterflies and were associated with improving the hydrophobicity of the wings (Kristensen & Simonsen 2003, Wanasekara & Chalivendra 2011). Furthermore, SEM analysis revealed the presence of quasi-randomly positioned, high-aspect-ratio nanopillars on the transparent parts, while 2D Fourier power spectra of the top-view SEM images unveiled the random height and width distribution of those nanopillars. Reflection-and-transmission spectrophotometry was used to confirm that this quasi-random nanostructure is responsible for the remarkable broadband and omnidirectional anti-reflection properties of the transparent parts of the wings (Siddique et al. 2015).

An interesting and rare example of a clear-winged butterfly found in Ecuador, Peru, and Bolivia is *Chorinea sylphina* of the Riodinidae family, known also as *sylphina angel*. It was first classified as *Zeonia sylphina* by H. W. Bates in 1867–68 but has been known by its present name after a reclassification that took place earlier than 1910. The *Chorinea* species live in the subtropical broadleaf forests of the Andean mountains at 2000–3000 m altitudes. All four wings of *C. sylphina* have distinctive patterns that feature transparent regions contoured by dark brown, almost black, "veins" and peripheral regions. The other seven existing species in the *Chorinea* genus have clear wings with the same basic pattern as *C. sylphina*, but differ in the configuration and extent of the red/yellow markings on their hindwings. Their beauty is revealed in flight in full sunshine, when the transparent parts of their wings glitter with iridescent green, blue, magenta and golden hues.

The intriguing combination of natural transparency and shimmering colors of the wings of *C. sylphina* drew our attention. Since, to our knowledge, the structure and the origin of the coloration on *C. sylphina* wings seem to have not been studied yet, they became the subject of our investigation reported in this paper.

We used optical and scanning electron microscopy, ultra-violet (UV) photography, spectrophotometry, and polarimetry to analyze the structure and coloration of the wings of *C. sylphina*. Our findings about the *C. sylphina* transparency are compared here with the results for *G. oto* reported by Binetti et al. (2009) and Siddique et al. (2015). In addition, we found that the transparent areas of the *C. sylphina* wings function as UV reflectors and linear polarizers of light.

MATERIALS AND METHODS

Specimen. Ten dead *C. sylphina* specimen were bought from Insect Design (Redbank, QLD, Australia; www.insectdesigns.com). All specimen had red markings on the hindwings. Dorsal and ventral views of the butterfly are shown in Figs. 1a and 1b, respectively. The butterfly has four wings (wingspan of 4.5 cm) with distinct pattern of dark-lined transparent areas on both forewings and hindwings (areas numbered 1, 2, 4 and 6 in Fig. 1a) and red areas on the hindwings (area numbered 5 in Fig. 1a).

Optical Microscopy. The wing structure was observed using (i) an optical microscope (Model 420T-430PHF-10, National Optical Instruments, Schertz, TX, USA) with 10X magnification and (ii) a polarization microscope (Nikon Eclipse LV100D-U, Tokyo, Japan) with objectives Lu Plan Fluor 10X/0.30 (with calibration 0.340 µm/pixel) and Lu Plan Fluor 20X/0.45 (with calibration 0.170 µm/pixel). The samples were observed in epi- and dia- illumination when placed between parallel and crossed polarizers.

Scanning Electron Microscopy. The fine structure of the butterfly wings was studied by a scanning electron microscope (FEI Nova NanoSEM 630, Hillsboro, Oregon, USA). For that purpose, pieces of the different parts of the butterfly wings were processed in a liquid-nitrogen environment and the mounted samples were coated with a gold film of thickness of few nanometers.

Spectrophotometry. The transmittance and reflectance spectra of the transparent areas of the forewings and hindwings of *C. sylphina* were measured with a custom-made spectroscopic system shown in Fig. 2 and described in detail in the Appendix. The spectra were obtained for illumination with unpolarized, linearly polarized, and circularly polarized light. All four linear transmittances (T_{ss}, T_{pp}, T_{sp}, and T_{ps}), linear reflectances (R_{ss}, R_{pp}, R_{sp}, and R_{ps}), circular transmittances transmittances (T_{RR}, T_{LL}, T_{RL}, and T_{LR}), and circular reflectances (R_{RR}, R_{LL}, R_{RL}, and R_{LR}) were measured. Transmittances and reflectances with both subscripts different denote depolarization (Collett 2005, Lakhtakia & Messier 2005).

Polarimetry. Transmission measurements were carried out over the visible regime in the electromagnetic spectrum using a modification of the custom-made spectroscopic system shown in Fig. 2, as described in the Appendix.

RESULTS AND DISCUSSION

Wing anatomy by optical and scanning electron microscopy. Figures 3–5, obtained using optical and scanning electron microscopies, revealed that on both

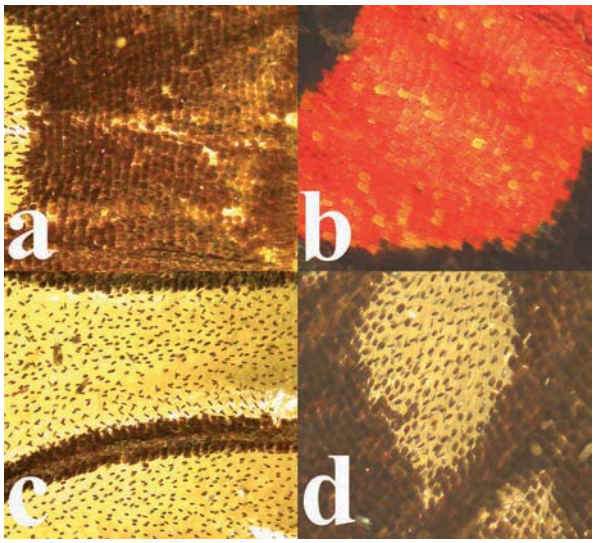

FIG. 3. Photographs of *C. sylphina* wings viewed through an optical microscope (10× magnification). (**a**) Dark area 3 of the forewing, (**b**) red area 5 of the hindwing, (**c**) outer transparent area 2 with black "veins" of the forewing, and (**d**) outer transparent area 6 of the hindwing. The numbered areas are identified in Fig. 1a.

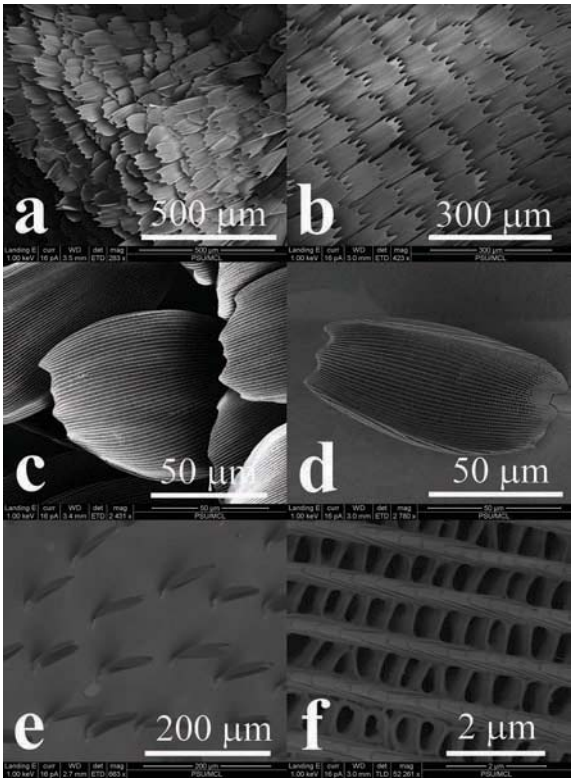

FIG. 4. SEM images of (**a**) the outermost black part of area 3, (b) arrays of scales on the red parts of area 5, (**c**) the black part close to the transparent area 2, (**d**) a single scale on the inner transparent area 4, (**e**) scales on the inner transparent area 1, and (**f**) the microstructure of a scale from the black region of the hindwings. The overlapping lamellae on the ridges function as Bragg mirrors, and the crossribs between adjacent ridges function as diffraction gratings.

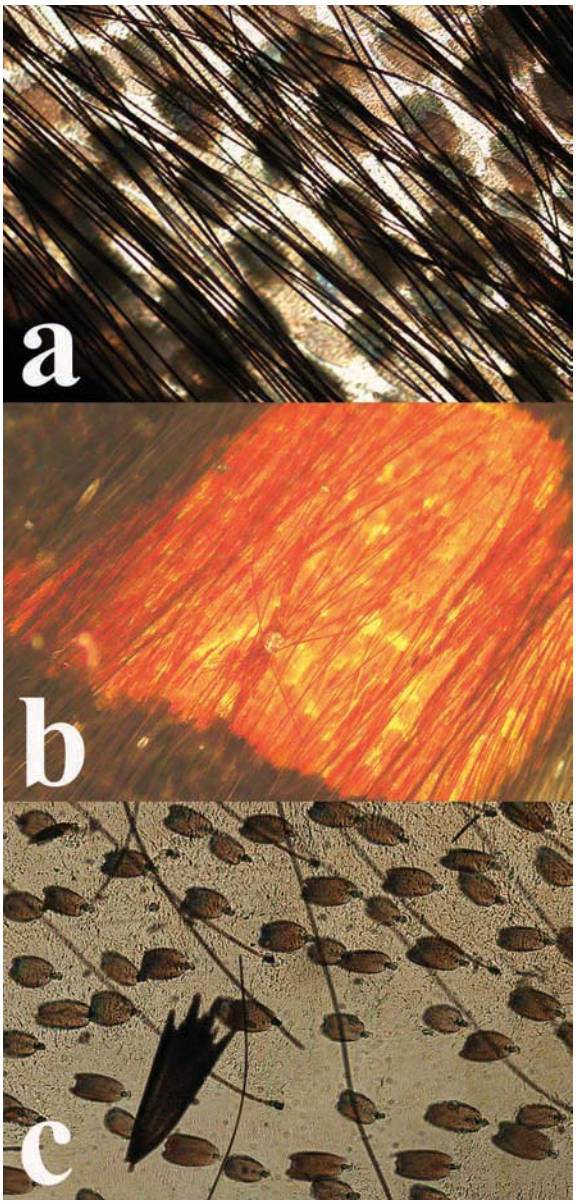

FIG. 5. (**a**) Photograph of hair on the semi-transparent area 6, taken through a polarization microscope with objective Lu Plan Fluor 10x/0.30 and a digital camera; image size 1275×1020 µm. (**b**) 10× magnified photograph of red area 5 taken with an optical microscope. (**c**) Photograph of hair on the large transparent area 4, taken through a polarization microscope with objective Lu Plan Fluor 10x/0.30 and a digital camera; image size 1275×1020 µm.

the ventral and dorsal sides, the wing membrane is covered with flat scales of similar dimensions and microstructure. The inner nontransparent areas, i.e., the dark area 3 (Fig. 3a) and the red area 5 (Figs. 3b and 4b), display nearly perfect arrays of almost identical flat rectangular scales of 127±33 µm length and 79±6 µm width, partially overlapping like shingles on a roof (Fig. 4b). The dark outermost areas of the wings, as well as the veins, are densely covered with loosely stacked rectangular fork-shaped scales (Figs. 3c, 3d, and 4a). In contrast, the scales on the hindwing tails are triangular in shape, with the largest median being 196±8 µm in length, as exemplified by the large scale shown in Fig. 5c. These scale dimensions are typical for Lepidoptera (Ghiradella 1989, 1991; Simonsen & Kristensen 2010) and agree with the dimensions of about 50 µm along the minor axis and 200 µm along the major axis of the brown and white oval scales that cover the nontransparent parts of the *G. oto* wings (Binetti et al. 2009, Siddique et al. 2015).

The wing membrane in the clear transparent areas on both the dorsal and ventral sides of the *C. sylphina* wings is sparsely dusted with semitransparent scales (Figs. 3c and 3d) which do not differ in morphology from the scales that cover the nontransparent black and red areas. Single scales are loosely aligned as checkers on a chessboard, separated by a distance of about 140 µm (about the width of two scales) from their closest neighbors. All scales are aligned in the same direction (Fig. 4e), which is the direction of the black veins, i.e., from the butterfly's body towards the edge of the wings. In the large transparent areas, the single scales bend upward from the glass membrane surface and curl inward along the length of the scale (Figs. 4d and 4e). Therefore, their presence does not impair significantly the visual transparency of the clear areas. Since no structural and dimensional difference were observed between the scales on the clear transparent and the nontransparent areas, one could assume that the scales on the former are the result of evolutionary shredding that left behind a transparent membrane.

The smallest area in the transparent parts of the *C. sylphina* wings that we were able to analyze reliably with SEM was 15×15 µm. At this magnification, we did not observe fine nanopillars. In contrast, nanopillars were seen on the transparent parts of *G. oto*'s wings, but those were imaged with roughly 36 times higher magnification (Siddique et al. 2015). We are planning further investigation by transmission electron microscopy (TEM) and SEM at higher resolution in order to clarify the membrane morphology for *C. sylphina*.

The hindwings of *C. sylphina* are entirely covered with brown hair of cross-sectional thickness about 6 µm and length exceeding 1 mm (Figs. 5a–c). The hair cover both the transparent areas 4 and 6 as well as the black and red areas, and are more densely distributed in the portions of area 6 closer to the dark boundaries than on the central part of area 4 (Fig. 5c). Such hair were not observed on the forewings of *C. sylphina*. The *C. sylphina* hair are longer, thicker, and more densely

distributed than the microtrichia found on the wings of *G. oto* (Binetti et al. 2009, Siddique et al. 2015). Similar microtrichia are also found on the wings of *Actias luna*, a lime-green, nearctic saturniid moth in the family Saturniidae (Wanasekara & Chalivendra 2011). In addition, the *A. luna* microtrichia cover fork-shaped scales similar in shape and size to those on the wings of *C. sylphina*. While the microtrichia in both *G. oto* and *A. luna* are randomly oriented, the much longer and thicker hair on the hindwings of *C. sylphina* are well aligned along the direction of the fork-shaped scales. The presence of microtrichia and hair, respectively, on the scales must cause optical scattering (Bohren & Huffman 1983) and result in the translucence of the wings of *A. luna* and the hindwings of *C. sylphina*.

Morphology of single scales. Single scales from both the clear transparent areas and the colored areas of the *C. sylphina* wings comprise well-defined longitudinal parallel ridges connected by crossribs (Figs. 4d and 4f), as is known for numerous lepidopterans (Ghiradella 1989, 1991). The average distance between adjacent ridges is 1.65±0.04 µm for the scales found in the black areas and 1.42±0.09 µm for the scales found in the transparent areas. The average distance between neighboring crossribs is 0.62±0.02 µm. The crossribs between two adjacent ridges function together as diffraction gratings (Dushkina & Lakhtakia 2013). The ridges are ornamented by partially overlapping lamellae (Fig. 4f). These stacks constitute a multilayered structure which functions as a Bragg mirror (Dushkina & Lakhtakia 2013) to produce interference colors, as becomes clear from Fig. 6a.

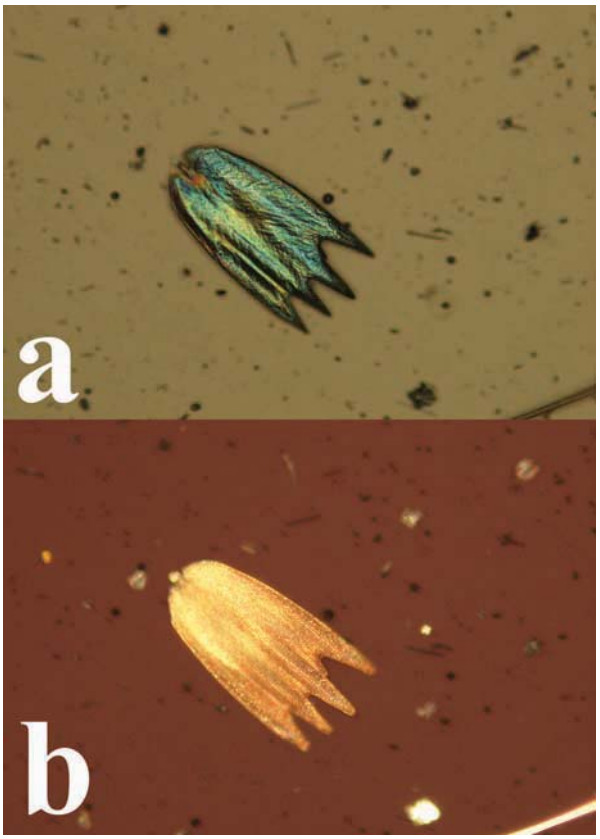

Fig. 6. Color of a single scale from a hindwing of *C. sylphina*, as viewed in epi illumination through a polarization microscope with 20× magnification when the scale was placed between either (**a**) a polarizer and an analyzer with parallel axes of light transmission, or (**b**) two crossed polarizers.

Colors of scales. When viewed in transmitted light, the single scales of *C. sylphina* display a deep brown color indicative of dopamine-derived melanin, a pigment commonly found in lepidopterans (Koch 1995). The scales are semitransparent (Fig. 5c) but, when piled up as in Fig. 4a, produce the darkness of the veins and the outer/peripheral areas of the wings. In the wings of pierid butterflies, pterin pigments are present as granules attached to the crossribs across the scale ridges (Ghiradella 1991, Wijnen et al. 2007, Giraldo & Stavenga 2008). SEM micrographs of the *C. sylphina* wings with four times higher magnification than in Fig. 4f did not show any pigment granules attached to the crossribs. This implies that pigment is distributed throughout the cuticle, which is common in some butterfly families (Allyn & Downey 1977, Stavenga et al. 2004, Wilts et al. 2011).

Yellow, orange, and red colors in many species are of pigmentary origin (Nijhout 1997, Giraldo 2007, Wijnen et al. 2007). For example, xanthopterin and erythropterin produce yellow and orange/red wing coloration by absorbing light in the blue and green portions of the visible regime, respectively (Wijnen et al. 2007). The orange/red coloration of area 5 (Fig. 1a) on the *C. sylphina* wing is also due to the presence of a light-absorbing pigment, as we proved by soaking a hindwing for 3 h in a 5% aqueous solution of sodium bicarbonate ($NaHCO_3$). Within the first half hour of soaking, the red area became milky white and the black areas became light brown.

Erothropterin fluoresces orange/red and is found in butterfly wings. *Vespa orientalis*, a wasp with a large yellow stripe across the body, uses xanthopterin as a light-harvesting pigment to transform solar radiation into electrical energy (Plotkin et al. 2010). Experiments with radiolabelled tryptophan have revealed that red and some brownish pigments are ommochromes such as ommatin (Martel & Laws 1991, Koch 1995). The red bands on the dorsal hindwings of the *Precis coenia* butterfly are almost pure ommatin D, while the orange red marks on the dorsal forewings of the same species are composed of xanthommatin and dihydro-

FIG. 7. Photograph of a wing of *C. sylphina* illuminated with 365-nm UV light.

xanthommatin (Nijhout 1997). The red pigment in area 5 (Fig. 1a) might be one of these pigments, but detailed chemical analysis is necessary for identification.

The black and red areas of the wings of *C. sylphina* exhibit brilliance and shimmer at large viewing angles, an effect which is distinctive for structural colors. The brilliance arises from the orderly arrangement of the scales in the colored areas (Figs. 3a, 3b, and 4b), causing multilayer-interference enhancement of the red color along with diffraction shimmer by the ridges on the scales when viewed at large angles (Dushkina & Lakhtakia 2013).

When bathed in direct sunlight and viewed at large angles, the clear and black areas of the wings of *C. sylphina* shine with a bluish-purplish shimmer (Fig. 1b). The effect is more pronounced in area 4 of the hindwings than on the forewings. However, at normal incidence, the clear parts are highly transparent (Fig. 1a). The play of colors at large viewing angles is typical for structural (i.e., non-pigmental) colors (Dushkina & Lakhtakia 2013) and is due to the ridged morphology of the individual scales (Zhang et al. 2014). The ridged morphology and dimensions of the *C. sylphina* scales are very similar to those of scales of butterflies in the *Morpho* genus (Kinoshita & Yoshioka 2005, Saito 2011) and produce similar bluish iridescence (Fig. 6a). The single scales on the clear areas of the wings of *C. sylphina* are sparsely distributed in a somewhat ordered unidirectional manner creating a pseudo-structure of tiny diffraction gratings. The sunlight glancing on this microstructure is subjected to wavelength-selective coherent scattering from the diffraction micropattern (Berthier 2007, Dushkina & Lakhtakia 2013), thereby producing the brilliant blue reflections.

Some lepidopterans with bright colored wings have scales with UV patterns for attracting mates (Allyn & Downey 1977, Aardema & Scriber 2014). To check the occurrence of that phenomenon in *C. sylphina*, a forewing was illuminated with UV light of wavelength λ_0 = 365 nm from a UV lamp (Model UVGL-25, Mineralight® Lamp multiband UV 254/365 nm, 115V, 60 Hz, 0.16 A). Figure 7 shows strong bluish reflectance from area 1 (Fig. 1a), similarly to the effect reported by Aardema & Scriber (2014).

Reflection and transmission spectrophotometry. Angle-resolved spectroscopy was used to study the optical reflection and transmission characteristics of the clear transparent areas 1, 2, and 4 (Fig. 1a). Figure 8 compares the linear transmittances $T_s = T_{ss} + T_{ps}$ and $T_p = T_{pp} + T_{sp}$ of area 4 for $\lambda_0 \in [400, 900]$ nm, when light hits the sample at an angle $\theta \in [0^o, 70^o]$ with respect to the normal to the plane of the sample. Both T_s and T_p increase gradually and smoothly as λ_0 increases from the UV regime to the near-infrared regime in the electromagnetic spectrum, regardless of the angle of incidence θ. The measured transmittance T_{unpol} for unpolarized incident light displays the same characteristic. The fact that T_p exceeds T_s throughout the whole visible regime indicates an optical-polarization phenomenon discussed later in this section.

Figure 9a compares spectra of the transmittance T_{unpol} of the clear transparent areas 1 and 2 for normally incident ($\theta = 0^o$) unpolarized light. Viewed through an optical microscope, area 2 has fewer scales compared to area 1, which justifies the higher transmittance for area 2. Most likely, the clear transparent areas of the wing membranes of *C. sylphina* are made of α-chitin plus proteins and possibly alkaloids depending on the butterfly's food, just as has been found for *G. oto*, *Godyris duillia*, and *Vanessa cardui* (Binetti et al. 2009). Indeed, the negligible light-absorption characteristics of bulk α-chitin for $\lambda_0 \in [250, 750]$ nm (Azofeifa et al. 2012) are responsible for the uniformly high transmittance of the clear transparent areas in the *G. oto* wings (Binetti et al. 2009).

According to Fig. 9a, the transmittance T_{unpol} of the clear transparent areas of *C. sylphina* wings increases by about 20% through the visible regime wherein bulk α-chitin exhibits negligible light-absorption characteristics (Azofeifa et al. 2012). The adult *C. sylphina* sips nectar from flowers of evergreen plants such as *Prionostemma* spp. (Hippocrataceae) and *Maytenus* spp. (Celastraceae), and migrates up and down mountains to

follow the seasonal changes and the corresponding floral blooms. It is possible that specific alkaloids in the food of *C. sylphina* increase the absorption of light at the blue end of the visible regime, and thereby enhance transmission at the red end of the visible regime. A similar explanation has been proffered for *G. duillia* and *V. cardui* (Binetti et al. 2009). Moreover, light absorption by melanin, the dark brown pigment presumably present in the scales, decreases steadily as λ_0 increases (Riesz 2007), which could also assist in enhancing transmission of red light over blue light.

Figure 9b shows the measured values of the transmittance T_{unpol} of area 4 for $\theta \in \{0^o, 10^o, 20^o, 30^o, 40^o, 50^o, 60^o, 70^o\}$ when λ_0 = 500 nm and the incident light is unpolarized. Also shown in that figure are the values of the sum $(T_s + T_p)/2$ calculated from the standard Fresnel formulas (Born & Wolf 1980) for a 550-nm-thick film (Siddique et al. 2015) of α-chitin of refractive index 1.552 (Leertouwer et al. 2011); the transmittance values from the Fresnel formulas were multiplied by a factor of 0.755 $cos^2\theta$ to account for (i) the absorption and scattering of light from the individual scales sparsely distributed in area 4 and (ii) the elliptical area of illumination of the sample when θ is large. Transparency requires weak absorption, weak reflection, and weak scattering of light. Absorption by melanin and scattering by the single scales distributed sparsely on the clear transparent areas, as well as reflection from the chitin-rich membrane, reduce the transmittance T_{unpol} at normal incidence to about 0.37 for λ_0 = 500 nm (Fig. 9b).

For incident unpolarized light, the measured reflectance of the clear transparent area 1 for $\theta \in \{10^o, 60^o, 70^o\}$ was below 0.01 through the whole visible spectrum. When viewed obliquely at large angles θ in direct sunlight, the dark areas of the wings display the visually intense blue iridescence shown in Fig. 1b. We hoped to detect a blue shift in the reflectance spectrum from the dark parts on increasing θ, but conclusive results were not obtained since the intensity levels of the experimentally obtained reflectance spectra were very small (< 0.004) and quite noisy. The ultralow reflectances of the clear transparent area 1 for a wide range of θ over the entire visible spectrum are in agreement with the 0.02 value of reflectance at $\theta = 65^o$ reported for *G. oto* wings (Siddique et al. 2015).

Spectra of the linear transmittances (T_{pp}, etc.) and linear reflectances (R_{pp}, etc.) of the dorsal transparent parts of the wings of *C. sylphina* were found to be the same, within experimental uncertainty, as those measured of the ventral transparent parts. This result was expected since our optical and SEM investigations showed no morphological differences between the ventral and dorsal transparent areas of the wings of *C. sylphina*.

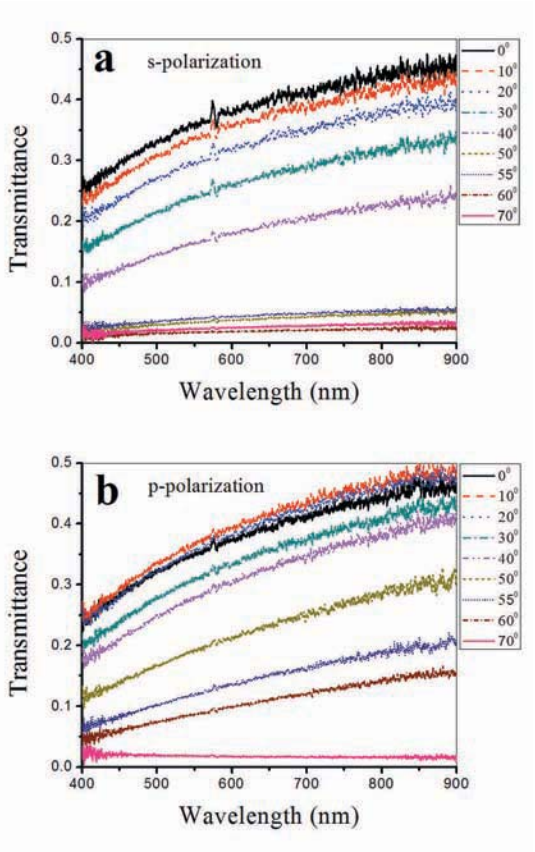

FIG. 8. Measured spectra of the linear transmittances (**a**) $T_s = T_{ss} + T_{ps}$ and (**b**) $T_p = T_{pp} + T_{sp}$ of the clear transparent area 4 for angles of incidence $\theta \in [0^o, 70^o]$.

Optical-polarization phenomenon. Biophotonic structures found on the wing scales of some iridescent beetles (such as *Chrysochroa fulgidissima*) and butterflies (such as *Papilio blumei*) can polarize incident sunlight due to multiple inner reflections from multilayered structures (Berthier 2007, Stavenga et al. 2011). The polarization phenomenon and intrinsic color-mixing properties of bionanostructures are of special interest for anti-counterfeiting measures (Poncelet et al. 2015).

The surfaces of the wing membranes of *G. oto* are decorated with high-aspect-ratio nanopillars, which are responsible for reflection reduction and clear transparency (Binetti et al. 2009, Siddique et al. 2015). However, transmission electron microscopy did not reveal any structural details in the cross-section of the wing membrane, which would imply that no optical-polarization phenomenon is likely to be observed in the *G. oto* wings.

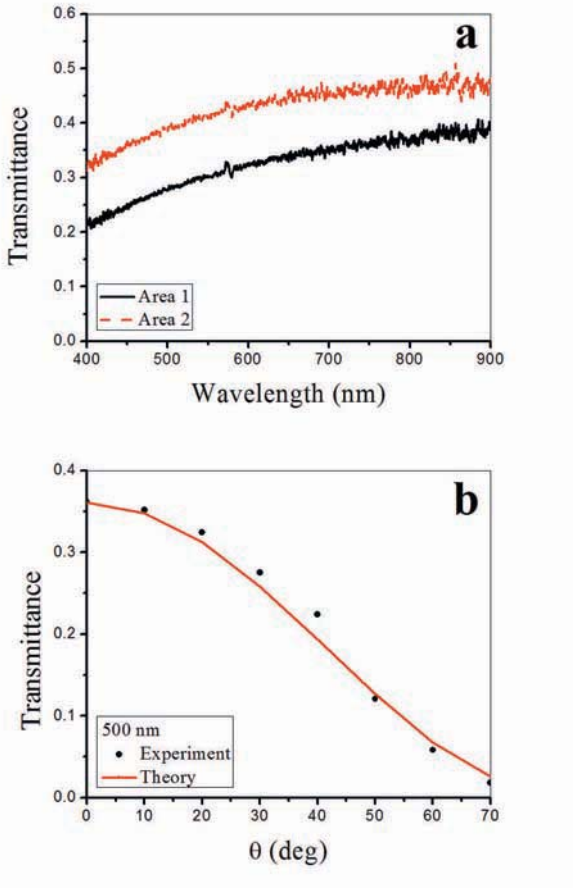

FIG. 9. (**a**) Measured spectra of the transmittance T_{unpol} of the clear transparent areas 1 and 2 for normally incident ($\theta = 0^o$) unpolarized light. (**b**) Circles: measured values of T_{unpol} of area 4 for $\theta \in [0^o, 70^o]$ and $\lambda_0 = 500$ nm, when the incident light is unpolarized. Solid line: predicted values of $(T_s + T_p)/2$ of a 550-nm-thick layer of chitin for $\theta \in [0^o, 70^o]$ and $\lambda_0 = 500$ nm.

In contrast, our studies using a polarization microscope showed that the wings of *C. sylphina* display an optical-polarization phenomenon. In general, polarized colors are produced by materials with periodic variation in refractive index in one or two dimensions. In butterflies, this effect is mainly related to the ridged microstructure of the wing scales (Zhang et al. 2014). In order to produce Fig. 6a, the scale was placed between a polarizer and an analyzer with parallel axes of transmission; the same scale was placed between crossed polarizers in order to produce Fig. 6b. Thus, these two figures show that the intense iridescent color of a single scale is linearly polarized by the periodic array of parallel ridges.

Next, we performed experiments with normally incident ($\theta = 0^o$) circularly polarized light. We found that the circular reflectances (R_{LL}, etc.) were minuscule in magnitude. Furthermore, $T_{LL} = T_{RR}$ and $T_{RL} = T_{LR}$, within experimental uncertainty, for $\lambda_0 \in [400, 900]$ nm. These results were expected since cross-sectional SEM images had not revealed any helical structures (De Silva et al. 2005) in the wing membranes that could discriminate between incident left- and right-circular polarization states (Lakhtakia & Messier 2005).

To further study the optical-polarization effect from the *C. sylphina* wings, we carried out polarimetry experiments (Collet 2005). Measured values of T_{pol} of the transparent area 1 for normally incident ($\theta = 0^o$) linearly polarized light are shown in Fig. 10 as functions of the angle of polarization $\theta_p \in [0^o, 360^o)$ for $\lambda_0 \in \{480, 520, 600\}$ nm, with $\theta_p = 0^o$ corresponding to the *p*-polarization state. Clearly, T_{pol} is an oscillating function of θ_p. As the forewing sample was positioned with the black veins almost parallel to the initial position of the transmission axis of the linear polarizer ($\theta_p = 0^o$) and T_{pol} is maximum at about $\theta_p = 80^o$ for all three wavelengths, the polarization axis of the wing membrane is almost perpendicular to the black veins.

In order to model the experimental data in Fig. 10, we adopted the following methodology. When unpolarized light is incident upon two ideal linear polarizers with transmission axes at an angle θ_p, the irradiance *I* transmitted through the second polarizer is given by Malus' law $I = I_0 \cos^2 \theta_p$, where I_0 is the irradiance of the incident unpolarized light (Born & Wolf 1997). In our polarimetry experiments, the transparent wing membrane, playing the role of a second polarizer, served as an analyzer. In addition, the measured values of T_{pol} were normalized with the irradiance measured after the first polarizer, i.e., *p*-polarized light (not the irradiance of the incident unpolarized light). To account for this and also for the fact that the wing membrane is not an ideal polarizer, the experimental data in Fig. 10 were fitted to the modification

$$T_{pol} = T_o \cos^2(\theta_p) + T_b \quad (1)$$

of Malus' law, where T_b represents the transmittance of the wing membrane which is not influenced by the state of polarization of the incident light and T_0 is the amplitude of modulation due to the wing's optical polarization characteristics. The experimental data were fitted with $T_0 = 0.029$ for all three wavelengths, and $T_b = 0.3$, 0.33, and 0.37 for $\lambda_0 = 480$, 520, and 600 nm, respectively. The differences between the experimental data and the corresponding solid curve for the same value of λ_0 in Fig. 10 indicates that the transparent wing membrane has an optical role additional to that of a polarizer.

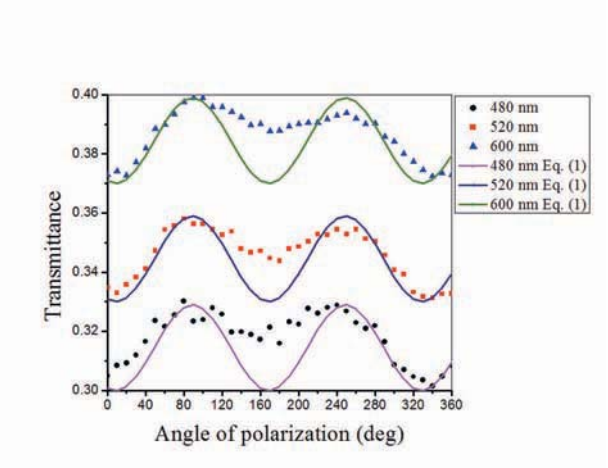

FIG. 10. Transmittance T_{pol} of area 1 measured as a function of the angle of polarization θ_p for normally incident linearly polarized light.

In conclusion, we have investigated the morphology of the wings of the glass-winged butterfly *C. sylphina* to determine the origin of transparency. Spectrophotometry experiments showed that a minuscule fraction of the intensity of normally incident light is reflected whereas about 40% of the intensity of normally incident light is transmitted by the clear transparent areas in the wings, over the entire visible regime. This transparency is a result of: (1) the sparse distribution of single scales on the wing membrane, (2) the semitransparency of the individual scales, and (3) the upright orientation of the scales that leaves much of the wing membrane uncovered. It is possible that a large number of scales fell off the transparent areas of the wings during eclosion. The red and dark brown colors of the nontransparent areas of the wings arise from pigments. The scales' blue iridescence and shimmer at large viewing angles result from coherent scattering from the slanted and overlapping lamellae in the scale ridges and the diffraction from every scale's longitudinal network of parallel ridges. Polarimetry experiments showed that the transparent areas of the wings function partially as linear polarizers, due to both the parallel ridges and the almost unidirectional orientation of individual scales on those areas. The same areas also function as partial absorbers of incident light. Further studies with transmission electron microscopy and analytical chemistry are necessary to clarify all the mechanisms of the coloration of the wings of *C. sylphina*.

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SEE APPENDIX ON NEXT PAGE

APPENDIX

Spectrophotometry. The transmittance and reflectance spectra of the transparent areas of the wings of *C. sylphina* were measured with a custom-made computer-controlled variable-angle spectroscopic system depicted in Fig. 2. A halogen light source (HL-2000, Ocean Optics, Dunedin, FL, USA) was used to illuminate the sample. For the transmittance measurements, a butterfly wing was mounted on a metal holder with a circular opening. This setting allowed us to obtain various transmittances of the sample directly without using a glass substrate for the sample. The sample was mounted on a rotatable stage that allowed sample positioning in a specific orientation with angular precision of 5 arc minutes. Measurements were obtained from all transparent areas of the forewings and hindwings illuminated with unpolarized, linearly polarized, or circularly polarized light. For illumination with unpolarized light, white light from the halogen source was guided through an optical fiber and made to impinge directly on the sample. For illumination with linearly polarized light, the sample was positioned between two Glan–Taylor linear polarizers. The first polarizer (GT10, ThorLabs, Newton, NJ, USA) was used to select the linear polarization state (either p or s) of the light impinging on the sample. In the s-polarization (p-polarization) state, the electric (magnetic) field vector of the incident light is oriented perpendicular to the plane of incidence. The second polarizer (GT5, ThorLabs) was used as an analyzer and was set so that the exiting light was either p-polarized or s-polarized.

The exiting light was detected using a CCD spectrometer (HRS-BD1-025, Mightex Systems, Pleasanton, CA) for free-space wavelength $\lambda_0 \in [400, 900]$ nm. For normalization, the light intensity received by the CCD camera in the absence of the sample and the second polarizer was used. All four linear transmittances (T_{ss}, T_{pp}, T_{sp}, and T_{ps}) were measured. The first subscript in T_{sp} denotes the s-polarization state of the transmitted light and the second subscript denotes the p-polarization state of the incident light, and similarly for the other three linear transmittances. Thus, transmittances with both subscripts the same are co-polarized transmittances, whereas those with both subscripts different are cross-polarized transmittances. A non-zero cross-polarized transmittance (or reflectance) indicates depolarization of incident light by the sample (Collet 2005; Lakhtakia & Messier 2005). By virtue of the principle of conservation of energy, no reflectance or transmittance can exceed unity, and there is a similar constraint on appropriate sums of reflectances and transmittances (Lakhtakia & Messier 2005).

The experimental setup for illumination with circularly polarized light is shown in Fig. 2a, with two Fresnel rhombs (LMR1, Thorlabs) used right before and after the sample in addition to the two linear polarizers. All four circular reflectances (R_{RR}, R_{LL}, R_{RL}, and R_{LR}) were measured. The first subscript in R_{RL} abbreviation denotes the right-circular polarization (RCP) state of the reflected light and the second subscript denotes the left-circular polarization (LCP) state of the incident light, and similarly for the other three circular reflectances. The intensity of light received by the CCD spectrometer in the absence of the sample and the second polarizer-rhomb combination was used as the reference. The Fresnel rhombs were removed and the linear polarizers were set appropriately to measure the linear reflectances R_{ss}, R_{pp}, R_{sp}, and R_{ps}. All eight reflectances of the clear transparent areas turned out be less than 0.01, indicating extremely weak reflection of light by the clear transparent areas on the wings.

Figure 2b presents the configuration used to measure the circular transmittances T_{RR}, T_{LL}, T_{RL}, and T_{LR}. Appropriate changes were made to measure the linear transmittances T_{ss}, T_{pp}, T_{sp}, and T_{ps}. For unpolarized incident light, no polarizers and Fresnel rhombs were used, and the transmittance T_{unpol} measured by the CCD spectrometer was recorded.

Polarimetry. For polarimeteric measurements, the incident light was polarized, with the intensities of the p- and s-polarized components in the ratio $\cos^2\theta_p$:$\sin^2\theta_p$, where the angle of polarization $\theta_p \in [0^o, 360^o)$ (Collet 2005). The first polarizer was set to select the angle of polarization θ_p, while the second polarizer was absent. Also, neither Fresnel rhomb was present. The CCD spectrometer measured the transmittance T_{pol} as a function of θ_p and λ_0.

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REPORT OF DIURNAL ACTIVITY IN MIMALLONOIDEA WITH NOTES ON THE SEXUAL BEHAVIOR OF *LACOSOMA CHIRIDOTA* GROTE, 1864

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ABSTRACT. We report diurnal behavior in the mimallonid *Lacosoma chiridota* Grote, 1864. Female pheromone releasing behavior was recorded in the late afternoon in Gainesville, Florida. A single diurnal male was recorded responding to a "calling" female at 1745 h. We discuss the unique case of sexual dimorphism exhibited by *L. chiridota*, such that most Mimallonidae generally do not display pronounced sexual dimorphism, and suggest that this may be related to the likewise unique diurnal behavior of this species.

Additional keywords: Lepidoptera of Eastern North America, Sexual dimorphism

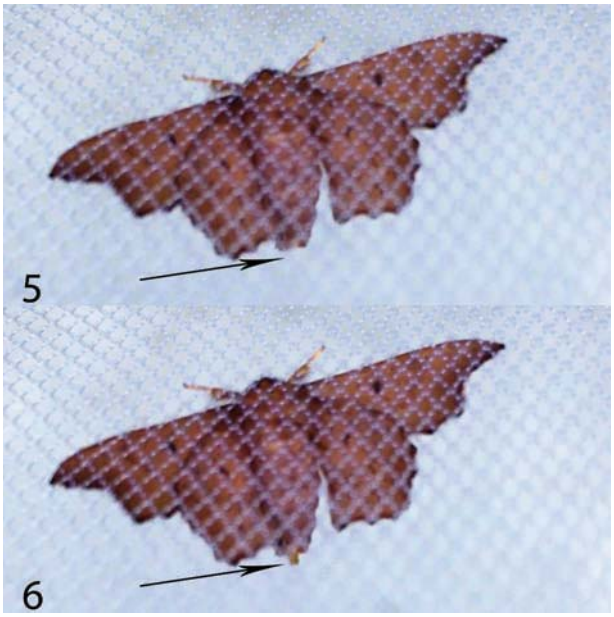
The superfamily Mimallonoidea containing the single family Mimallonidae, which is restricted to the New World, includes about 300 species (Becker 1996, St Laurent unpublished), with four species occurring north of Mexico (Franclemont 1973). One of the two North American *Lacosoma* Grote, 1864 species, *L. chiridota* Grote, 1864 (Figs 1, 2, 5–7), has a broad range in eastern North America and can be expected anywhere with oak (*Quercus* L., Fagaceae), the host plant, east of the Great Plains. Records exist from southern Ontario, southern New England, south to Florida, west to the Plains and south-central Texas (Franclemont 1973, Moth Photographers Group 2017). The natural history of *L. chiridota* was described by Dyar (1900), with a focus primarily on the immature stages. *Lacosoma chiridota* is often listed and reported in many Lepidoptera-related publications that cover eastern North America (Covell 2005, Wagner 2005, Handfield 2011). This species displays strong sexual dimorphism which is more pronounced than in most other Mimallonidae (compare Figs 1, 2 to 3, 4 for example). There was no study focused on the adult behavior of this species or any other Mimallonidae. No species of Mimallonidae were known to be diurnal, and data regarding the sexual behavior of this superfamily are not published.

The purpose of the research was to determine if *L. chiridota* exhibits diurnal behavior. Our hypothesis was that males of *L. chiridota* are infrequent at lights because they are diurnal. Females of *L. chiridota* are more often taken at light than the males (Franclemont 1973, Covell 2005, St Laurent pers. obs.). In all major entomological collections visited by the first author, the number of female *L. chiridota* far outnumber the males. The opposite has been observed for the majority of Mimallonidae species in collections.

MATERIALS AND METHODS

Utilizing captive reared females of *L. chiridota*, we investigated the period of pheromone release by the female, and a subsequent wild male response. All specimens utilized in this study resulted from eggs collected from a wild-caught female at UV light on 20.VII.2016, from USA: Florida: Alachua County: Gainesville, Austin Cary Forest, 29.732039°, -82.219913°. We reared the larvae on a potted *Quercus shumardii* Buckland through to the final two instars, at which time the larvae were transferred to cut *Q. shumardii* branches placed in water. Pupae were formed within the larval sacks, which are structures consisting of leaves, silk, and frass. The sacks containing the pupae were maintained outdoors until adults eclosed. Five males were killed and vouchered before their activity commenced. Females were moved to separate screened cages after emerging. On different days in October 2016, three captive reared females were monitored in order to register pheromone release periods and male response in Gainesville, Florida at the University of Florida, Natural Area Teaching Lab (NATL), 29.633191°, -82.368669° or in a residential area near NATL.

FIGS. 1–4. Adults of *Lacosoma*, a=recto, b=verso. **1.** *L. chiridota* male, USA, Texas, Anderson Co., Bethel, Engling Wild Life Management Area, 16.IV.1968, J.G. Franclemont leg. (Cornell University Insect Collection, Ithaca, NY, USA [CUIC]). **2.** *L. chiridota* female, USA, Virginia, Arlington Co., Arlington, 21.VI.1950, J.G. Franclemont leg. (CUIC). **3.** *L. arizonicum* male, USA, Arizona, Santa Cruz Co., Santa Rita Mts, Santa Rita Canyon, 4880 ft., 7.VII.1959, J.G. Franclemont leg. (Carnegie Museum of Natural History, Pittsburgh, PA, USA [CMNH]). **4.** *L. arizonicum* female, data as for Fig. 3 but 2.VII.1959 (CMNH). Scale bar= 1 cm.



FIGS. 5, 6. Adult female of *Lacosoma chiridota*, pheromone releasing behavior, arrow points to ovipositor. USA, Florida, Alachua Co., Gainesville, 14.X.2016, about 1815 h. **5.** Ovipositor retracted. **6.** Ovipositor extended.

Specimens from this experiment are deposited in the research collection of Ryan St Laurent, Gainesville, Florida, USA, the research collection of Richard Peigler, San Antonio, Texas, USA, and in the molecular collection of the McGuire Center for Lepidoptera & Biodiversity, Gainesville, Florida, USA.

RESULTS

Both males and females of *L. chiridota* eclosed from pupae generally between 1400 h and 1600 h EDT. Depending on the time when the females emerged, they would begin a brief flight within the cage starting between 1500 and 1650 h. Eventually the females would alight at the top of the cage and immediately commence pheromone releasing behavior (usually around 1700 h), consisting of a cyclic extension/retraction of the ovipositor from the tip of the abdomen (Figs 5, 6). A single cycle of extension to retraction to re-extension occurred roughly once every second. If unmated, the female would continue this behavior for several hours, until sunset. Throughout the night and morning hours this behavior was not detected, although we did not monitor the females between 2400 h and 0800 h.

On October 14th, 2016, we began to monitor the female at 1745 h, and this was when a single male was observed hovering around the caged female (Fig. 7) for several minutes. Daylight was still obvious during this observation, which took place more than an hour before sunset (sunset on 14.X.2016 was 1859 h). We did not allow the male to enter the closed cage and eventually he flew away. Observation continued until 1808 h and no additional males were attracted.

On October 15th, 2016, the same female was set in the same location in Gainesville, FL, and she started releasing pheromones at 1658 h. Pheromone release did not start until after ongoing rains subsided. No males arrived despite continuous observation. Intensifying rain resulted in the female performing rapid flight within the cage, with pheromone release behavior not restarting until after rain weakened substantially.

DISCUSSION

Prior to this work, diurnal behavior in Mimallonidae was not mentioned in the literature. However, we are aware of anecdotal reports from Carlos and Olaf Mielke of Brazil that males of *Cicinnus funebris* (Schaus, 1896) are also diurnal, where numerous individuals were witnessed in response to a virgin, calling female during the daytime (C. Mielke pers. comm.). We report here the diurnal pheromone releasing behavior of female *L. chiridota* as well as the diurnal male response to a

FIG. 7. Adult male of *Lacosoma chiridota* responding to "calling," caged female, USA, Florida, Alachua Co., Gainesville, University of Florida, Natural Area Teaching Lab, 14.X.2016, 1749 h.

"calling" female. This behavior confirms our hypothesis and suggests a possible explanation for this unique case of sexual dimorphism in Mimallonidae. In most other species of Mimallonidae, such as those of the genera *Menevia* Schaus, 1928 and other *Lacosoma*, females are larger than males, with broader wings and larger bodies. However, maculation usually does not differ substantially between the sexes as it does so dramatically in *L. chiridota* (St Laurent pers. obs.). For comparative purposes, we here illustrate *L. arizonicum* Dyar, 1898 (Figs 3, 4), a species that exhibits the degree of sexual dimorphism more commonly encountered in Mimallonidae. It is interesting to note that sexual dimorphism and very dark (nearly black) coloration of males is also apparent in the only other mimallonid known to be diurnal, *C. funebris*.

Research suggests that sexual dimorphism may be a result of male-limited mimicry and/or divergent selection pressures on males and females due to the sexes experiencing different ecological interactions (e.g. flying during the day versus the night) (Allen et al. 2011). Similarly, dark, diurnal males and lighter colored, nocturnal conspecific females are known in other families of moths, such as in the Saturniidae genus *Callosamia* Packard, 1864 (Tuskes et al. 1996). We therefore consider the possibility that sexual dimorphism, specifically the dark color of the male, and diurnal behavior in *L. chiridota* may be related.

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THE SPATIAL DISTRIBUTION AND OVIPOSITION PREFERENCE OF THE RANCHMAN'S TIGER MOTH, *PLATYPREPIA VIRGINALIS* (LEPIDOPTERA: EREBIDAE)

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ABSTRACT. Despite decades of research on Ranchman's tiger moth (*Platyprepia virginalis*), little is known about the behavior and ecology of the adult life stage. To address this knowledge gap, we conducted surveys to quantify the spatial distribution of moths, and conducted laboratory and field oviposition assays as well as a field oviposition survey. We found that *P. virginalis* exhibits hilltopping behavior, a mate-locating strategy where individuals congregate on hilltops to increase the likelihood of sexual encounters. This behavior is common across many insect orders, but there are few examples of moths exhibiting this behavior. We found no evidence supporting our hypothesis that bush lupine (*Lupinus arboreus*), the primary larval hostplant within our study site, is the preferred oviposition hostplant. The opportunistic discovery of egg clutches on seaside daisy plants (*Erigeron glaucus*) led us to conduct a no-choice larval feeding assay to determine its suitability as a hostplant. We found that larvae reared on *L. arboreus* were more likely to survive compared to those reared on *E. glaucus*.

Additional key words: Hilltopping, Erebidae, mate choice, oviposition, larval survival

The Ranchman's tiger moth, *Platyprepia virginalis* (Lepidoptera: Erebidae) (Boisduval 1852), is a large, aposematically colored diurnal moth. The larva is covered with setae; late-instars are usually orange with a black band across the center part of the body with many long white hairs. *P. virginalis* ranges from Monterey Bay, California to Southern British Columbia and as far inland as Colorado (Ferguson 2000). Along the coast of California, the flight period most commonly extends from June through August. Eggs hatch shortly after oviposition in summer, but caterpillars do not become conspicuous until the following March and April, when they become large and mobile. To date, most of our ecological knowledge of *P. virginalis* comes from study of larval populations occurring within the Bodega Marine Reserve (BMR), Sonoma County, California. Researchers studying the population dynamics of the larval stage have assumed that the population is spatially structured into isolated sub-populations that correspond with the fragmented distribution of the primary larval hostplant, bush lupine (*Lupinus arboreus*: Fabaceae; English-Loeb et al. 1993, Karban and English-Loeb 1997). The density of caterpillars is highest and most temporally stable (i.e., less prone to localized extinction events) in patches of *L. arboreus* around low-lying, marsh habitat (Karban et al. 2012). This is due in part to lower predation rates of early instar caterpillars by ants in *L. arboreus* patches adjacent to marshes. Experiments demonstrated that the complex habitat substrate associated with marshes provides more predation refuges for young caterpillars (Karban et al. 2013).

Despite decades of research on this species, little is known about the biology and behavior of the adult life stage. Though a metapopulation framework has been used to understand the population dynamics of this system (e.g., Karban et al. 2012), the spatial distribution and dispersal ability of adult moths have never been determined. Similarly, despite the tight association between caterpillars and *L. arboreus*, it is not known whether this is the preferred oviposition plant. To address these knowledge gaps, we conducted surveys to quantify the spatial distribution of moths. We also conducted laboratory and field oviposition assays as well as an oviposition survey. Additionally, the opportunistic discovery of egg clutches on seaside daisy plants (*Erigeron glaucus*: Asteraceae) led us to conduct a no-choice larval feeding assay to determine its suitability as a hostplant.

MATERIALS AND METHODS

Adult Surveys

The initial distribution of tiger moths within BMR was determined using the line transect method (Pollard 1977). A network of transects was established across the reserve using established trails and walked weekly at a constant pace (10 m/min) between 10:00 and 15:00 hours during the 2010 and 2011 flight seasons (2010, June–September; 2011, June only). The GPS coordinates of moths encountered (within a 20m radius of observer) along transects were recorded as well as proximity to areas of local topographic prominence (hereafter hilltops). We manually classified observed individuals into those found on hilltops and those at least 20 m from the base of the hill (i.e., the lowest approximate contour line). To determine if moths occurred more frequently on hilltops, we compared the proportion of moths on hilltops to the proportion of surveyed habitat classified as hilltop using a Chi-square test.

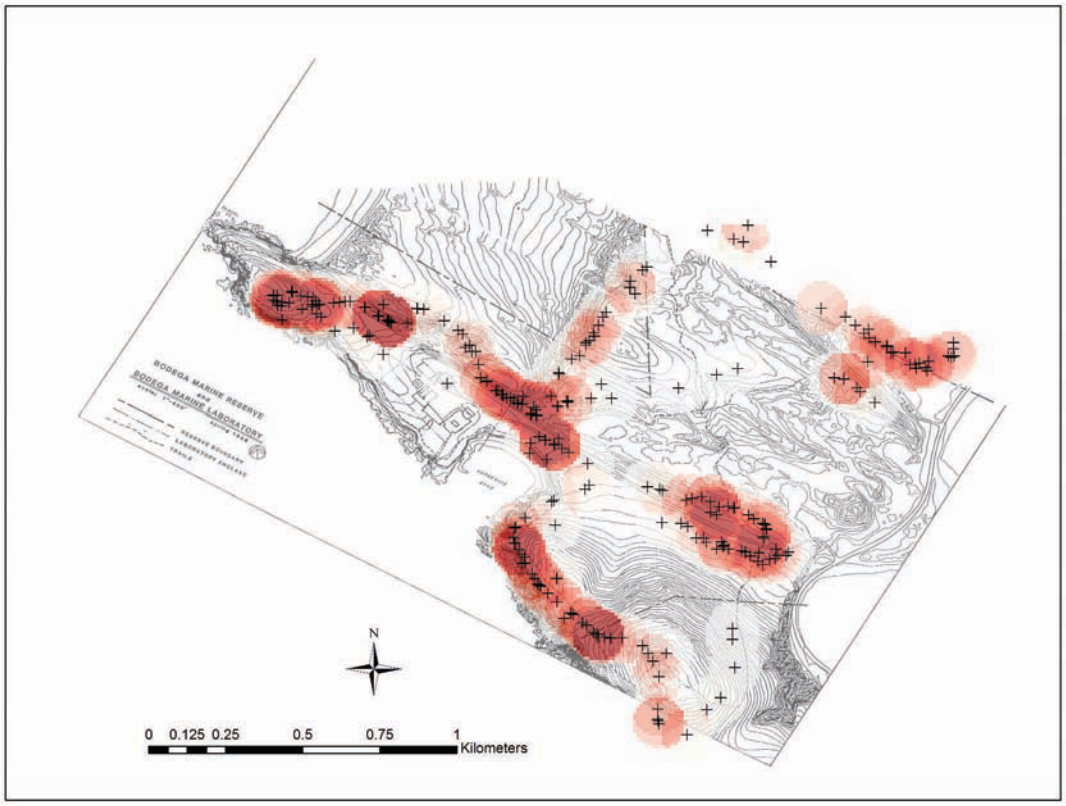

FIG. 1.Topographic map of the Bodega Marine Reserve showing moth encounters from Pollard transects. Shading intensity is directly proportional to moth density, as each encounter symbol may represent several individuals..

Oviposition preference and host plant suitability

Egg clutches are cryptic and we have rarely observed oviposition. Despite our working hypothesis that *L. arboreus* is the preferred oviposition plant due to the tight association of *P. virginalis* caterpillars with this plant, we observed three egg clutches on *E. glaucus* at one hilltop site. To test the oviposition preference of *P. virginalis* and the suitability of *E. glaucus* as a hostplant, we conducted laboratory and field oviposition assays, an egg transect survey and a no-choice larval performance assay.

We first tested oviposition preference using a choice assay in the laboratory. In June 2012, mating pairs of moths (n=11) were placed in small polyspun fiber cages with a plywood floor (30×30×50 cm). Bouquets of *E. glaucus* and *L. arboreus* of approximately equal mass were randomly placed on opposite sides of the cages and replaced every week. Moths were misted daily to prevent desiccation. Upon death of a female occupant (see Results for lifespan mean ± SD), cages were thoroughly searched for eggs. Similarly, bouquets were searched for eggs prior to replacement with fresh bouquets. Upon cage searches, we found that the majority of oviposion occurred on the walls of the cage and not on either plant species. Consequently this experiment was only used to estimate average female lifespan and total egg production.

In July 2012, we conducted an experiment examining oviposition preference. One large cage (1 m^3), with an open bottom, was imbedded into the ground at 10 areas previously found to contain high densities of caterpillars (not hilltop sites). Within each cage, at least one small *L. arboreus* plant was present along with other naturally occurring plants. One pair of mating moths was placed into each cage. Upon death or escape of a female occupant, the cage and the naturally occurring vegetation was thoroughly searched for eggs. We recorded the number of eggs and the plant species on which they were found.

To determine natural oviposition preference, we conducted an egg survey by searching stems and leaves on all plants encountered along 23, 2×20 m transects. Transect locations within BMR are described by Grof-Tisza et al. (2015). To quantify larval performance on *E. glaucus* and *L. arboreus*, we conducted a no-choice field feeding assay from 30 July to 14 September 2012 in a

common garden experiment. Two-hundred and twenty, 2nd instar caterpillars were randomly caged on 11 *E. glaucus* and 11 *L. arboreus* plants (10 caterpillars/plant) where the ranges of these two plant species overlap within the reserve. Caterpillars were collected from a colony maintained in our laboratory at BMR prior to the experiment. Cages consisted of 10×15 cm sewn bridal veil mesh with a Velcro closure to prevent caterpillar escape. At the termination of the experiment, we recorded the number of caterpillars surviving. We used a generalized linear model (glm) to determine the relationship between the proportion of caterpillars surviving and host plant identity. The glm was fitted in R (version.3.0.2) using a quasibinomial error distribution (to account for over-dispersion) and logit link function (Crawley 2007).

RESULTS

Adult surveys

The density of moths censused in areas classified as hills (190 moths/ 56,540 m^2) was approximately three-times greater than the density of moths in non-hill areas (65 moths/51,820 m^2; $\chi^2 = 61.3$, df = 2, P= <0.0001; Fig. 1).

Oviposition preference and host plant suitability

The average number of eggs laid per female over her life (21.1±7.4 days (mean ± SD)) was 355.0 ± 260.5 eggs (mean ± SD) across all treatments. High winds at our study site damaged all but 4 of our large cages, allowing moths to escape before oviposition. Due to the resulting low sample size, statistical analysis was not conducted. However, within the intact cages, eggs were found on *Potentilla anserina* ssp. *pacifica, Artemisia douglasiana*, and *Stachys rigida*. No eggs were found on *L. arboreus* in any of the cages. Similarly, no eggs were found on *L. arboreus* (n=81 plants) nor on any other plants during the oviposition field survey (the opportunistic finding of eggs on *E. glaucus* did not occur during the survey). In the no-choice larval assay, caterpillars were almost 2× more likely to survive when caged on *L. arboreus* than on *E. glaucus* (df=19, t=2.22, P=0.039; Fig. 2).

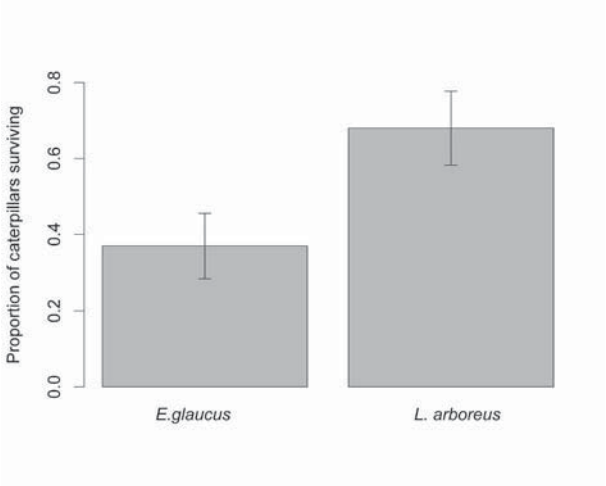

FIG. 2. Proportion of second-instar caterpillar survival in a no choice field feeding assay on *E. glaucus* and *L. arboreus*.

DISCUSSION

Platyprepia virginalis moths appear to exhibit hilltopping behavior, a mate-locating strategy where individuals congregate on hilltops to increase the likelihood of sexual encounters in populations occurring at a low density (Shields 1967). Hilltopping is common in butterflies and has also been observed in many other insect orders (Alcock 1987). Few examples of moths exhibiting this behavior have been documented (but see McFarland 1976, Holoway 1977). For most hilltopping species, males typically arrive first at summits and await the arrival of virgin females. After mating, females descend to lower elevations to lay eggs. This behavior is consistent with the distribution of larvae within the BMR. Caterpillars are generally found at non-hilltop sites and often reach their highest densities in low-lying marsh habitat (Karban et al. 2012). Similar to what Baughman and Murphy (1988) found in their study of what constitutes a hill to a hilltopping butterfly , the hilltop sites varied in topographical prominence, from large hills rising 200 m above the surrounding landscape to small dunes of "seemingly insignificant topographical relief."

The opportunistic discovery of *P. virginalis* eggs at a hilltop site on *E. glaucus* deviates from the classic model of mated females descending from hilltops to oviposit on suitable hostplants. The no-choice feeding assay demonstrated that survival on *E. glaucus* was lower than on *L. arboreus*. The distribution of *E. glaucus* is generally restricted to coastal bluffs (i.e., hilltops), and *L. arboreus* is generally found within the coastal prairie (i.e., non-hilltop sites; Barbour et al. 1973). Indiscriminate oviposition across the landscape and across the hostplants available, coupled with habitat-dependent survival could account for the observed spatial distribution of larvae. Indeed, predator exclusion experiments showed that early-instar survival varies across habitats within BMR and is higher in marsh habitats (Karban et al. 2013). An alternative explanation for this seemingly suboptimal oviposition preference for laying eggs on *E. glaucus*, is that the few females involved could not return to non-hilltop larval patches due to injury, predation risk, or other limiting factors (Scheirs et al. 2000, Gripenberg et al. 2010).

Despite the close association between caterpillars and *L. arboreus*, we found no evidence to support the hypothesis that *L. arboreus* is the preferred hostplant for oviposition. In the field oviposition choice assay, only plants other than *L. arboreus* were chosen for oviposition, and no eggs were found on 81 *L. arboreus* plants that we destructively searched. During annual winter surveys, we generally observed 2nd -instar caterpillars on understory plants and not in the canopy of *L. arboreus* . Taken together, this evidence suggests that though late-instar caterpillars may prefer *L. arboreus*, it is likely not the preferred oviposition hostplant.

Most lepidopteran species are specialists (Futuyma and Moreno 1988). However, there are generalist lepidopteran species that use a broader range of hosts. Generalists tend to be fairly indiscriminate regarding hostplant choice (Shappes et al. 2015). Though late-instar *P. virginalis* caterpillars are predominantly found on *L. arboreus* at our study site, they are known to be polyphagous, and we have observed the species thriving at many sites throughout their range that lack this hostplant. Further, Karban et al. (2010) found that diet mixing enhances the performance of late-instar *P. virginalis* caterpillars. Considering that *P. virginalis* is not only polyphagous, but performs better on a mixed diet, it is not surprising that oviposition preference is not highly constrained. This is consistent with many generalist erebids that use a wide range of hosts for oviposition (Conner 2009).

Conclusion

This is the first documentation of hilltopping in *P. virginalis* and a rare example of a moth exhibiting this behavior. The multiple hilltop aggregation sites used by moths within the Bodega Marine Reserve are non-overlapping with larval habitat. We infer that our observation of the disproportionate distribution of moths on hilltops results from preference for those hilltop sites and not random movement (Baughman, Murphy and Erhlich 1988). Despite the tight association of *P. virginalis* caterpillars with *L. arboreus*, it is likely not preferred for oviposition.

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THE HYPOTHETICAL GROUND PLAN OF THE ZYGAENIDAE, WITH A REVIEW OF THE POSSIBLE AUTAPOMORPHIES OF THE PROCRIDINAE AND THE DESCRIPTION OF THE INOUELINAE **SUBFAM. NOV.**

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ABSTRACT. The hypothetical ground plan of the lepidopterous family Zygaenidae is reconstructed based on a review of the apomorphic characters of the Zygaenoidea. Five subfamilies are recognised and their characters discussed in detail: **Inouelinae new subfamily**, Procridinae, Chalcosiinae, Callizygaeninae and Zygaeninae. A review of the possible autapomorphies of Procridinae is provided. The autapomorphic structure in the receptaculum seminis in the spermatheca of the Procridinae females is newly described as **bursa utricularis**.

Additional key words: Zygaenoidea, Zygaenidae, Zygaeninae, Chalcosiinae, Callizygaeninae, Procridinae, Inouelinae **new subfamily**, autapomorphies, morphology, bursa utricularis (**new structure**), chaetotaxy, anal combs, larval spined tubercles, karyotypes, character variability, taxonomy, systematics.

The lepidopterous family Zygaenidae has a worldwide distribution and hitherto has been divided into the four subfamilies: Procridinae, Chalcosiinae, Callizygaeninae and Zygaeninae (Tarmann 2004; Efetov et al. 2014a), to include more than 1,000 species. Alberti (1954) published the first comprehensive revision of the Zygaenidae of the world. However, a new review of the zygaenid characters is urgently needed because a number of new and significant characters of this family have been discovered in the last 60 years. Our views on the phylogeny of the group have improved and many new taxa have been described. Moreover, the status of some groups has changed. In the present work we provide an overview of our contemporary knowledge. We also describe Inouelinae as a new subfamily within the Zygaenidae, and propose a name (bursa utricularis) for a newly discovered and outstanding autapomorphic character of the subfamily Procridinae.

Apomorphies of Zygaenoidea

The definition of the superfamily Zygaenoidea is based on only a few characters that might only represent autapomorphies. The discussed characters are: head retractibility, heteromorphosis, forward migration and reduction of head setae, position of microsetae and pores, labral sensilla (see Vegliante & Zilli 2004: 181). However, as Vegliante & Zilli (2004) correctly state most of these characters have not been sufficiently studied throughout all families.

Two characters that may represent possible autapomorphies are:

1. Head of larva (at least in the later instars) retractile (Minet 1986: 300) (Figs. 1–3). It can be discussed if this applies also for Epipyropidae and Cyclotornidae. Our information is based on literature (Common 1990: 304; Epstein et al. 1998: 159) and personal communication with the late Clas M. Naumann and the late I. F. B. Common who both have dealt with these two families in detail. We have not examined the larvae of Epipyropidae and Cyclotornidae ourselves. The parasitic life habits and the morphology of both larvae are unique. The first instar larva of both families is attacking the host (Homoptera) by their piercing mandibles. In this position, the head is extended. When safely fixed to the host, in the later instars, the head is permanently retracted (Davis 1987: 460). Our conclusion is therefore that the head is principally retractile, but this habit is only used once.
2. Position of the 2nd abdominal spiracle of the pupa, which is covered by the wings whereas the 1st abdominal segment is visible (only verified for Epipyropidae, Megalopygidae, Limacodidae, Heterogynidae, Zygaenidae) (Minet 1986: 300; Nielsen & Common 1991: 877; Fänger et al. 2002: fig. 2) (Fig. 4).

The hypothetical 'ground plan' of the family Zygaenidae

We are aware that the term 'ground plan' is a hypothetical construction. It is an assembly of

characters that are supposed to represent plesiomorphies. In comparison with other groups (that are more primitive) many of these characters can, of course, represent apomorphies. For us the 'ground plan' of Zygaenidae is a hypothetical reconstruction of a 'primitive' Zygaenidae that most probably has never existed in that form. However, this hypothetical construction helps to understand the currently observed characters in the different groups of Zygaenidae. Therefore a list of so-called 'primitive characters' for this hypothetical 'ground plan' of the Zygaenidae is compiled below. It is summarized from all known characters of the superfamily Zygaenoidea and in comparison with other Lepidoptera:

A. Head with ocelli and chaetosemata (Epstein et al. 1998: 171) (Figs. 5, 6) (for discussion, see below).
B. Compound eyes with interommatidial setae (= interfacetal hairs sensu Scoble 1992: 27) (Tarmann 2004: 13, fig. 18; 19, fig. 53; 25, figs. 90, 91). This character is seen so far only in the Procridinae: in the Artonini in *Pollanisus* Walker, 1854, *Onceropyga* Turner, 1906, and *Homophylotis* Turner, 1904 (Fig. 7) and in some Procridini (e.g. present in *Adscita* Retzius, 1783) (Figs. 8, 9), but not observed in *Illiberis* Walker, 1854 (Procridini) (Figs. 10, 11) and *Thyrassia* Butler, 1876 (Artonini) (see also Davis 1978: 9, figs. 6, 8, 9). This character is not sufficiently studied in Inouelinae **subfam. nov.** (description see below), Chalcosiinae, Callizygaeninae and Zygaeninae but it has not been found in the so far examined species.
C. Maxillary palps present, short, with 2 segments (Fig. 12) (Tarmann 2004: 13, fig. 15; 16, fig. 31) (primary situation in Lepidoptera is 5 segments, as still observed in Micropteroidea—see Kristensen 1998: 41) (see Heppner 1998).
D. Labial palps present, consisting of 3 segments, with sensory organ at tip (Figs. 13–15) (Epstein et al. 1998: 171; Tarmann 2004: 9, 11, 20, figs. 2, 4, 60) (compared with other groups).
E. Mandibles reduced as in all higher Lepidoptera but still present as small lobes which connect to the anterior margin of the lower subgena by an intersegmental membrane. From the position of this membrane cyanogenic droplets can be released (Fig. 16) (Yen 2003: 305; Tarmann 2004: 23, fig. 76; Yen et al. 2005: 239, fig 53a).
F. Proboscis present, unscaled, long, spirally enrolled with muscles (myoglossat), tip with sensory hairs (Figs. 13, 17) (Naumann et al. 1999: 25, text-fig. 22), unpigmented and therefore yellowish.
G. Antenna biserrate or bipectinate (Figs. 18, 19; Efetov 2001c: figs. 42–67; Efetov 2005b: figs. 2–12).
H. Antenna tapering towards and pointed at apex (Fig. 20).
I. Pectinations with setae (sensillae) (Figs. 21, 22) (e.g. Tarmann 2004: 19, fig. 56).
J. Scapus short, without pecten (Figs. 5, 15) (Epstein et al. 1998: 171; e.g. Tarmann 2004: 19, figs. 2, 12, 57, 59, 77, 87).
K. Prothorax with paired, strongly sclerotized sac-like patagia and parapatagia (Figs. 23, 24) (Naumann et al. 1999: 26, text-fig. 23; Yen 2003: 307).
L. Mesothorax with tegulae (Figs. 23, 24) (Naumann et al. 1999: 26, text-fig. 23; Yen 2003: 307).
M. Metathorax with a wing-thorax-coupling mechanism, as in most ditrysian Lepidoptera (Fig. 25) (Yen 2003: 308; Tarmann 2004: 10).
N. Forelegs with epiphysis (Fig. 26) (Naumann 1977: 22; Efetov & Tarmann 1994: 91, fig. 29; Efetov 1994a: 56, fig. 5; Efetov 1994b: 119, fig. 6; Efetov 2001a: 42, fig. 1; Efetov 2001c: figs. 70, 71, 81; Efetov 2005a: fig. 101.1).
O. Tibial spurs 0–2–4 (Fig. 27) (Epstein et al. 1998: 171; Efetov 2001c: figs. 68–82).
P. Frenate wing coupling mechanism with frenulum and retinaculum, with variable sexual dimorphism (as in most ditrysian Lepidoptera) (Fig. 28) (Yen 2003: 308).
Q. Wing venation with full set of veins (forewing: C, Sc, R_1–R_5, M_1–M_3, CuA_1, CuA_2, CuP, 1A+2A+3A (as basal fork); hindwing: Sc+R_1, Rs, M_1–M_3, CuA_1, CuA_2, CuP, 1A, 2A, 3A) (Fig. 29) (Alberti 1954: pls 44–55; Tarmann 1984: figs. 219–245; Efetov 2001c: figs. 83–87; Efetov 2005b: fig. 13; Yen 2003: 310).
R. All veins arising evenly spaced from cell (Fig. 29) (Alberti 1954: pls 44–55; Tarmann 1984: figs. 219–245; Efetov 2001c: figs. 84–87; Efetov 2005b: fig. 13).
S. Medial stem as fully developed vein present in both wings (Fig. 29) (Alberti 1954: pls 44–55; Tarmann 1984: figs. 219–245; Efetov 2001c: figs. 83–87; Efetov 2005b: fig. 13).
T. CuP completely present (Fig. 29) (Alberti 1954: pls 44–55; Tarmann 1984: figs. 219–245; Epstein et al. 1998: 171; Efetov 2001c: figs. 83–87; Efetov 2005b: fig. 13).
U. Sc and Rs free in hindwing (Fig. 29) (Alberti 1954: pls 44–55; Tarmann 1984: figs. 219–245; Efetov 2001c: figs. 83–87; Efetov 2005b: fig. 13).
V. Ultrastructure of wing scales on the 'abwing side' (upperside) with longitudinal ribs and with transverse striae (Fig. 30) (Naumann et al. 1999:

30, text-fig. 29l; Yen 2003: 312). In the most primitive Zygaenidae no perforation is developed. This character combination is similar to that of most primitive Lepidoptera (non-glossatan families) (Simonsen 2001; Kristensen & Simonsen 2003: 11).

W. Abdomen with tortricoid apodemes on sternite 2 (Fig. 31) (Epstein et al. 1998: 172).

X. Abdomen of larva, pupa and imagines with a pair of lateral protuberances on segments 2 and 7 (Figs. 32, 33, 34) (Tarmann 1994; Epstein et al. 1998: 172; Tarmann 2004: 33, 50, 218–219, figs. 431, 433, 434; Efetov & Tarmann 2004: 301–303, figs. 1–7; Efetov 2005b: pl. 25, fig. 6).

Y. Larva feeding freely on the host-plant (Figs. 35, 36, 37). Not leaf mining.

Possible apomorphies of the family Zygaenidae

1. Cyanogenesis and resistance against cyanides. This character is possibly shared with other groups. Whether it is based on true relationship or just a parallel development is still unclear.
2. Head with ocelli and chaetosemata present (as in some other more primitive ditrysian groups, e.g. Yponomeutoidea and Tortricoidea). This character combination is absent in all other Zygaenoidea. It has also been reported for the Heterogynidae (Scoble 1992: 37, fig. 39). However, Scoble used the South African *Janseola titaea* Druce, 1896, as a model for his figure. Whereas *Heterogynis* species have only sensillae trichodea present (Jordan's first type of chaetosema), *Janseola* has the typical zygaenoid type (Jordan's second type of chaetosema) (Jordan 1923). Zilli (1998: 111) proposed the transfer of *Janseola* to the Zygaenidae, a change that has never been done officially in a separate paper but is correct and the authors agree with A. Zilli's arguments. Moreover, groups that were placed in the Zygaenidae earlier (e.g. Alberti 1954) are now excluded from that family, viz. Anomoeotidae, Himantopteridae, Phaudidae (that are now treated as families) and the genus *Chalcosiopsis* as well as the *Burlacena*-group (see also Yen et al. 2005: 184). Consequently, within the Zygaenoidea the character combination 'head with ocelli and chaetosemata present' applies to the Zygaenidae only (with the five included subfamilies: Inouelinae **subfam. nov.** (see below), Procridinae, Chalcosiinae, Callizygaeninae and Zygaeninae) (Figs. 5, 6).

   The chaetosema was first described by Jordan (1923). He found two different forms that he described as 'type one' and 'type two'. Eltringham (1925) examined the enervation and found that the chaetosema must be a sensory organ. Since then, the chaetosema is also known as Jordan's or Eltringham's organ (Scoble 1992: 36).

   In the Zygaenidae the chaetosemata have a special structure consisting of erected scales (that look similar to those that cover the head at the vertex and genae) and setose bristles (sensilla trichodea) situated on a ground-plate and arranged in a typical way (Figs. 6, 38, 39) ('zygaenoid chaetosema', similar to 'second type' of chaetosema sensu Jordan (1923: 7), who figured the chaetosema of a *Micronia* sp. (Uraniidae) (Jordan 1923: pl. 2; Alberti 1954: 164; Naumann et al. 1999: 24, text-fig. 19; Tarmann 2004: 9, 11–25, figs. 6, 17, 26, 33, 44, 52, 61, 88; Yen 2003: 306–307; Yen et al. 2005: 186–187, figs. 15, 16). This 'zygaenoid chaetosema' is here considered to be unique and different from all other chaetosemata known, it could be an apomorphy of the Zygaenidae and Lacturidae (Lacturidae without ocelli!) (Yen et al. 2005: 186, fig. 15G). It differs from the chaetosemata of Uraniidae and Geometridae, by the large number of vertical-standing scales within or beside the sensilla trichodea, arranged either in honeycomb-like clusters around them (Procridinae), with the upright scales and the sensillae scattered between (Chalcosiinae, Callizygaeninae) (Yen et al. 2005: 184, figs. 15, 16) or side by side (Zygaeninae). In Inouelinae **subfam. nov.** (see below) the broad upright scales are arranged in the centre and the papillae trichodea beside them (Yen et al. 2005: 186, fig. 15C). In Uraniidae and Geometridae (both also referred to the 'second type' sensu Jordan (1923)) the sensillae trichodea are also arranged in groups in which some very slender upright scales are scattered. Most other Lepidoptera that have a chaetosema present belong to Jordan's first type without upright scales included beside the sensilla trichodea (Jordan 1923: 7, pl. 2) (see e.g. Horak 2006: 22, fig. 4 for Tortricidae; Scoble 1992: 37, fig. 39 for Heterogynidae).
3. Development of Petersen's gland (a pair of glands close to the ooporus in Zygaeninae and Procridinae of not exactly known function) (Fig. 40). This character is considered to be secondarily reduced in Inouelinae **subfam. nov.** (see below), Chalcosiinae and Callizygaeninae. This gland seems to have an importance for the protection of the eggs against predators, as it is reduced in three Australian genera of Procridinae (viz. *Pollanisus, Onceropyga,* and *Hestiochora*), species of which

protect their eggs with poisonous dart-like scales from an abdominal hair tuft and in Chalcosiinae. The latter have an ovipositor and deposit their eggs into clefts of the bark of twigs and stems and obviously do not need additional protection (Bode & Naumann 1987; Naumann 1988; Naumann et al. 1999: 35, 37, text-fig. 38; Yen 2003: 316; Tarmann 2004: 36).

Possible apomorphies of the subfamily Chalcosiinae

1. Presence of a specialised hindwing-abdominal scent organ consisting of a fold on the first to second abdominal pleurite and a bundle of hair tufts that arises from the anal axillary sclerite of the hindwing and inserts into the pleural fold (Figs. 42–44) (Haase 1888; Tarmann 1992: 34, figs. 42–45; Yen et al. 2005: 229–230, 273, figs. 43–46).
2. Capability of releasing a large amount of cyanogenic protective fluid (often with a hissing sound) as foam from a dorsolateral opening situated between the patagia and parapatagia (Fig. 41). Whether this fluid is stored in cavities within the patagia or parapatagia is not known yet (Yen et al. 2005: 188, 239, fig. 53b).
3. Compound eyes without interommatidial setae (Yen 2003: 306). This character is considered to be a secondary reduction (see ground plan of Zygaenidae above and Figs. 7–11).
4. Chaetosema with upright scales and sensillae trichodea arranged in a mixture amongst each other (see above and for comparison Figs. 38, 39).
5. Tegumen in male genitalia with specialised apodemes (Yen 2003: 314–315, fig. 4).
6. In the females abdominal segments 8–10 transformed into an ovipositor (Yen 2003: 319, fig. 5).
7. Accessory gland on utriculus in receptaculum seminis absent (Yen et al. 2005: 221)

Possible apomorphies of the subfamily Zygaeninae

1. Compound eyes without interommatidial setae (Yen 2003: 306). This character is considered to be a secondary reduction (see ground plan of Zygaenidae above and Figs. 7–11).
2. Chaetosema placed on an elongate oval plate that is situated dorsad (Pryeriini) or laterodorsad/dorsad of ocellus (like an encaved triangle behind the ocellus) (Zygaenini), with a trichose part (main part) and upright scales arranged around or along side (see Yen et al. 2005: 186, fig. 15D).
3. Development of a characteristic abdominal coremata organ (a pair of eversible brushes on the intersegmental integument between abdominal segments 8 and 9) in the male. This character is secondarily reduced in *Zygaena anthyllidis* Boisduval, 1828, and *Z. loti*-group (Kames 1980; Efetov 2004; 2005b).

Possible apomorphies of the subfamily Callizygaeninae

We know three plesiomorphic characters of the group:

A. Medial stem in wing venation fully developed.
B. Lagena in receptaculum seminis present.
C. Petersen's gland absent (based on examination of *Callizygaena aurata* only).

Possible apomorphies:

1. Compound eyes without interommatidial setae (Yen 2003: 306). This character is considered to be a secondary reduction (see ground plan of Zygaenidae above and Figs. 7–11).
2. Foretibial epiphysis reduced (Alberti 1954: 217).
3. Bulla seminalis in form of a globular bulb that is connected with the ductus seminalis by a stick-like tubular connection (Alberti 1954: 217) (not sufficiently examined in further species).
4. Development of signum-plates in corpus bursae (Alberti 1954: 217).
5. Abdominal segments 8–10 transformed to form a short ovipositor (Alberti 1954: 375, pl. 15, fig. 3b).

Possible autapomorphies of the subfamily Procridinae

Based on the comparison of all the above-mentioned characters with those found in the Procridinae we consider the following to represent autapomorphies of Procridinae:

1. In the females of Procridinae the spermatheca (= receptaculum seminis) is not divided into a bulb-like lagena and a tube-like utriculus, as in most other known ditrysian Lepidoptera (including the Zygaenidae subfamilies Zygaeninae, Chalcosiinae and Callizygaeninae; not yet clear in Inouelinae **subfam. nov.** due to lack of material), and represented by long tube (utriculus) (Fig. 45), as found in the monotrysian Lepidoptera families. However, we interpret this as a secondary reduction of the lagena, or the lagena is fused with the utriculus (Naumann 1988; Epstein et al. 1998: 173, 175, fig. 10.3.P). Yen (2003: 317), citing Naumann (1988), mentions that a lagena is also absent in the Zygaeninae. This is incorrect, as

in all examined Zygaeninae we found a well-developed lagena present. The terminology for these parts of the spermatheca is slightly confusing, as different authors have transposed these terms (see Kristensen 2003: 436–437). The form of the utriculus (at the position where the lagena is situated in non procridine zygaenids and other Lepidoptera) is always slightly or prominently broadened in Procridinae, forming a bag-like structure. We propose to denote this unique structure in the Procridinae as '***bursa utricularis***' (Fig. 45).

2. Development of a trend to inflate the posterior part of the ductus bursae to form a praebursa (Figs. 45, 46, 47) (Alberti 1954: 155–156, 210, text-figs. 14, 15; Tarmann 1984: figs. 268–270, 275, 278, 279, 282, 319, 320; Efetov 1994a: figs. 6, 7; Efetov 1996b: fig. 2; Efetov 1997a: figs. 6, 12, 16, 18; Efetov 1997b: figs. 16, 22; Efetov 1998a: figs. 9, 15, 21; Efetov 1998b: fig 3; Efetov 2000: figs. 6, 10; Efetov 2001a: fig. 15; Efetov 2001c: pl. 27, figs. 2, 3, pl. 32, fig. 12, pl. 34, fig. 16, pl. 44, fig. 43; Efetov 2005a: figs. 105.8, 107.1–107.4; Efetov 2005b: pl. 53, figs. 9, 10, pl. 54, figs. 11, 12, pl. 55, fig. 15, pl. 56, figs. 16, 17, pl. 57, fig. 18; Efetov 2006: figs. 8, 11, 17, 26, 32, 47; Efetov & Tarmann 1999b: figs. 3, 4; Efetov & Tarmann 2013b: figs. 7, 8; Efetov & Tarmann 2014a: fig 5; Efetov & Tarmann 2014b: figs. 26, 27; Efetov & Tarmann 2016a: fig. 10; Parshkova 2007: figs. 1–3; Tarmann 2004: figs. 240–272, 373–377, 419–422, 425). This praebursa can accommodate the spermatophore and functionally partly replaces the corpus bursae. There are often special crests, teeth and spines developed or folded sclerotized walls that help to rupture the spermatophore mechanically. Their structure has little variability within a species but there are remarkable differences between species; therefore they are of high diagnostic value (see e.g. Figs. 46, 47).
3. Wing scales with a specialised ultrastructure (Tarmann 1984a: 17–20; 1984b: 42–64, figs. 81–218; 2004: 28–30, figs. 99–118). The scale ultrastructure between the longitudinal ribs develops from the primitive 'transverse striae'-type (also still preserved in the most primitive Zygaeninae) through the implementation of cross connections first to a 'plate'-type sensu Tarmann (1984: 58–59, figs. 177–188) without perforation and then to a 'central hole'-type sensu Tarmann (1984: 42–47, figs. 81–116), or to a 'grid scale'-type or 'sieve scale'-type sensu Tarmann (1984: 48–49, figs. 117–128) equal to the '*Urania*-type' sensu Kristensen & Simonsen (2003: 11) (Figs. 48–51). The ultimate situation is a 'ladder'-type sensu Tarmann (1984: 51, fig. 140; 53, figs. 150, 152). On the body but not on the wings one can still find scales with the primary ultrastructure of primitive Lepidoptera in the form of 'transverse striae' also in the Procridinae (e.g. on the abdominal hair tuft of females in the Australian genera *Pollanisus, Onceropyga* and *Hestiochora*) (Fig. 30).
4. Female 'calling' with a pheromone distributing organ that is located on abdominal tergites 3, 4 and 5. The release of the pheromone is combined with a characteristic 'calling position' in which the female spreads the wings and exposes these tergites by bending the abdomen downwards (Hallberg & Subchev 1997; Efetov 2001c: 16, 24, 25, pl. 53, figs. 1–7; Nishihara & Wipking 2003: fig. 3). The location of the glands in *Th. ampellophaga* was confirmed by investigations with electron microscopy which showed that the sex pheromone glands are situated on the anterior part of the 3rd–5th abdominal tergites of the females (Hallberg & Subchev 1997). In Figs. 52–57 one can see examples from the tribe Procridini, viz. the genera *Theresimima, Rhagades, Zygaenoprocris, Jordanita, Acoloithus.* In the tribe Artonini only *Pollanisus* females (Fig. 58) were observed in such a calling position, which is in a slightly different way from that observed in Procridini females: they spread their wings and expose the dorsal part of the abdomen. The latter is not bent downward but is almost straight; in addition, they vibrate the abdomen at a high frequency, which most likely helps to diffuse the pheromone from the glands (Mollet & Tarmann, pers. obs., 2013; Subchev 2014: 149). An interesting habit was observed by R. Turrent (pers. comm.). In Mexico he has found groups of females calling in a tree or shrub with the males flying around in the species *Triprocris ruemelli* (Druce, 1884) and a so far unidentified species that is related to *Pyromorpha latercula* (H. Edwards, 1882). Zygaeninae have the pheromone-producing glands at the posterior end of the abdomen between abdominal segments 8 and 9 (Tremewan 1985: 97; Naumann et al. 1999: pl. 9, fig. 1). In Inouelinae subfam. nov., Chalcosiinae and Callizygaeninae no such glands have been found so far. Moreover, Yen et al. (2005: 230) reported that calling by females of *Aglaope* (Chalcosiinae) has been observed in

which the female expands and contracts the abdominal segments, possibly to release the pheromone. Koshio & Hikada (1995) studied the sexual behaviour in *Elcysma weswoodi* and found that calling females were motionless and without a distinct calling posture.

The composition of sex attractants is known only for the Procridinae and Zygaeninae. While in Zygaeninae sex attractants are esters of higher alcohols and acetic acid, in Procridinae they are esters of 2-butanol and fatty acids (Subchev et al. 2010; 2012; 2013; 2016; Efetov et al. 2010; 2011; 2014b; 2015b; 2016; Efetov, Hofmann & Tarmann 2014).

5. Chaetosema in a honeycomb-like arrangement. The upright body scales (mainly broad scales) encircle the sensillae trichodea (Figs. 6, 38, 39) (Yen et al. 2005: 186, figs. 15A, 15B; Tarmann 2004: 11–25, figs. 6, 17, 26, 33, 39, 44, 52, 61, 63, 78, 88).

Tribe Artonini

(includes species from the eastern Palaearctis, the Oriental, Australian and Afrotropical regions).

Possible apomorphies:

1. Form of head dorsoventrally compressed with flat occiput (Fig. 14) (Tarmann 2004: 11, fig. 2; 12, fig. 12; 19, fig. 51; 20, fig. 59).
2. Chaetosema extending forward between the compound eye and the ocellus (Fig. 14) (Tarmann 1994; 2004: 11–20, figs. 2, 6, 12, 14, 17, 26, 32, 33, 38, 39, 43, 44, 51, 52, 59, 61).
3. A single unpaired medial spur developed on hind tibia (Fig. 59) (Tothill et al. 1930: 39; Efetov 2005a: figs. 101.5–101.7; Efetov & Tarmann 1996: 202–203, figs. 8–10; Efetov & Tarmann 2008: fig. 4). This character is secondarily reduced in some species (Tarmann 2004: 31, fig. 121a, b; Efetov & Tarmann 2008: fig. 5). A similar unpaired spur is known in the Eriocraniidae and Acanthopteroctetidae (Eriocranioidea) (Kristensen 1998: 51).
4. Valva in male genitalia fan-shaped (Tarmann 1994; 2004: 108–116, figs. 136–185; 136–137, figs. 273, 274, 277, 278; 178–179, figs. 344–355; 205, figs. 402–404; 212, figs. 412, 415), the dorsal and ventral sclerotisations are close together when in a relaxed position but can be remarkably spread when everted from the abdominal end to hold the abdomen of the female (Tarmann, personal observation); the translucent membrane between the dorsal and ventral sclerotisations is folded; this gives the whole valva a fan-shaped appearance (Fig. 60).
5. Antenna with the pectinations very movable (they can be closed to the shaft when the specimen is disturbed).
6. First instar larva with only one dorsal seta on the first abdominal segment (Fig. 61) (Tarmann 2004: figs. 131.g–131.i; Efetov et al. 2006: figs. 3–7; Efetov & Hayashi 2008: fig. 3) (plesiomorphic variant – 2 dorsal setae on the first abdominal segment).

Tribe Procridini

(includes species from the Palaearctic, Nearctic, Neoptropical, Afrotropical regions and the northern parts of the Oriental region).

Possible apomorphies:

1. Lateral protuberances on abdominal segments 2 and 7 reduced in imagines. Only in *Pseudoilliberis kuprijanovi* (Efetov, 1995) and some primitive *Illiberis* species this character is still developed (but all other characters show that these species belong to Procridini). The reduction is considered to be a secondary loss (see ground plan of Zygaenidae above).
2. Larvae develop the ability to live as leaf miners. The more primitive groups of Procridini still have larvae that feed freely on the plants but, step by step, beginning with the earliest instars in *Adscita* Retzius, 1783, the leaf-mining habit starts to become the standard habit. In *Jordanita* Verity, 1946, leaf-mining until the last instar is developed in most species.
3. The integument of adult larvae with sclerotized spined micro- and macrotubercles (unispined or multispined) (Efetov 1994b; 2001c; 2004).

Recently discovered characters in Procridinae

The data on the chaetotaxy of the first instar larvae, structure of sclerotized spined tubercles and anal combs of the adult larvae, and karyotypes are very important for studying the phylogeny and systematics of the Procridinae (Efetov 2001c; 2004; 2005b). A review of these characters is included below.

Chaetotaxy of first instar larva.

The chaetotaxy of the first instar larvae of species of the Zygaenidae is important for the understanding of relationships within this group because there are significant differences in the combinations of setae of genera and some subgenera (Efetov 2001a; 2001c; 2004; 2005b; Efetov et al. 2000; Efetov & Tarmann 1999a).

This character has been studied in different species of the Procridinae, Chalcosiinae and Zygaeninae. In spite of slight variation within a species there are good differences between species groups. Two main types of setae occur: light (*l*) and stronger sclerotized, dark (*d*). The numbers and combinations of dorsal (D), subdorsal (SD) and lateral (L) *l* and *d* setae are apomorphic characters in some groups. Some examples are listed below.

More than two SD setae is an apomorphic character of the genus *Rhagades* Wallengren, 1863 (Fig. 62). Two dark SD setae is an apomorphic character of the subgenus *Tarmannita* Efetov, 2000 that differs it from all other subgenera of the genus *Adscita* Retzius, 1783 (Figs. 63, 64). More than two D setae is a unique character of the subgenus *Roccia* Alberti, 1954, in the genus *Jordanita* Verity, 1946 (Figs. 65, 66).

Multispined micro- and macrotubercles of the larvae of Procridinae

Sclerotized tubercles that are covered with spines (spined tubercles) and situated on the integument of the adult larvae of Procridinae have already been described (Efetov 1994b). These structures have nothing to do with the coronetted tubercles described in the genus *Heterogynis* Rambur, 1837 (Lepidoptera: Heterogynidae) by Chapman (1904) and mentioned also by Vegliante & Zilli (2004). Coronetted tubercles are sclerotized cylinders with the upper end open and bordered with spines (Vegliante & Zilli 2004: figs. 2f, 2g), while spined tubercles have no openings and their function is not secretory. We suppose that the function of spined tubercles is to protect the integument of the larva (like chain armour of soldiers in early times) from injury and from attacks by parasitoids and predators (Efetov 2004).

More detailed investigations (Efetov 2004; 2005a) have shown that there are two types of spined tubercles: micro-tubercles (height less than 0.02 mm) (Figs. 67–70) and macrotubercles (height more than 0.04 mm) (Figs. 67–72). Tubercles of both types can be with one spine (unispined tubercles) (Figs. 67–70) or numerous (3–12 and more) spines (multispined tubercles) (Figs. 67, 68, 70–72).

Efetov & Tarmann (1995; 1999a) showed that multispined macrotubercles (Figs. 70–72) are a synapomorphy of *Zygaenoprocris* Hampson, 1900, *Adscita* Retzius, 1783, and *Jordanita* Verity, 1946.

Studies undertaken by Efetov (2004) in the subfamilies Procridinae (tribes Procridini and Artonini), Chalcosiinae and Zygaeninae revealed that multispined microtubercles are a synapomorphic character of the genera *Theresimima* Strand, 1917, *Rhagades* Wallengren, 1863, and *Illiberis* Walker, 1854 (Procridinae, Procridini).

The cuticle of the larvae of *Theresimima* + *Rhagades* + *Illiberis* is covered with sclerotized multispined microtubercles and unispined macrotubercles (Figs. 67, 68), while in *Zygaenoprocris* + *Adscita* + *Jordanita*, the combination of unispined microtubercles and multispined macrotubercles is characteristic (Fig. 70). In the genus *Hedina* Alberti, 1954, there is a combination of unispined microtubercles and unispined macrotubercles (Fig. 69) (Efetov 2008).

Moreover, it has also been shown that very important characters are the position of the spines on the tubercles (symmetrical, asymmetrical) and the shape of the apices of the spines (pointed apices, crown-shaped apices). Symmetrical multispined macrotubercles with crown-shaped apices of the spines (Fig. 70) are considered to be a synapomorphic character of the subgenera *Adscita* Retzius, 1783, and *Tarmannita* Efetov, 2000, of the genus *Adscita*. The presence of strongly asymmetrical multispined macrotubercles with crown-shaped apices of the longest spines (Fig. 71) is an apomorphic character of the subgenus *Solaniterna* Efetov, 2004 (Efetov 2004), of the genus *Jordanita*.

Anal combs

Larvae of the Procridinae have a sclerotized comb situated above the anus (the so-called anal comb). While investigating early stages of the Procridinae, we found that the anal combs of the adult larvae in different genera and subgenera of Procridinae have different structures (Efetov 2001c; 2004: figs. 143–189). The anal comb consists of a plate-like base and setae. The most important criterion is the ratio of the height (H) of the base to the length (L) of the central setae (Figs. 73, 74).

It was found that in the genera *Pseudoilliberis* Efetov & Tarmann, 2012, *Illiberis* Walker, 1854, *Rhagades* Wallengren, 1863, and *Jordanita* Verity, 1946, the height of the base of the anal comb is greater than the length of the setae: H/L more than 1 (Fig. 73), while in the genus *Theresimima* Strand, 1917, and subgenera *Adscita* Retzius, 1783, and *Tarmannita* Efetov, 2000, of the genus *Adscita* Retzius, 1783, the situation is reversed: H/L less than 1 (Fig. 74). The high base of the comb (H/L more than 1) is probably a plesiomorphic character, as it is present in the more primitive genera *Pseudoilliberis, Illiberis and Rhagades*.

The larvae of the genus *Zygaenoprocris* Hampson, 1900, have a reduced anal comb (Fig. 76) (Efetov 2001c; 2004), while in the subgenus *Procriterna* Efetov & Tarmann, 2004, of the genus *Adscita* Retzius, 1783, the anal comb is double (without central part) (Fig. 75). We consider such a double anal comb to be an apomorphic character of *Procriterna*.

In *Zygaenoprocris*, as mentioned above, the anal comb in the adult larva is reduced. This character is here considered to be an autapomorphy of the genus because the anal comb is well developed not only in *Pseudoilliberis, Illiberis, Theresimima, Rhagades, Jordanita, Adscita* but also in some other genera of the Procridinae. The apomorphy of the reduction of the anal comb in adult larva of *Zygaenoprocris* is supported by the fact that it is well developed in larvae of early instars (including the first instar) and is reduced in the last instars (according to observations by the first author on *Z. taftana* (Alberti, 1939)). *Harrisina* Packard, 1864, has no comb at all but only a dark spot at the place where the comb should be. However, *Harrisina* is a very derived genus with even asymmetrical genitalia.

The larvae of Artonini have a combination of three anal combs, consisting of a larger dorsal comb and two smaller lateral combs (Mollet & Tarmann 2010: fig. 7) arranged around the anal orifice. This character was observed in the genera *Pollanisus* and *Thyrassia*.

A thoracic brush organ in males of Artonini

This organ was recently discovered by B. Mollet & G. M. Tarmann in the Australian Procridinae genera *Pollanisus* Walker, 1854, *Hestiochora* Meyrick, 1886, and *Onceropyga* Turner, 1906. The structure consists of a bunch of transparent hairs (looking like androconial scales) inserted laterally on each side of the thorax between the prothorax and mesothorax. These hairs are arranged in a weakly sclerotized translucent fold, connected to the posterior part of coxa 1 and the inner skeleton by a fine skin-like diaphragm. The fold covers the densely grouped hairs (20 to 30) and is set distally to allow the "blooming" of these hairs, in the form of a brush or a bouquet, laterally on the outer side of the thorax.

Similar hairs between the forelegs on the prothorax were found in *Amuria* Staudinger, 1887, and *Pseudoamuria* Tarmann, 2004.

During the last years a number of other characters have been discovered in different species and species-goupes of Procridinae, viz. specialized structures on the juxta (Efetov 1996a; Tarmann & Drouet 2015), unusual shapes of the uncus (Tarmann 1984; Tarmann & Drouet 2015), very long vesica with more than 70 cornuti arranged in a long spiral (Efetov & Tarmann 2013a), pulvinus represented by a long process with strongly sclerotized spines (Efetov 2010), well developed lamina dorsalis (Tarmann 1984), extraordinary shapes of the valvae (Tarmann 1984;Tarmann & Drouet 2015) and the ductus bursae (Efetov 2012) etc.

Karyotypes

As shown by investigations during recent years (Efetov 2001b; 2001c; 2004; Efetov & Tarmann 1999a; Efetov et al. 2004; Efetov et al. 2015a), a study of the karyotypes in the family Zygaenidae provides very interesting results. The modal haploid chromosome number for Lepidoptera is generally 30–31 (Robinson 1971; Lukhtanov & Kuznetsova 1988). In the majority of species studied in the genus *Zygaena* Fabricius, 1775 (subfamily Zygaeninae), the haploid chromosome number is 30 (only in one species it is 29 and for three species both numbers: 30 and 31, were recorded by different authors) (Lukhtanov & Kuznetsova 1988; Tremewan 2006; Efetov & Parshkova 2003; 2004). It seems that this number is ancestral for the genus *Zygaena* (possibly for the subfamily Zygaeninae).

Aglaope infausta (Linnaeus, 1767) (subfamily Chalcosiinae) also has the modal haploid chromosome number 31 (Efetov 2004: fig. 177; Efetov & Parshkova 2004).

However, we found that in many species of the Procridinae the haploid chromosome numbers differ from the modal. For example, in species of the subgenera *Roccia* Alberti, 1954, *Tremewania* Efetov & Tarmann, 1999, and *Jordanita* Verity, 1946, of the genus *Jordanita* Verity, 1946, the haploid chromosome number is 31, while in *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862) it is 27 (Efetov 2004: fig. 176). The same situation is found in the genus *Adscita* Retzius, 1783. While the majority of species of the subgenera *Adscita* Retzius, 1783, and *Tarmannita* Efetov, 2000, have the haploid chromosome number 31, in *Adscita* (*Adscita*) *jordani* (Naufock, 1921) it is 30 (Efetov 2004: fig. 168), in *Adscita* (*Adscita*) *geryon* (Hübner, 1813) it is 32 (Efetov 2004: fig. 170), and in *Adscita* (*Procriterna*) *subtristis* (Staudinger, 1887) it is 17 (Efetov 2004: fig. 167). The most dramatic differences in chromosome numbers were found in the genus *Rhagades* Wallengren, 1863 (Figs. 79–81). In the primitive species, *Rh.* (*Naufockia*) *brandti* (Alberti, 1938), it is modal (31), but in *Rh.* (*Wiegelia*) *amasina* (Herrich-Schäffer, 1851) it is 12, and in *Rh.* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775) it is 47! We consider this increase in the number of chromosomes in *Rh. pruni* to result from polyploidy (Efetov 2001c; 2004). It is also interesting that the number is reduced (compared with the modal) to 25 in *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907 (Fig. 77), and to 28 in *Theresimima ampellophaga* (Bayle-Barelle, 1808) (Fig. 78). Thus, with the exception of *Rh. brandti*, the modal number in the genera *Rhagades* Wallengren, 1863, *Theresimima* Strand, 1917, and *Illiberis* Walker, 1854, does not occur. Our results confirm that

Procridinae are a unique group in the Zygaenidae from a karyological point of view and investigation of the karyotypes in this subfamily is very important for understanding evolutionary relationships and the systematic position of species in this group.

Results of DNA sequencing

A DNA barcoding project on zygaenid moths (ZYGMO) was activated in 2009 and remains in progress (Efetov et al. 2013; Efetov & Tarmann 2016b; Mutanen et al. 2016). Work initially focused on the Palaearctic Zygaenidae, but species from other regions (South East Asia, Australia, Central and North America, etc.) have also been examined.

We obtained DNA results based on analysis of the 658-bp barcode region of the cytochrome *c* oxidase I (COI) mitochondrial gene involving 1031 sequences of 245 species from 64 genera. Sequence divergences for the barcode region were calculated using the Kimura 2 Parameter model by the analytical tools on BOLD (the Barcode of Life Datasystems – www.boldsystems.org). Our results demonstrate species level resolution of the COI sequences in most taxa. The barcoding provides only additional data for scientific discussions on Zygaenidae systematics. However, our DNA results coincide on the whole with the contemporary system of the family.

A new subfamily of Zygaenidae

In 1999 K. A. Efetov described the genus *Inouela* Efetov, 1999, and included the two species *Inouela formosensis* Efetov, 1999 (type-species of the genus) and *I. exiguitata* (Inoue, 1976) (described as *Clelea* in Procridinae). He placed this new genus in the subfamily Chalcosiinae as both species have a double uncus which is not typical for Procridinae. Yen (2003) and Yen et al. (2005) found that this genus cannot be placed in Chalcosiinae based on a number of characters. However, *Inouela* agrees with the main characters of Zygaenidae. Nevertheless, it can neither be placed in Procridinae, nor in Chalcosiinae, nor for that matter in the two other subfamilies Callizygaeninae or Zygaeninae. To accommodate this isolated taxon it is therefore necessary to describe a new subfamily **Inouelinae subfam. nov.**

Historical note

The history of this group began in 1910 when H. Sauter collected two male zygaenid specimens in 'Formosa, Taihorin' (Taiwan). These specimens are deposited in the Museum für Naturkunde in Berlin (Germany). The late Dr Burchard Alberti dissected one of these two specimens and intended to describe a new genus and species that he wanted to name 'Taihorina formosensis'. This is discernible from the handwritten pin-labels by Alberti under both specimens (Efetov 1999: 93, figs. 2–4). However, Alberti never described these taxa.

In 1976 Prof. Hiroshi Inoue described the nominal taxon *Clelea exiguitata* Inoue, 1976 (Zygaenidae: Procridinae) from Japan (9 male specimens from the three localities: Mikyo, Island of Tokunoshima (leg. H. Inoue), Yuwandake, Amami-oshima, and Sumiyoson, Amami-oshima (leg. T. Okada). The genus *Clelea* Walker, 1854, belongs to the subfamily Procridinae (Alberti 1954; Efetov & Tarmann 1995; 2012). However, the genitalia structures of '*Clelea*' *exiguitata* have nothing to do with the genitalia structures of *Clelea* Walker, 1854, but they are close to those of the dissected male from Taihorin examined by Alberti.

After reinvestigation of the material in Berlin with the additional dissection of the second male specimen from Taihorin and one paratype of *Clelea exiguitata* from the type locality in Japan (now deposited in the Natural History Museum in London) Efetov (1999) described *Inouela* Efetov, 1999, with the two species *Inouela formosensis* Efetov, 1999, and *Inouela exiguitata* (Inoue 1976).

Xue & Han (2003: 255) included *Inouela formosensis* in the check-list of Zygaenidae from China in Chalcosiinae. Also Horie (2013: 329) included *Inouela exiguitata* in Chalcosiinae. Yen (2003: 293) writes that the monophyly of the subfamily Chalcosiinae is doubtful when the genera *Inouela* Efetov, 1999, *Chalcosiopsis* Swinhoe, 1894, *Cleoda* Tremewan, 1973, and *Heteropan* Walker, 1854, are included. Yen et al. (2005) underline the necessity of these exclusions and suggest to place *Inouela* into 'Procridinae s. l.' However, as the characters of *Inouela* do not agree with the main autapomorphies of Procridinae (without *Inouela* this subfamily is monophyletic) we see no other possibility as to describe a new subfamily to accommodate this isolated group: **Inouelinae subfam. nov.**

Inouelinae subfam. nov.

Type genus *Inouela* Efetov, 1999 (here designated)

Diagnosis

Small Zygaenidae (length of forewing 4–7 mm) with unicolorous greyish brown wings and body (Fig. 82). Male and female similar in habitus. Head of male and female without sexual dimorphism. Male and female antenna tapered towards apex, with dorsoventrally compressed shaft and very long, large distances between pectinations, length of pectinations only slightly shorter in female, sensory setae of rami long. Proboscis reduced, developed as two short, only slightly

downward-curved lobes, labial palps very short (note that the mouthparts are not completely reduced, as mentioned by Yen et al. 2005: 183, 184). No sexual dimorphism in size of compound eyes (present in Chalcosiinae and Procridinae). Ocelli and chaetosemata present. Chaetosema situated dorso-laterally on an almost vertical part of head between (slightly posterad) compound eye and the small ocellus, with characteristic structure with upright scales in the centre and a group of sensillae trichodea at the anterior margin (Fig. 83) (Yen et al. 2005: fig. 15C). Inouelinae are not able to release protective fluid from the membrane between patagia and parapatagia (present in Chalcosiinae) (Yen et al. 2005: 188). Foreleg with long tibial epiphysis, hindtibia without spurs.

Wing venation with full number of veins in forewing, M_1 absent in hindwing; veins almost evenly spaced; in *I. formosensis* all veins free from cell, in *I. exiguitata* R_4+R_5 stalked; medial stem in both wings present as a vein but only distally; hindwing with a short cross vein between Sc and anterior margin of cell. Frenulum developed as one spine in male and female, retinaculum in male developed as a small fold at base of Sc, in female as a long row of filiform scales at base of CuP.

Androconial hairbrush in male hindwing and invagination of pleural membrane absent (present in Chalcosiinae).

Male genitalia (description based on the two species: *I. formosensis* and *I. exiguitata*) (Figs. 84, 85). Uncus broad, with two lobes. Valva with very weakly sclerotized, rounded distal part; sacculus with sclerotized process; transtilla dorsally with sclerotized bifurcated process (not true gnathos). Aedeagus not spiny or hooked; vesica with two cornuti, at least one of them serrate (see also additional comments below).

Female genitalia (description based on additional material from Taiwan collected by W. Mey and G. Ebert and deposited in Museum für Naturkunde, Berlin). Ovipositor absent (present in Chalcosiinae). Ductus bursae tubular, corpus bursae translucent, not double-lobed, without signa. Pseudobursa absent (present in Chalcosiinae) (see also additional comments below).

Differential diagnosis when compared to Procridinae and Chalcosiinae.

The description of Inouelinae has to be based on only a few existing specimens. However, the characters are clear. The fact that there is no sexual dimorphism in the head capsule in Inouelinae (breadth of frons and size of compound eyes are equal in male and female) separates this subfamily from the Procridinae and Chalcosiinae. Moreover, the chaetosema in the Inouelinae is a unique character in the Zygaenidae and is considered to represent a good autapomorphy of this newly described subfamily. It differs from the chaetosemata of Procridinae and Chalcosiinae. The procridine chaetosema and the chalcosiine chaetosema are considered to represent autapomorphies for each of these two subfamilies. The antenna of Inouelinae is also unique within Zygaenidae. The shaft is dorsoventrally compressed and the long and slender pectinations are arranged with large spaces between them, reminiscent of similar antennae in some Psychidae. The wing venation of the Inouelinae shows an ancestral character combination with the full number of veins present in forewing and all veins arising evenly spaced from the cell. This character is shared with the more primitive groups in Procridinae but is in contrast to this character situation in Chalcosiinae (with several radial veins stalked together).

Additional comments.

Some important characters could not be sufficiently studied owing to lack of material. It would be especially interesting to know how the receptaculum seminis is constructed in the female. We also studied additional specimens from the Philippines (deposited in the Museum für Naturkunde, Berlin) with the external habitus of Inouelinae and strong similarities in the characters of the head and wing venation. However, these specimens have very different genitalic structures from those of the two species *I. formosensis* and *I. exiguitata.*

Nevertheless, these specimens may belong to Inouelinae and represent a separate group in this subfamily. Extreme variation in genitalic morphology (for example in the American genus *Neoprocris* Jordan, 1915 (Tarmann 1984)) is known in the Procridinae. The same situation can be present also in the Inouelinae. In future more species could be included in this subfamily.

So far this new subfamily is only known from South East Asia. The biology of Inouelinae species is unknown.

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FIGURES

Black and white figures on following pages are in numerical order.
However, figures 23, 32, 33, 35, 36, 37, 41, 52, 53, 54, 55, 56, 57, 58 and 82 are color images included in separate color plates at the end of the paper.

Photos taken by Konstantin A. Efetov – (KAE), by Gerhard M. Tarmann – (GMT).

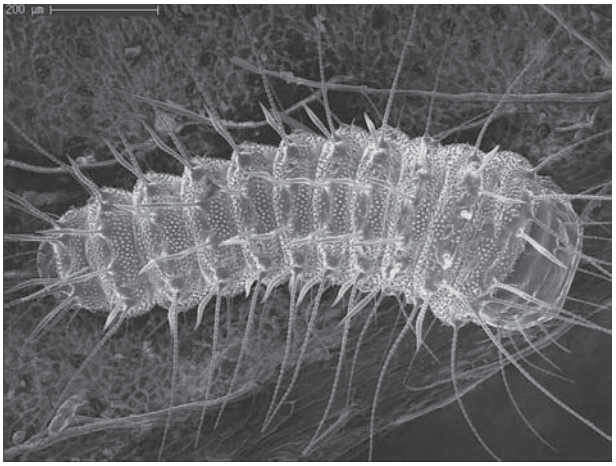


Fig. 1. First instar larva of *Pollanisus subdolosa clara* Tarmann, 2004 (Procridinae, Artonini), Australia, New South Wales. (Photo A. Zwick). (after Tarmann 2004)

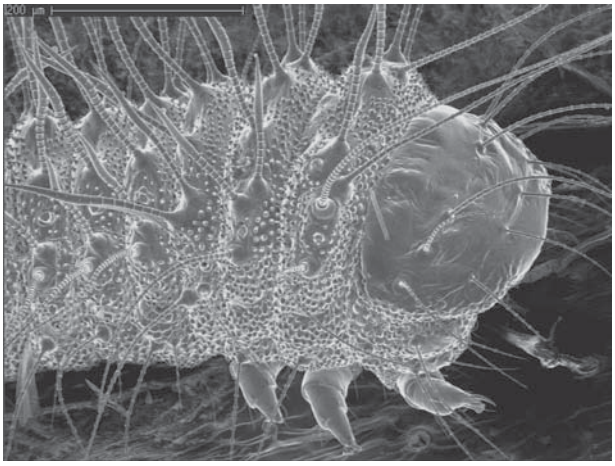


Fig. 2. Idem, head and thorax in higher magnification. (Photo A. Zwick). (after Tarmann 2004).

Fig. 3. Head hidden (retracted) below the thoracic shield in first instar larva of *Pollanisus subdolosa clara* (same larva as figs 1, 2). (Photo A. Zwick).

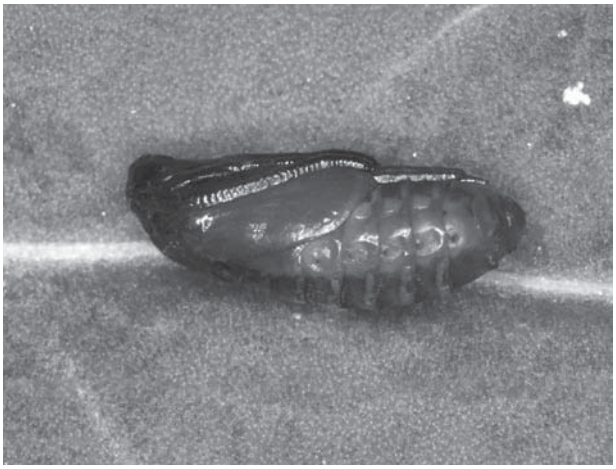


Fig. 4. Pupa of *Adscita mannii* (Lederer, 1853) (Procridinae, Procridini), lateral view. The second spiracle is covered by the wing. (GMT).

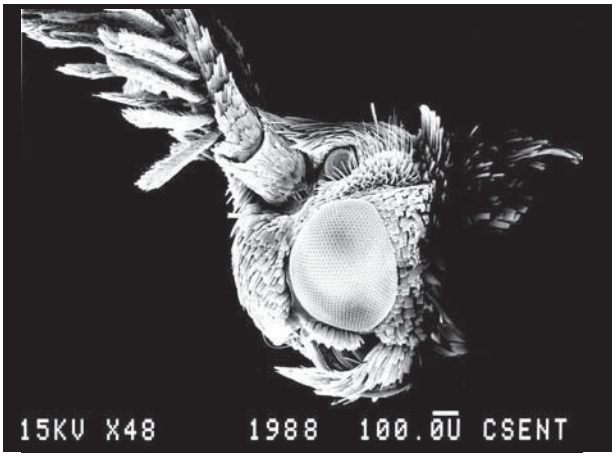


Fig. 5. Head of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), male, lateral view, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

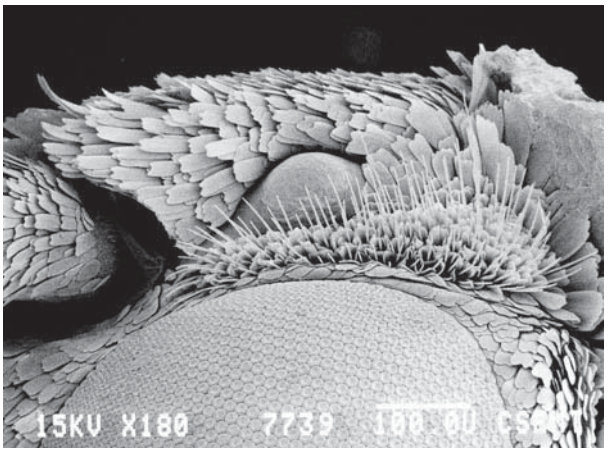


Fig. 6. Head of *Pseudoamuria uptoni* Tarmann, 2004 (Procridinae, Artonini), male, lateral view, with ocellus, chaetosema and upper half of compound eye, Australia, Queensland. (Photo E. Hines). (after Tarmann 2004).

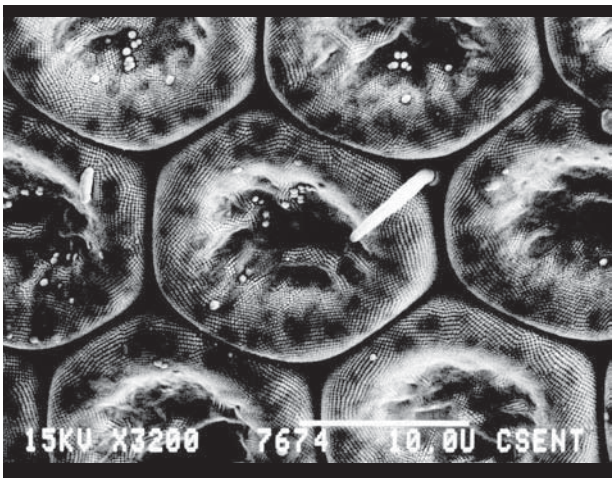


Fig. 7. Compound eye of *Homophylotis thyridota* Turner, 1904 (Procridinae, Artonini), with interommatidial seta, Australia, Queensland. (Photo E. Hines). (after Tarmann 2004).

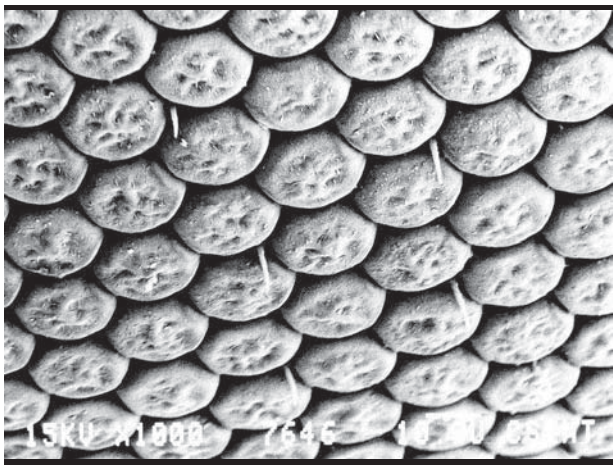


Fig. 8. Compound eye of *Adscita statices* (Linnaeus, 1758) (Procridinae, Procridini) with interommatidial setae, Austria, Tirol. (Photo E. Hines). (after Tarmann 2004).

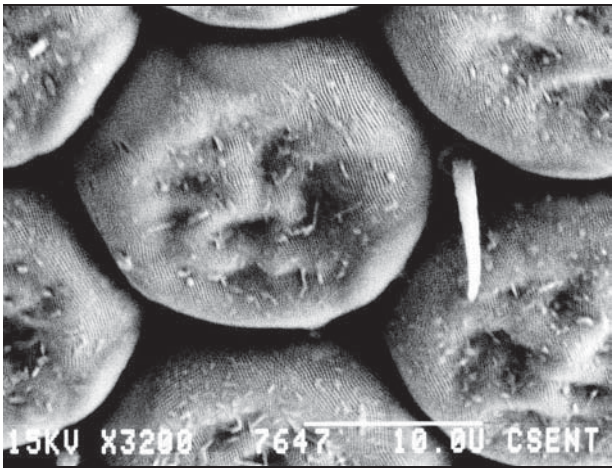


Fig. 9. Idem, higher magnification.

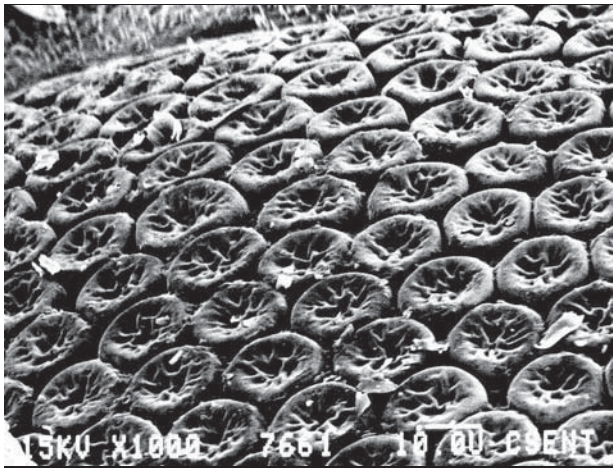


Fig. 10. Compound eye of *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905 (Procridinae, Procridini), without interommatidial setae, Japan. (Photo E. Hines). (after Tarmann 2004).

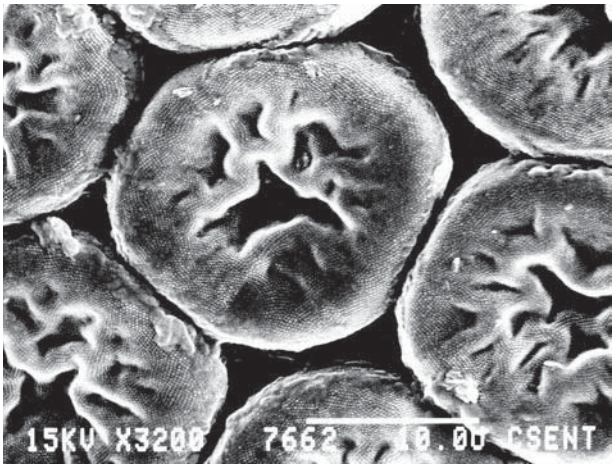


Fig. 11. Idem, high magnification.

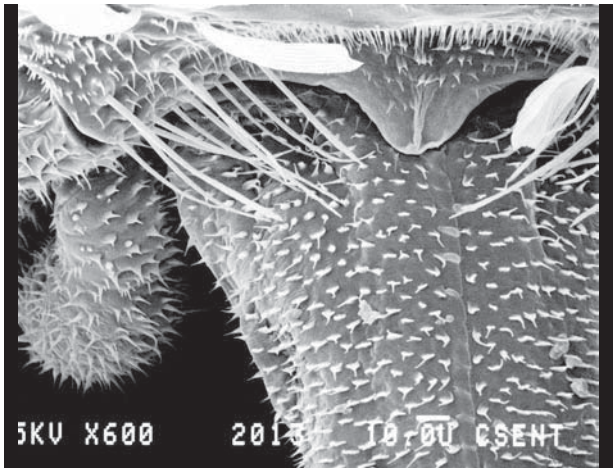


Fig. 12. Base of proboscis and maxillary palps of *Turneriprocris dolens* (Walker, 1854) (Procridinae, Artonini), male, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

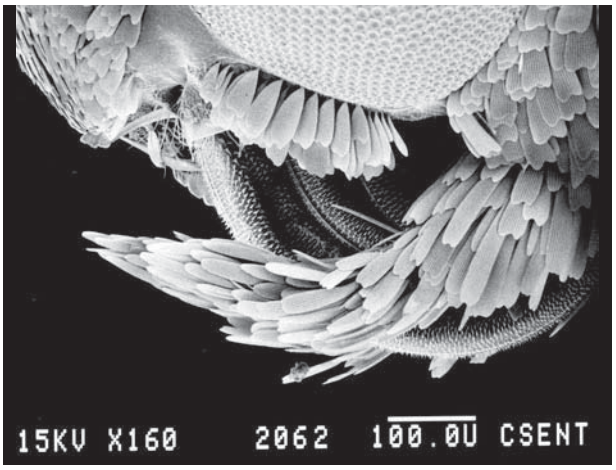


Fig. 13. Labial palp and eyelash below compound eye of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), male, lateral view, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

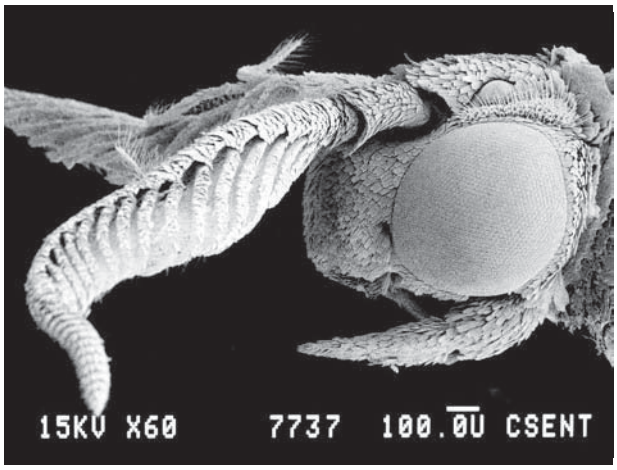


Fig. 14. Head in lateral view with labial palps of *Pseudoamuria uptoni* Tarmann, 2004 (Procridinae, Artonini), male, lateral view, Australia, Queensland. (Photo E. Hines). (after Tarmann 2004).

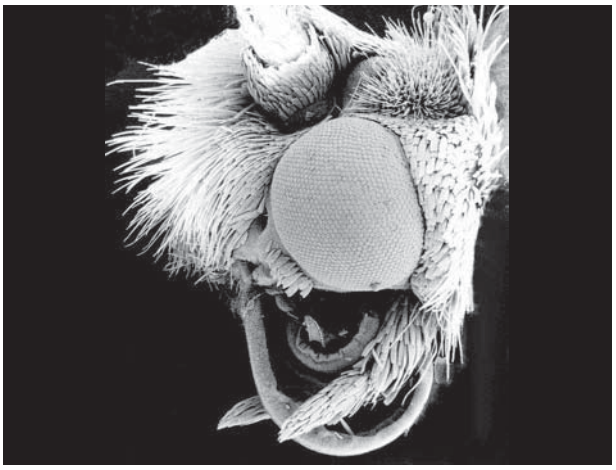


Fig. 15. Head in lateral view of *Adscita statices* (Linnaeus, 1758) (Procridinae, Procridini), Austria, Tirol. (Photo E. Hines). (after Tarmann 2004).

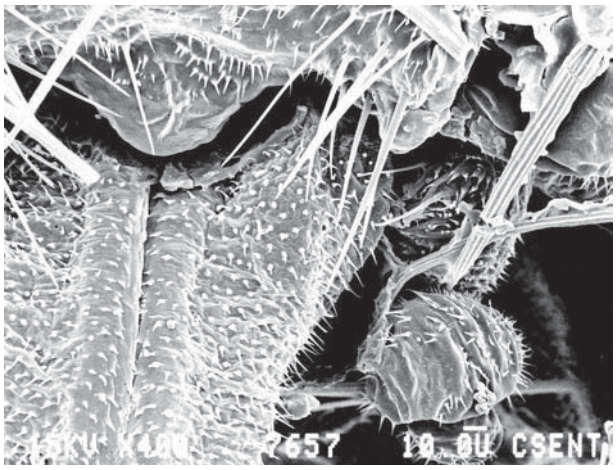


Fig. 16. Base of proboscis with clypeus and mandible lobe in *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905 (Procridinae, Procridini), Japan. (Photo E. Hines). (after Tarmann 2004).

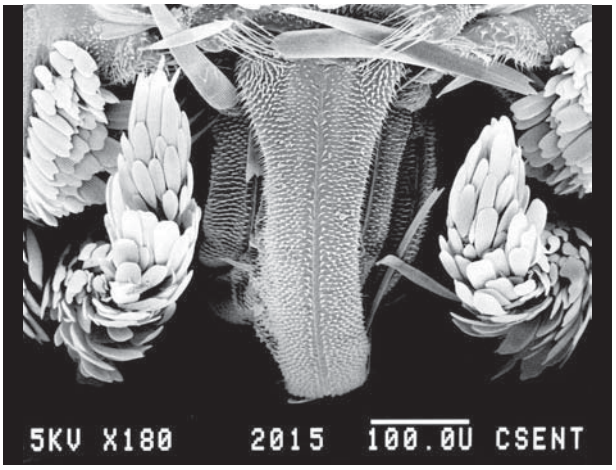


Fig. 17. Proboscis of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), male, frontal view, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

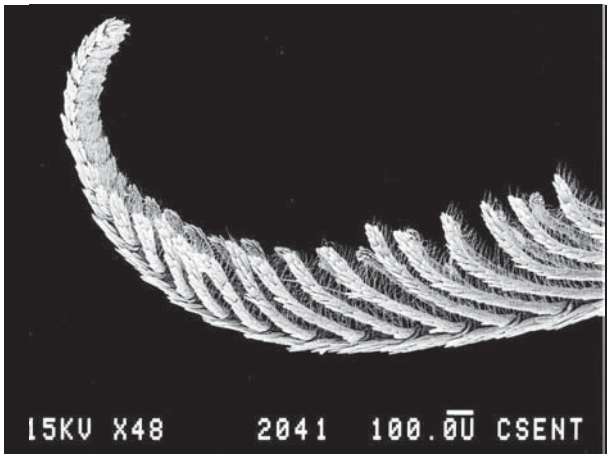


Fig. 18. Male antenna of *Australartona mirabilis* Tarmann, 2004 (Procridinae, Artonini), Australia, NSW. (Photo C. Beaton). (after Tarmann 2004).

Fig. 19. Distal part of male antenna of *Adscita statices* (Linnaeus, 1758) (Procridinae, Procridini), Austria, Tirol. (Photo E. Hines). (after Tarmann 2004).

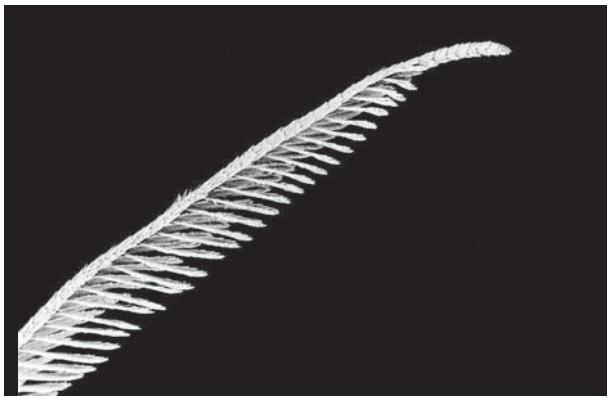
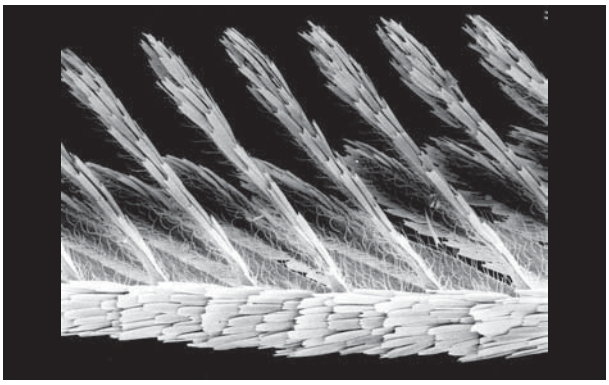


Fig. 20. Male antenna of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).



21. Pectination of male antenna of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

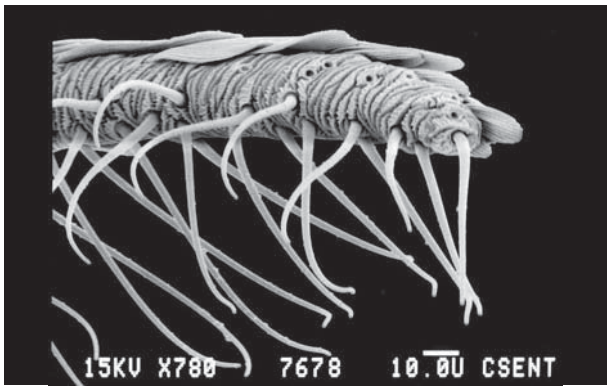


Fig. 22. Distal end of pectination of male antenna of *Homophylotis thyridota* Turner, 1904 (Procridinae, Artonini), Australia, Queensland. (Photo E. Hines). (after Tarmann 2004).

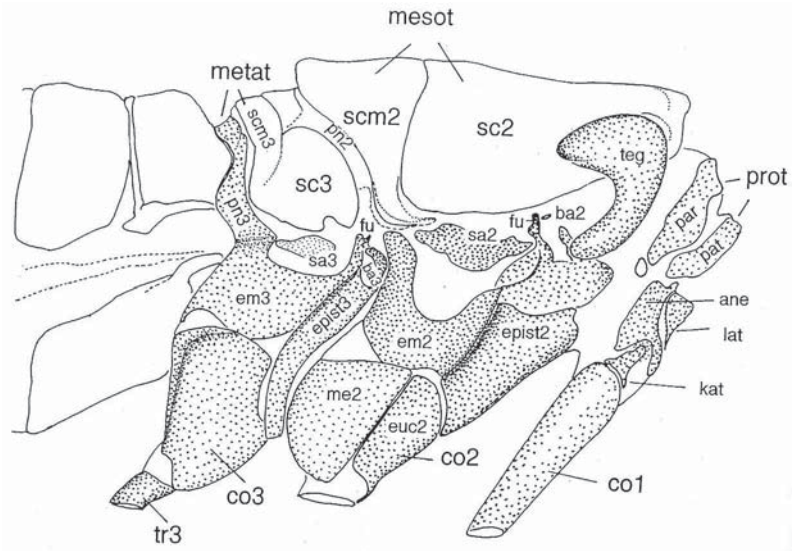


Fig. 24. Lateral view of thoracic segments of adult of *Zygaena trifolii* (Esper, 1783). **ane**: anepisternum; **ba**: basalare; **co**: coxa; **em**: epimeron; **epist**: episternum; **euc**: eucoxa; **fu**: fulcrum; **kat**: katepisternum; **lat**: laterocervicale (anterior arm omitted); **me**: meron; **mesot**: mesothorax; **metat**: metathorax; **par**: parapatagium; **pat**: patagia; **pn**: postnotum; **priot**: prothorax; **sa**: subalare; **sc**: scutum; **scm**: scutellum; **teg**: tegula; **tr**: trochanter; figures 1, 2, and 3 depict the pro- (1), meso- (2) and metathorac (3). (after Naumann et al. 1999).

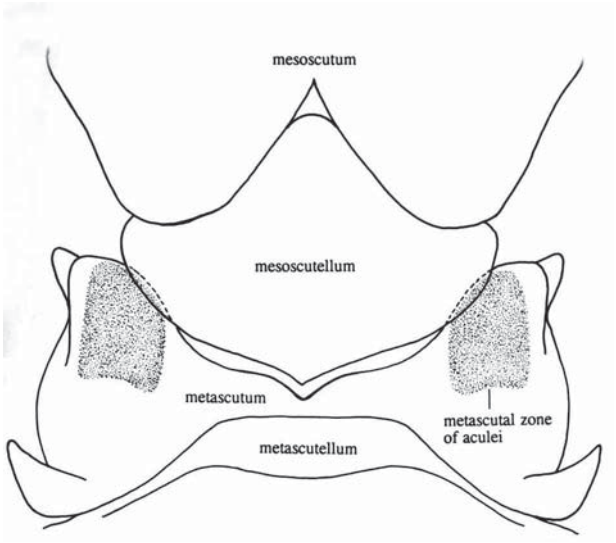


Fig. 25. Metathorax with wing-thorax-coupling mechanism, as in most ditrysian Lepidoptera. (after Scoble 1992).

Fig. 26. Right foreleg of *Zygaena* (*Zygaena*) *filipendulae* (Linnaeus, 1758). (after Efetov 2001c).

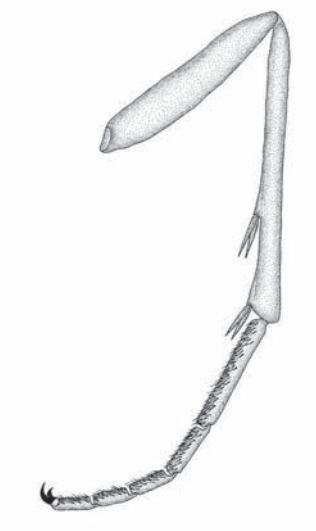


Fig. 27. Right hindleg of *Zygaena* (*Zygaena*) *filipendulae* (Linnaeus, 1758). (after Efetov 2001c).

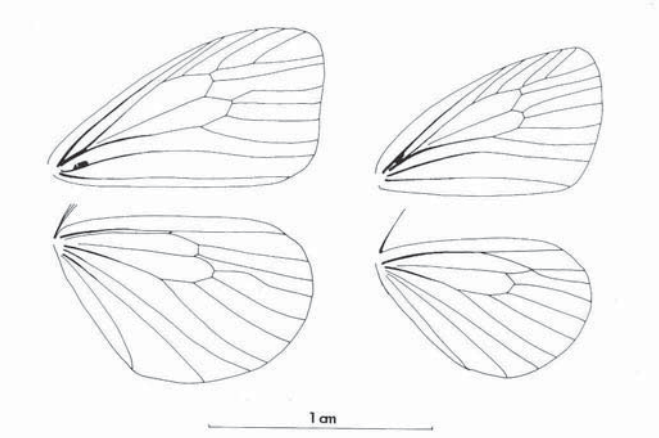


Fig. 28. Frenulum and retinaculum of female and male of two Chalcosiinae: *Pseudarbudas ochrea* (Elwes, 1890), female (left), *Heteropanula flavimacula* (Hampson, 1892), male (right). (after Tarmann 1992).

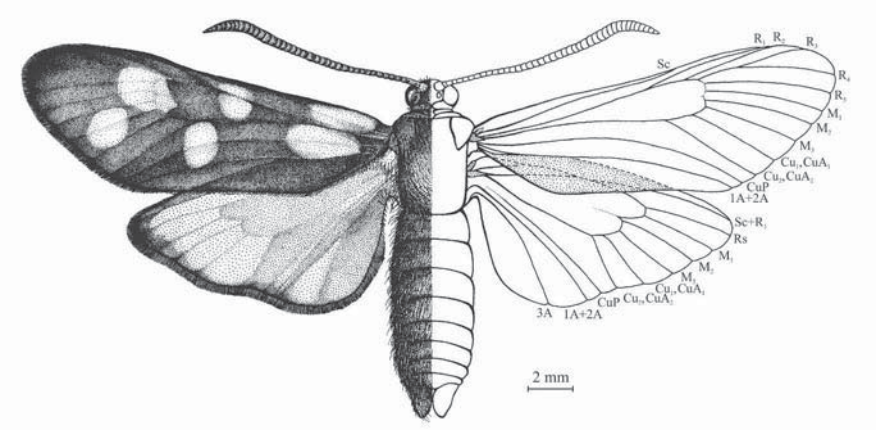
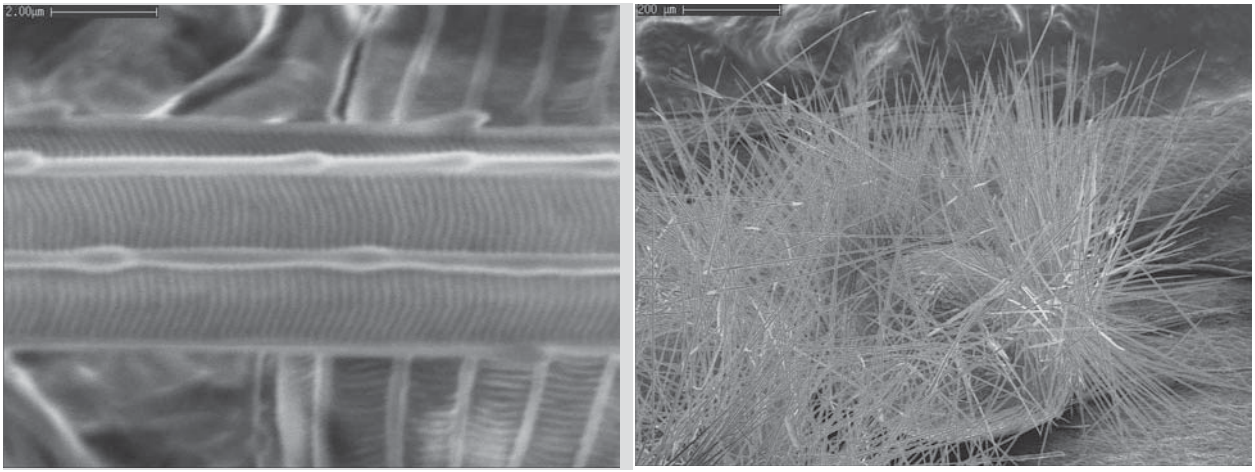


Fig. 29. *Zygaena* (*Zygaena*) *filipendulae* (Linnaeus, 1758). Male. Wing venation. (after Efetov 2005b).



Figs 30a, b. Scales with longitudinal ribs and transverse striae from the abdominal hairtuft of *Pollanisus subdolosa clara* Tarmann, 2004 (Procridinae, Artonini), protecting the egg, Australia, New South Wales. (Photo A. Zwick). (after Tarmann 2004).

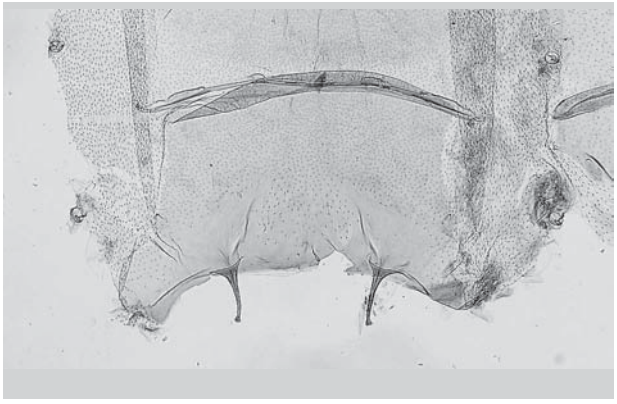


Fig. 31. Tortricoid apodemes on first abdominal segment of abdomen of *Pseudoamuria uptoni* Tarmann, 2004 (Procridinae, Artonini), Australia, Queensland. (after Tarmann 2004).

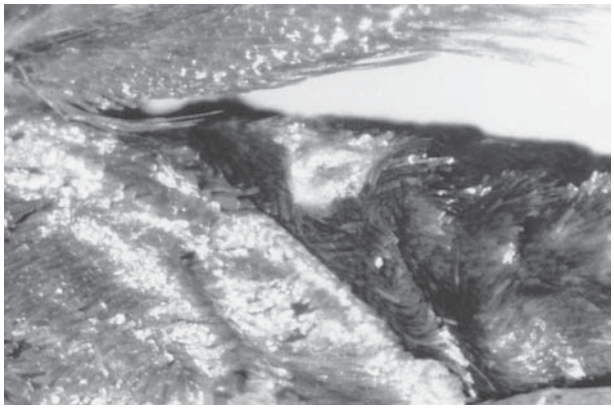


Fig. 34. Lateral gland on second abdominal segment of male of *Australartona mirabilis* Tarmann, 2004 (Procridinae, Artonini), Australia, NSW. (Photo J. Green). (after Tarmann 2004).

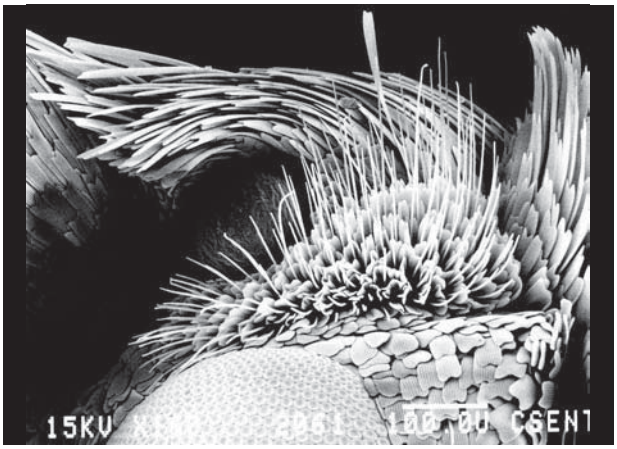


Fig. 38. Chaetosema of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini), male, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

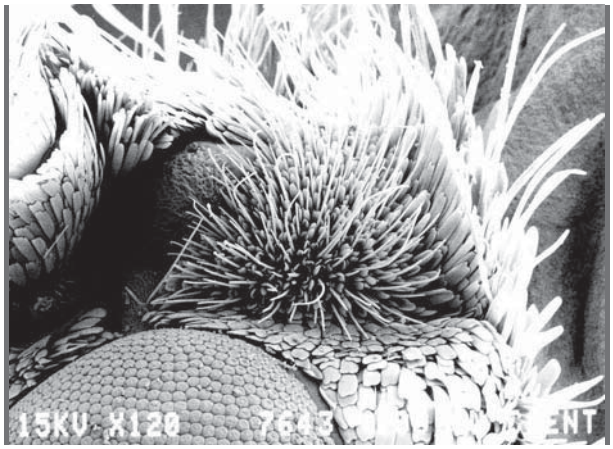


Fig. 39. Chaetosema of *Adscita statices* (Linnaeus, 1758) (Procridinae, Procridini), Austria, Tirol. (Photo E. Hines). (after Tarmann 2004).

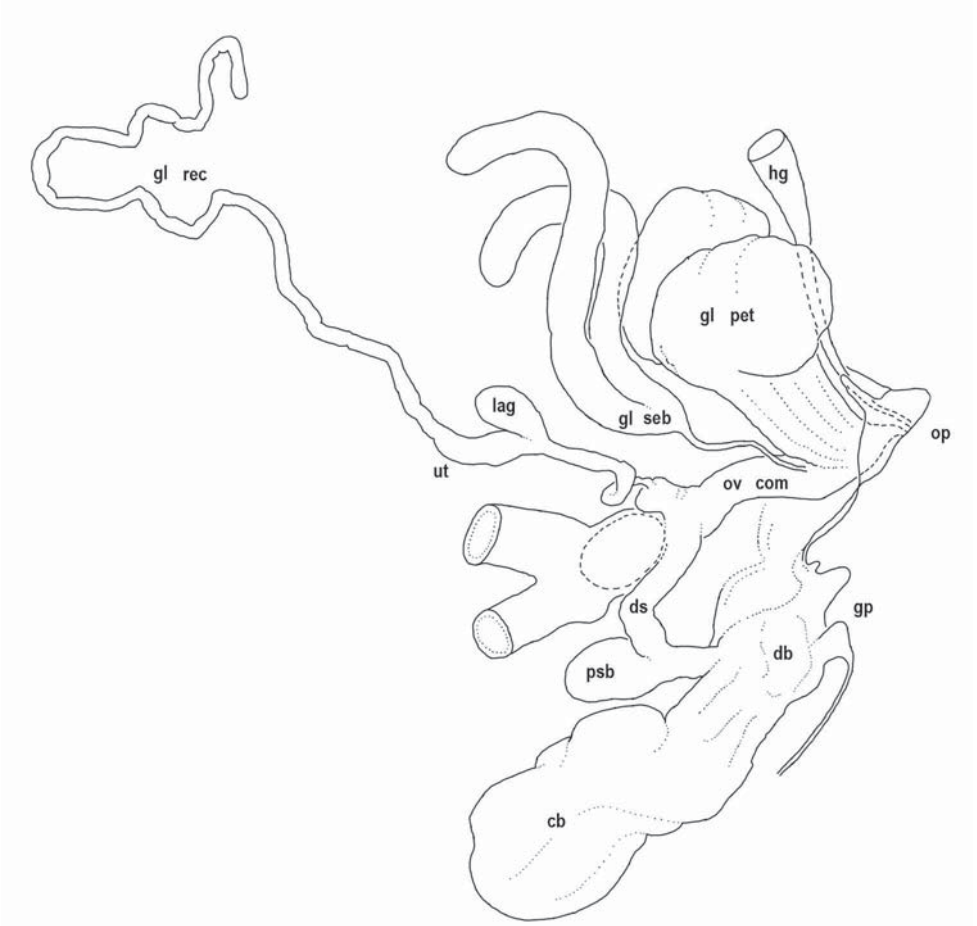


Fig. 40. Internal female genitalia of *Zygaena* (*Zygaena*) *trifolii* (Esper, 1783). **cb**: corpus bursae; **db**: ductus bursae; **ds**: ductus seminalis; **gl pet**: Petersen's gland; **gl rec**: glandula receptaculi; **gl seb**: sebaceous gland; **gp**: gonoporus; **hg**: hind gut; **lag**: lagena; **op**: ooporus; **ov com**: oviductus communis; **psb**: pseudobursa; **ut**: utriculus. (after Naumann et al. 1999).

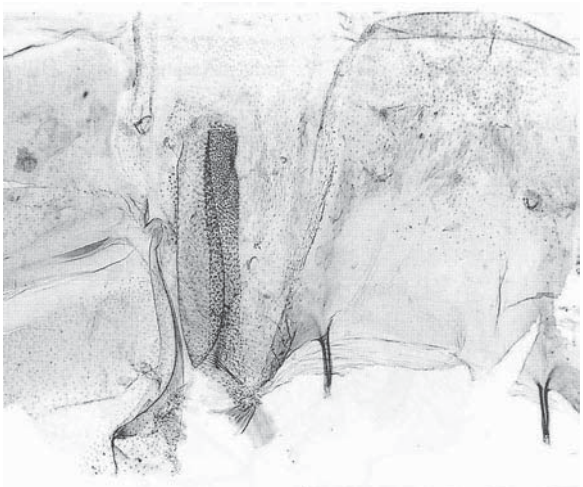


Fig. 42. Abdominal fold on the second pleurite of *Arbudas submacula* (Wileman, 1910) (Chalcosiinae), Taiwan. (after Tarmann 1992).

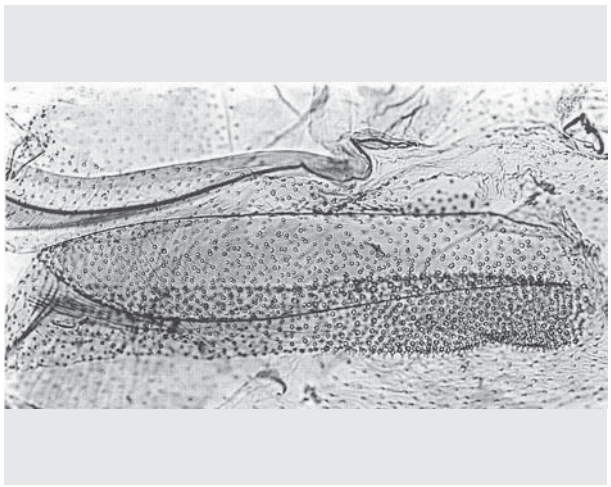


Fig. 43. Idem, higher magnification.

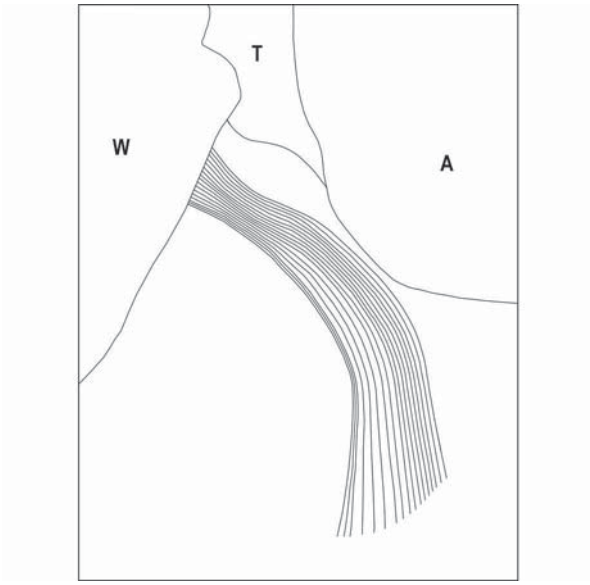


Fig. 44. Hindwing of *Arbudas submacula* (Wileman, 1910) with S-shaped bristles. **A**: abdomen; **T:** thorax; **W**: hindwing.

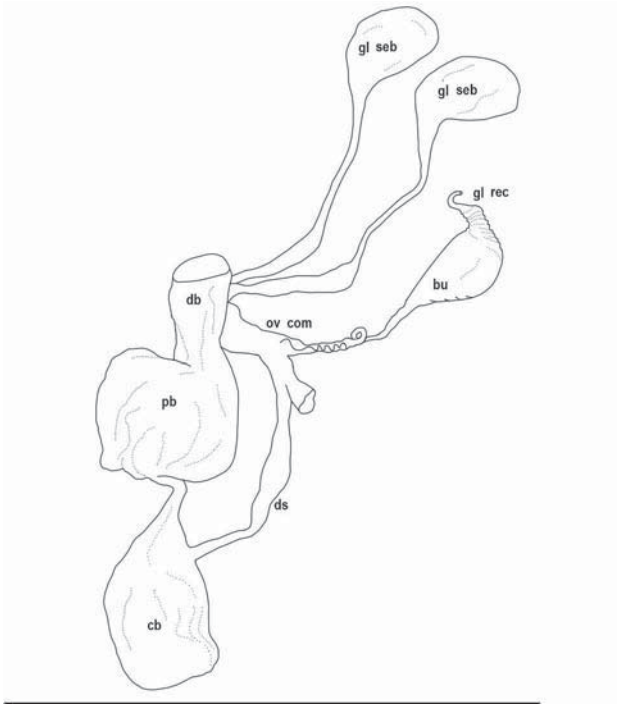


Fig. 45. Internal female genitalia of *Myrtartona coronias* (Meyrick, 1886) (Procridinae, Artonini) with bursa utricularis. **bu**: bursa utricularis; **cb**: corpus bursae; **db**: ductus bursae; **ds**: ductus seminalis; **gl rec**: glandula receptaculi; **gl seb**: sebaceous gland; **ov com**: oviductus communis; **pb**: praebursa.

Fig. 46. Female genitalia of *Pollanisus apicalis* (Walker, 1854) (Procridinae, Artonini), with well-developed praebursa, Australia, ACT. (after Tarmann 2004).

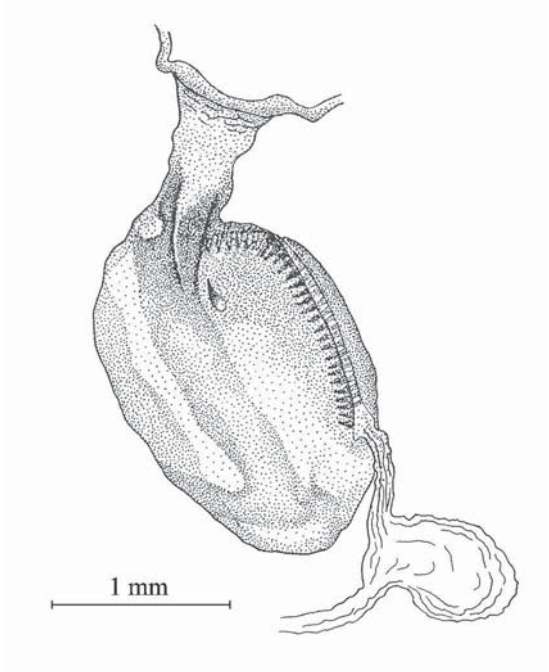


Fig. 47. Female genitalia of *Artona* (*Fuscartona*) *martini* Efetov, 1997 (Procridinae, Artonini), with well-developed praebursa, China. (after Efetov 1997a).

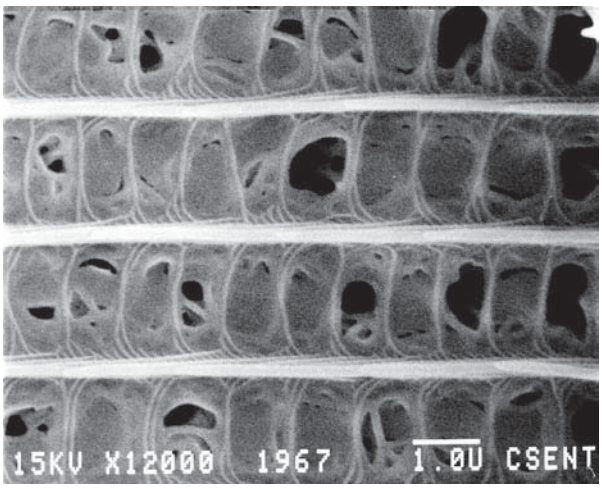


Fig. 48. Ultrasturcture of forewing scale of *Turneriprocris dolens* (Walker, 1854) (Procridinae, Artonini), male, Australia, ACT. (Photo C. Beaton). (after Tarmann 2004).

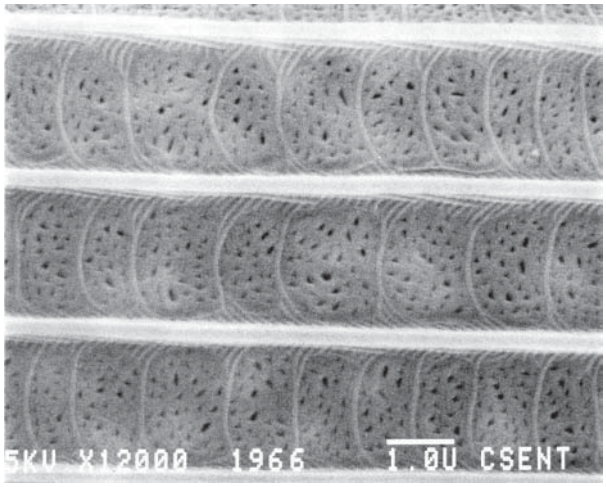


Fig. 49. Idem, shiny scale.

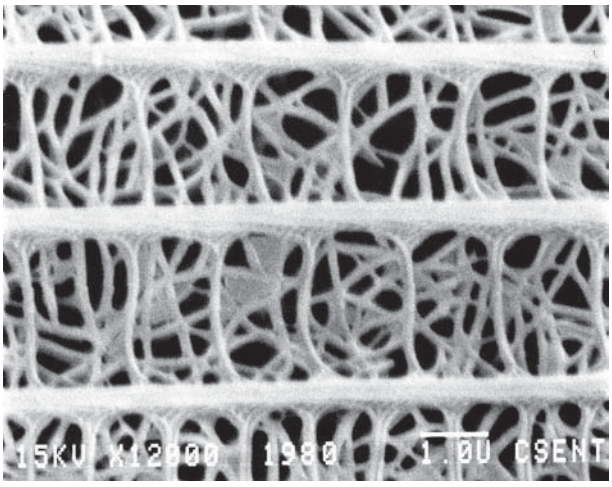


Fig. 50. Ultratructure of forewing scale of *Myrtartona leucopleura* (Meyrick, 1886) (Procridinae, Artonini), male, Australia, NSW. (Photo C. Beaton). (after Tarmann 2004).

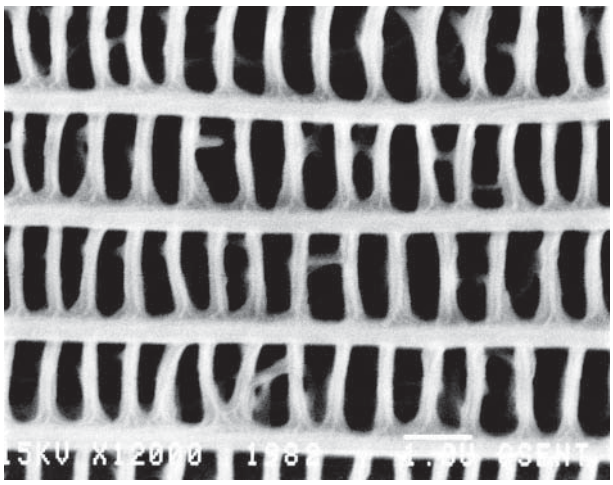


Fig. 51. Idem, hindwing scale.

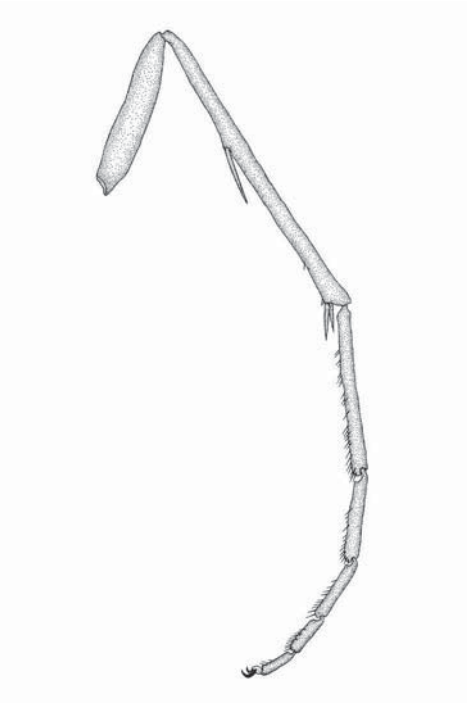


Fig. 59. Right hindleg of *Artona* (*Balataea*) *gracilis* (Walker, 1865). (after Efetov 2005a).

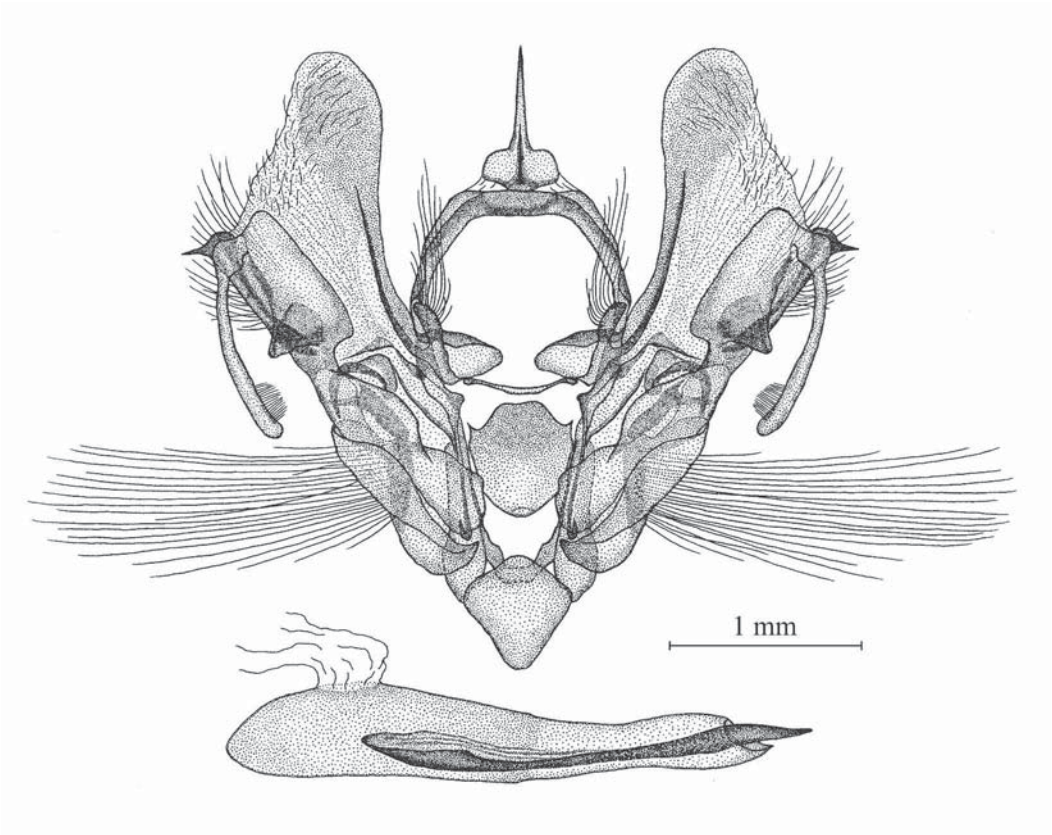
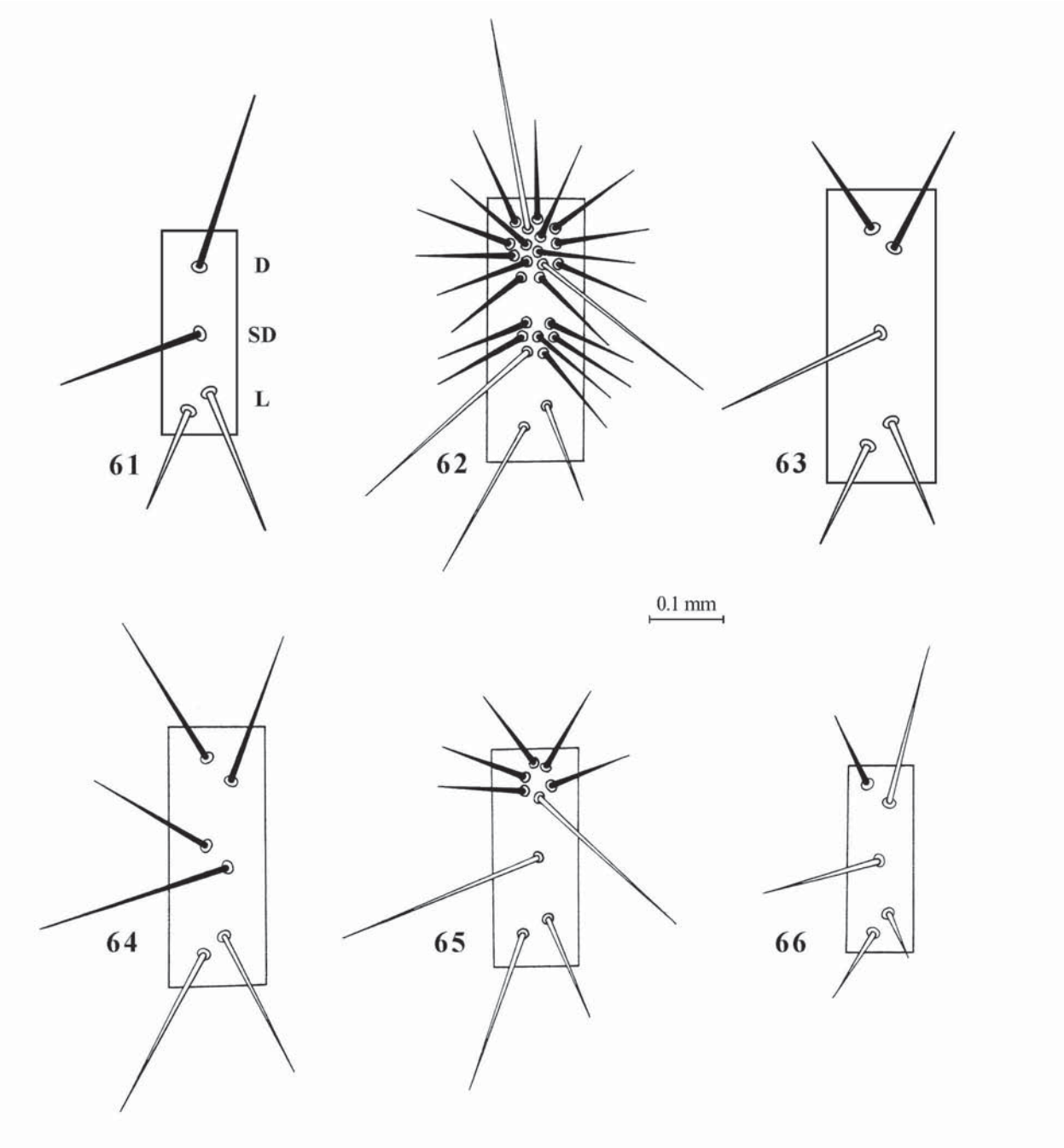


Fig. 60. Male genitalia of *Artona* (*Balataea*) *gracilis* (Walker, 1865). (after Efetov 2005b).



Figs. 61–66. Diagrams of chaetotaxy of the first abdominal segment of first instar larvae of Procridinae. Anterior end to the left. **D** – dorsal, **SD** – subdorsal, **L** – lateral setae. **61**. *Artona* (*Fuscartona*) *martini* Efetov, 1997. **62**. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775). **63**. *Adscita* (*Adscita*) *statices* (Linnaeus, 1758). **64**. *A.* (*Tarmannita*) *mannii* (Lederer, 1853). **65**. *Jordanita* (*Roccia*) *budensis* (Speyer & Speyer, 1858). **66**. *J.* (*Jordanita*) *chloros* (Hübner, 1813). (after Efetov 2001c; Efetov & Hayashi 2008).

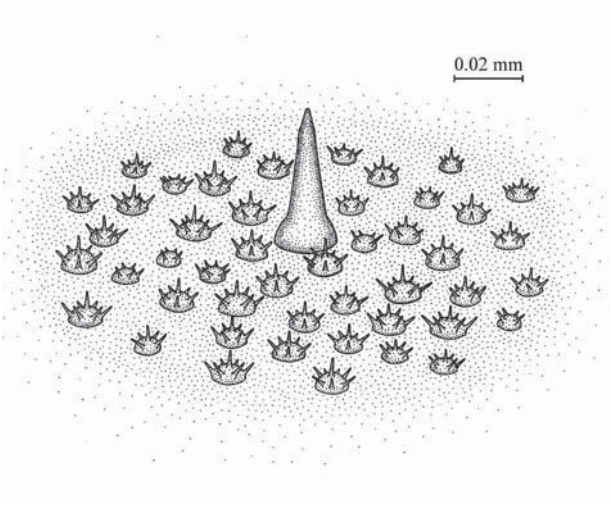


Fig. 67. Combination of the unispined macrotubercle and multispined microtubercles on the cuticle of the last instar larva of *Theresimima ampellophaga* (Bayle-Barelle, 1808). (after Efetov 2004).

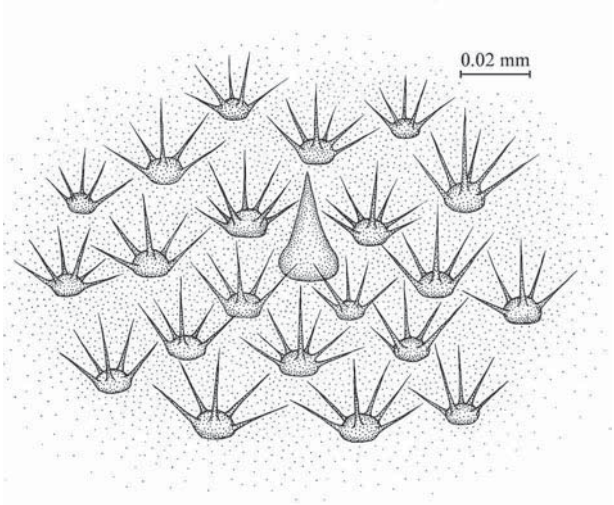


Fig. 68. Combination of the unispined macrotubercle and multispined microtubercles on the cuticle of the last instar larva of *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907. (after Efetov 2004).

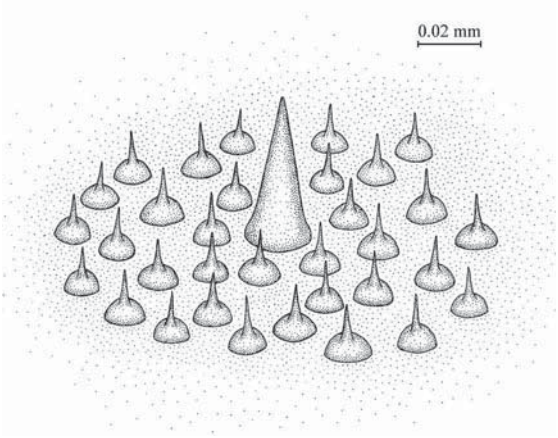


Fig. 69. Combination of the unispined macrotubercle and unispined microtubercles on the cuticle of the last instar larva of *Hedina consimilis* (Leech, 1898).

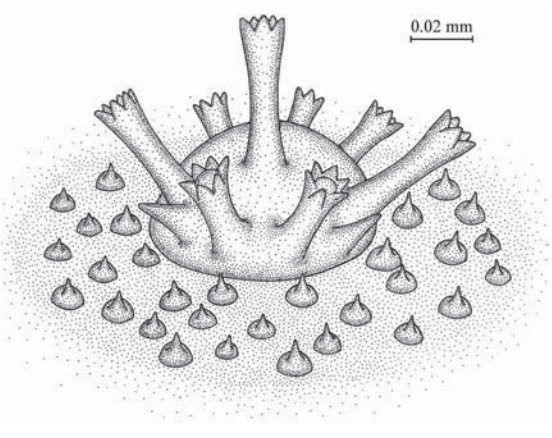
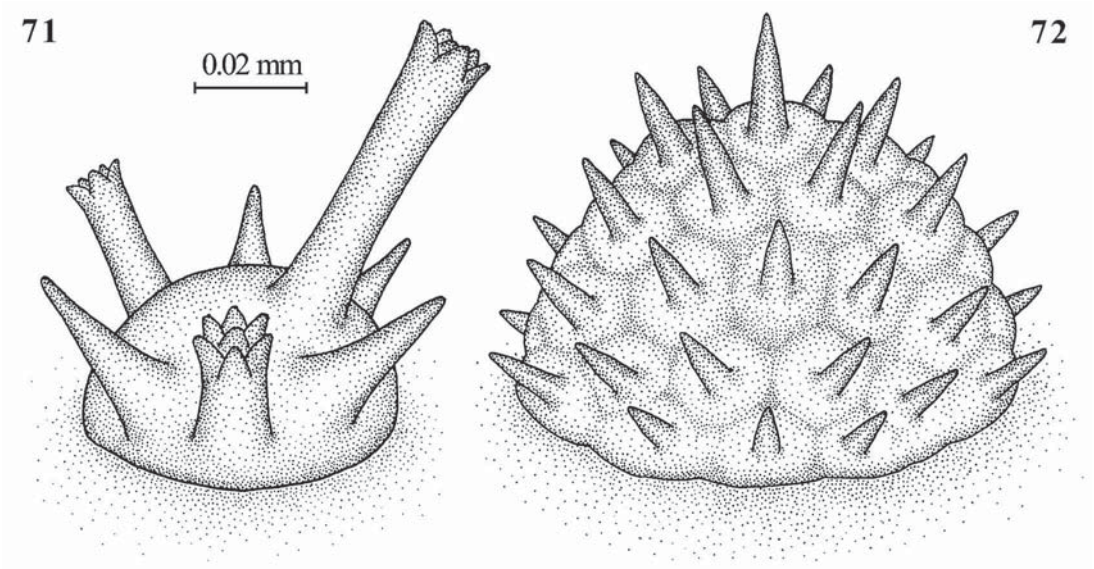
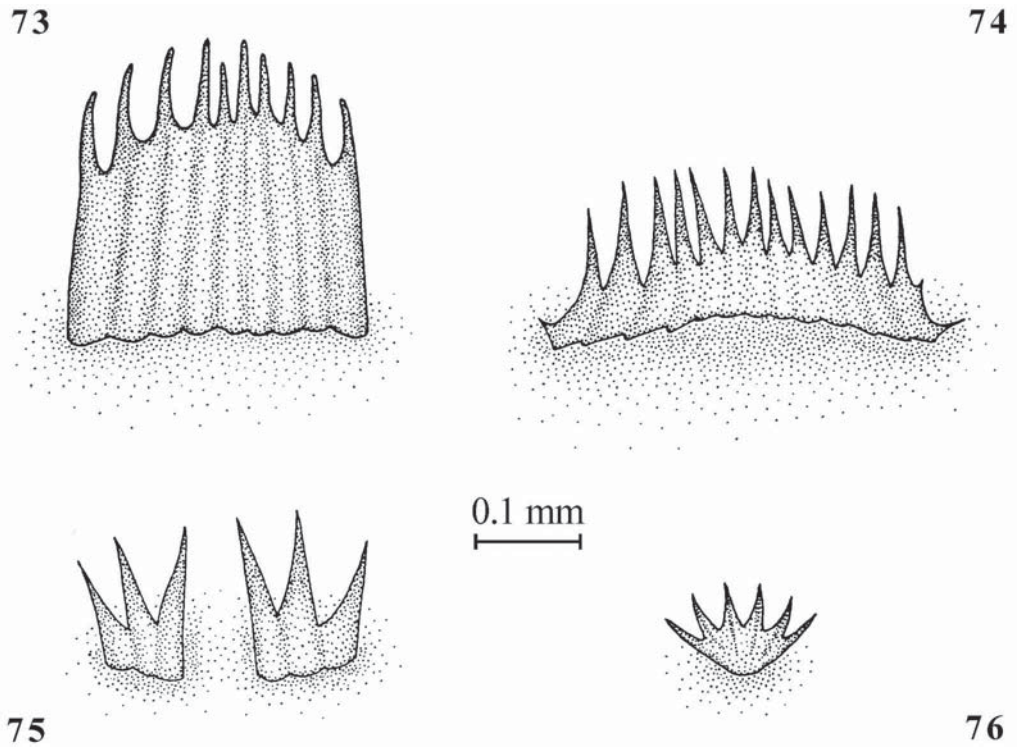


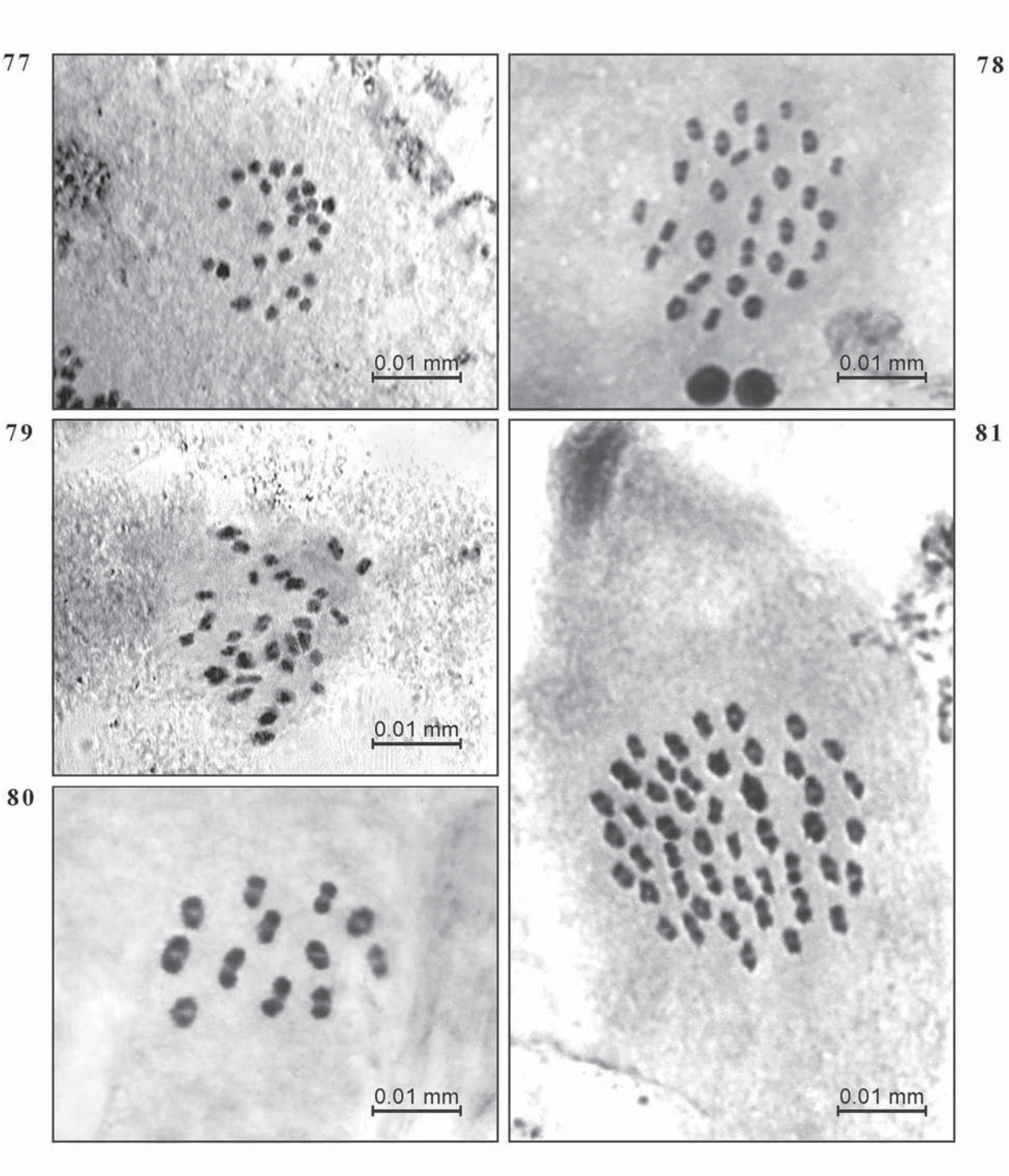
Fig. 70. Combination of the multispined macrotubercle and unispined microtubercles on the cuticle of the last instar larva of *Adscita* (*Adscita*) *geryon* (Hübner, 1813). (after Efetov 2004).



Figs. 71, 72. Macrotubercles of adult larvae. **71**. *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862). **72**. *J.* (*Rjabovia*) *horni* (Alberti, 1937). (after Efetov 2004).



Figs. 73–76. Anal combs of adult larvae. **73**. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775): anal comb with high base. **74**. *Adscita* (*Adscita*) *capitalis* (Staudinger, 1879): anal comb with low base. **75**. *A.*(*Procriterna*) *subtristis* (Staudinger, 1887): anal comb double. **76**. *Zygaenoprocris* (*Keilia*) *minna* (Efetov, 1991): anal comb reduced. (after Efetov 2004).



Figs. 77–81. Meiotic metaphase of spermatogenesis in some species of the Procridinae. **77**. *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907: haploid chromosome number (n) = 25. **78**. *Theresimima ampellophaga* (Bayle-Barelle, 1808): n = 28. **79**. *Rhagades* (*Naufockia*) *brandti* (Alberti, 1938): n = 31. **80**. *Rh.* (*Wiegelia*) *amasina* (Herrich-Schäffer, 1851): n = 12. **81**. *Rh.* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775): n = 47. (KAE). (after Efetov 2004).

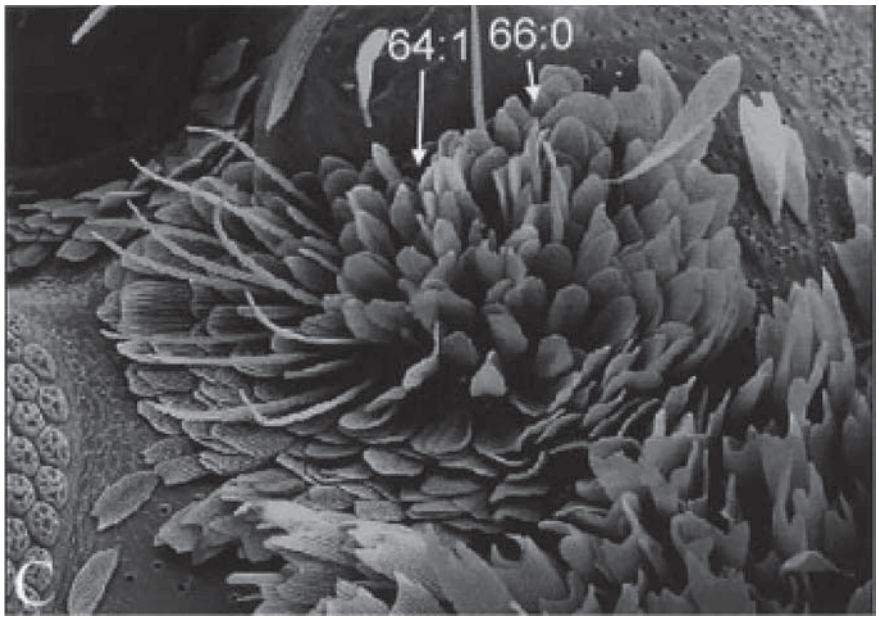


Fig. 83. Chaetosema of *Inouela formosensis* Efetov, 1999. (after Yen et al. 2005).

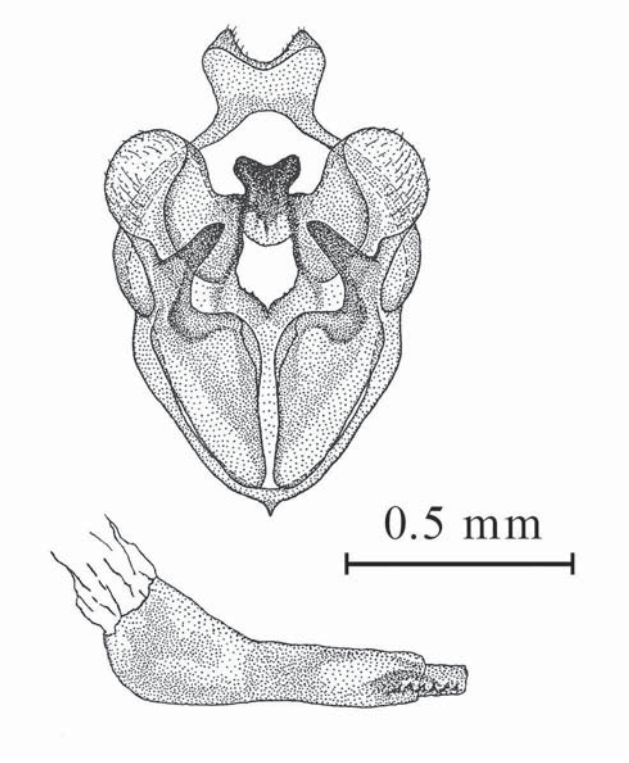


Fig. 84. Male genitalia of paratype of *Inouela formosensis* Efetov, 1999. (after Efetov 1999).

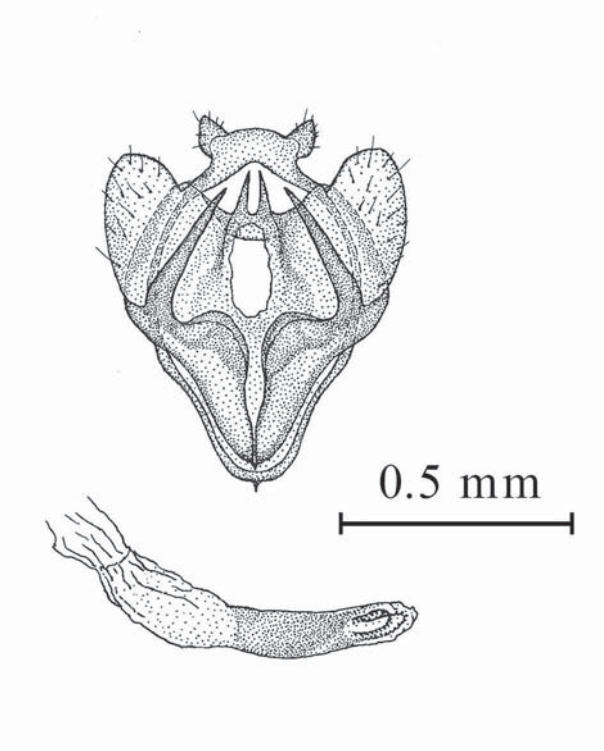


Fig. 85. Male genitalia of paratype of *Inouela exiguitata* (Inoue, 1976). (after Efetov 1999).

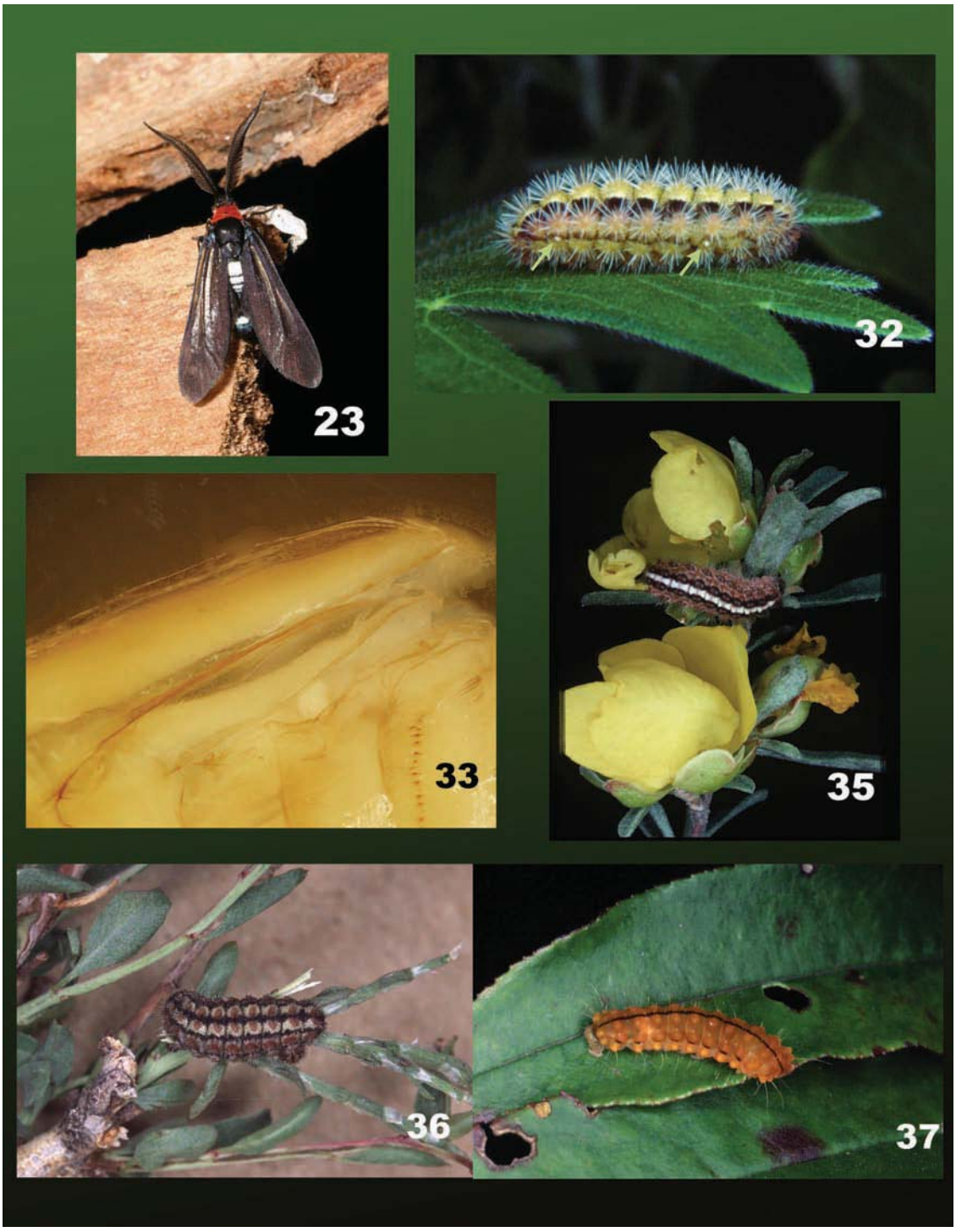


Fig. **23**. Male of *Hestiochora tricolor* (Walker, 1854) (Procridinae, Artonini), with coloured patagia and parapatagia, Australia, NSW. (GMT). Fig. **32**. Adult larva of *Adscita bolivari* (Agenjo, 1937) with lateral protuberances. (KAE). (after Efetov & Tarmann 2004). Fig. **33**. Pupa of *Pollanisus viridipulverulenta* (Guérin-Méneville, 1839) (Procridinae, Artonini) with opened wing with the abdominal gland 2 visible. (after Tarmann 2004). Fig. **35**. Adult larva of *Pollanisus apicalis* (Walker, 1854) (Procridinae, Artonini) freely feeding on *Hibbertia obtusifolia* DC. Australia, ACT. (GMT). Fig. **36**. Adult larva of *Zygaenoprocris persepolis* (Alberti, 1938) (Procridinae, Procridini) freely feeding on *Polygonum spinosum* H. Gross, Iran. (GMT). Fig. **37**. Adult larva of *Soritia costimacula battakorum* (Dohrn, 1906) (Chalcosiinae) freely feeding on *Eurya* sp., Indonesia, Sumatra. (GMT).

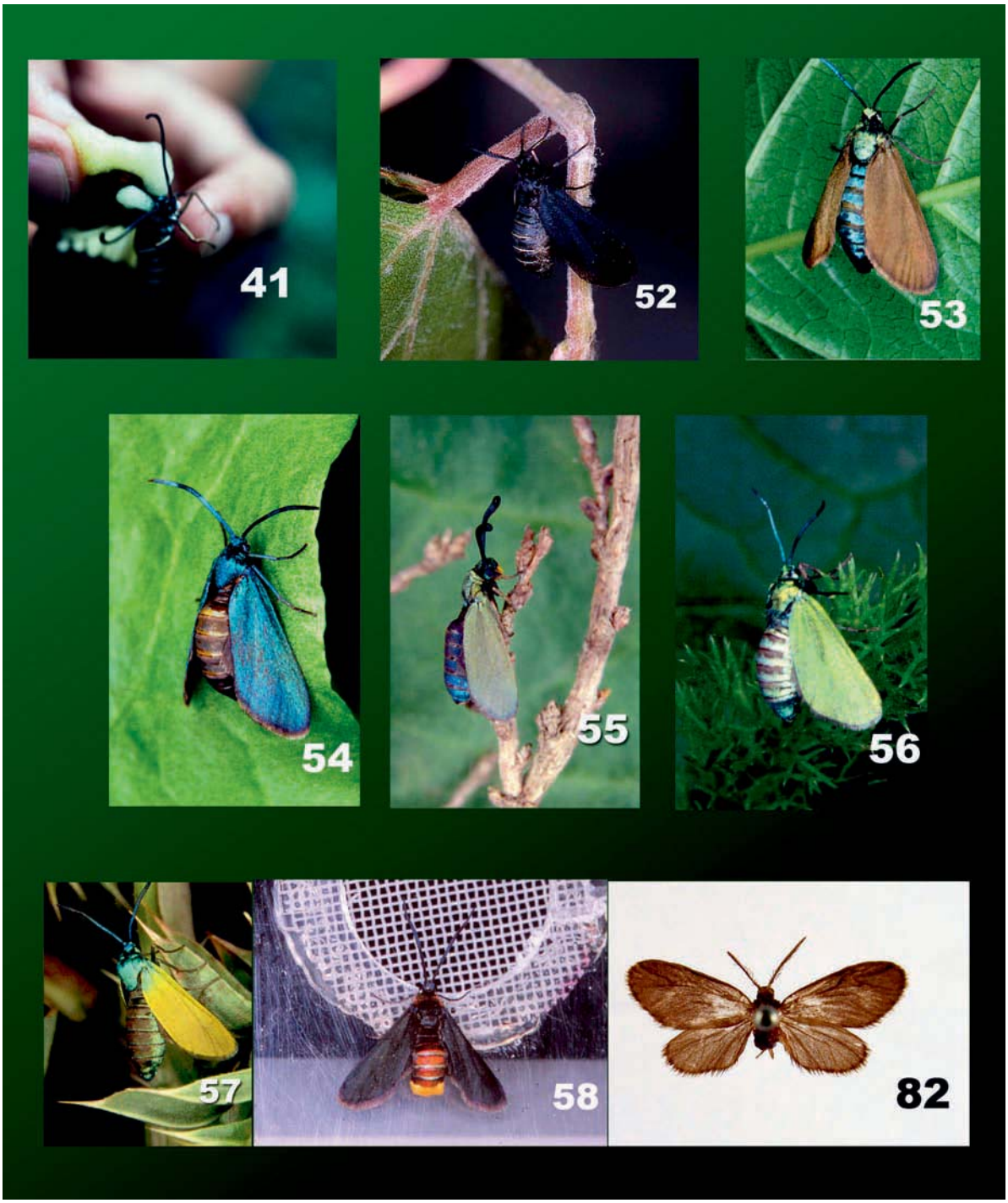


Fig. **41.** Defensive foam from head of *Erasmia pulchella* Hope, 1841 (Chalcosiinae). (GMT). Fig. **52**. Female of *Acoloithus rectarius* Dyar, 1898 (Procridinae, Procridini) in calling position waiting for male, U.S.A., Arizona. (GMT). Fig. **53**. Female of *Theresimima ampellophaga* (Bayle-Barelle, 1808) in calling position waiting for male, Crimea. (KAE). (after Efetov 2001c). Fig. **54**. Female of *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775) in calling position waiting for male, Crimea. (KAE). (after Efetov 2001c). Fig. **55**. Female of *Zygaenoprocris* (*Molletia*) *taftana* (Alberti, 1939) in calling position waiting for male, Armenia. (KAE). (after Efetov 2001c). Fig. **56.** Female of *Jordanita* (*Roccia*) *budensis* (Speyer & Speyer, 1858) in calling position waiting for male, Crimea. (KAE). (after Efetov 2001c).Fig. **57**. Female of *Jordanita* (*Jordanita*) *chloros* (Hübner, 1813) in calling position waiting for male, Crimea. (KAE). (after Efetov 2001c). Fig. **58**. Female of *Pollanisus commoni* Tarmann, 2004, in calling position waiting for male, Australia, Queensland. (GMT). Fig. **82**. *Inouela formosensis* Efetov, 1999. Holotype male (KAE).

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EGG AND FIRST INSTAR OF THE NEOTROPICAL GEOMETRID MOTH *PERO OBTUSARIA* PROUT (GEOMETRIDAE: ENNOMINAE: AZELININI)

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ABSTRACT. The external morphology of the egg and first instar of the little-known Neotropical geometrid moth *Pero obtusaria* Prout, 1928 (Lepidoptera: Geometridae: Ennominae: Azelinini) is described and illustrated based on light and scanning electron microscopy. This is the first morphological study dealing with immature stages of a Neotropical species of the highly diverse New World moth genus *Pero*.

Additional key words: Aeropyle, Chaetotaxy, Immature stages, Micropyle, SEM

The highly diverse New World moth genus *Pero* Herrich-Schäffer (Lepidoptera, Geometridae, Ennominae, Azelinini) includes more than 300 described species, most of which are Neotropical (Poole 1987, Pitkin 2002, Ferris 2003, Lévêque 2006, Brown 2007, Vargas 2007). Despite the enormous diversity of this genus in the Neotropics, the natural history of the species has been scarcely documented in this area, and only a few records based on research done on their host plants suggest that their larvae are mainly host specialists (Poole 1987, Barros 2007, Bodner et al. 2010, Vargas 2011). In contrast, the host plants of the Nearctic species are better known, and many cases of polyphagy have been described (Comstock 1963, McGuffin 1963, Poole 1987, Robinson et al. 2010). Detailed morphological descriptions are available for the immature stages of some Nearctic *Pero* (Comstock 1963, McGuffin 1963, Salkeld 1983), but the morphology of the immature stages of the Neotropical species remain unknown.

Pero obtusaria Prout, 1928, is a little-known Neotropical geometrid moth described from Peru. The southern limit of its geographic range extends to the coastal valleys of the Atacama Desert of northern Chile (Vargas & Hausmann 2008), where the native shrub *Pluchea chingoyo* (Asteraceae) is the only host plant recorded so far for its folivorous and mostly nocturnal-feeding larvae (Vargas 2011).

Detailed knowledge of external morphology of immature stages is widely recognized as an important tool in studies dealing with the evolution and systematics of Lepidoptera (Dias et al. 2015, Hernández-Mejía et al. 2015, Salik et al. 2015, Dolinskaya 2016, Neves & Paluch 2016, Nieves-Uribe et al. 2016). Therefore, the objective of this study is to describe and illustrate the external morphology of the egg and first instar of *P. obtusaria* based on light and scanning electron microscopy, providing the first morphological observations of immature stages of a Neotropical species of Pero.

MATERIALS AND METHODS

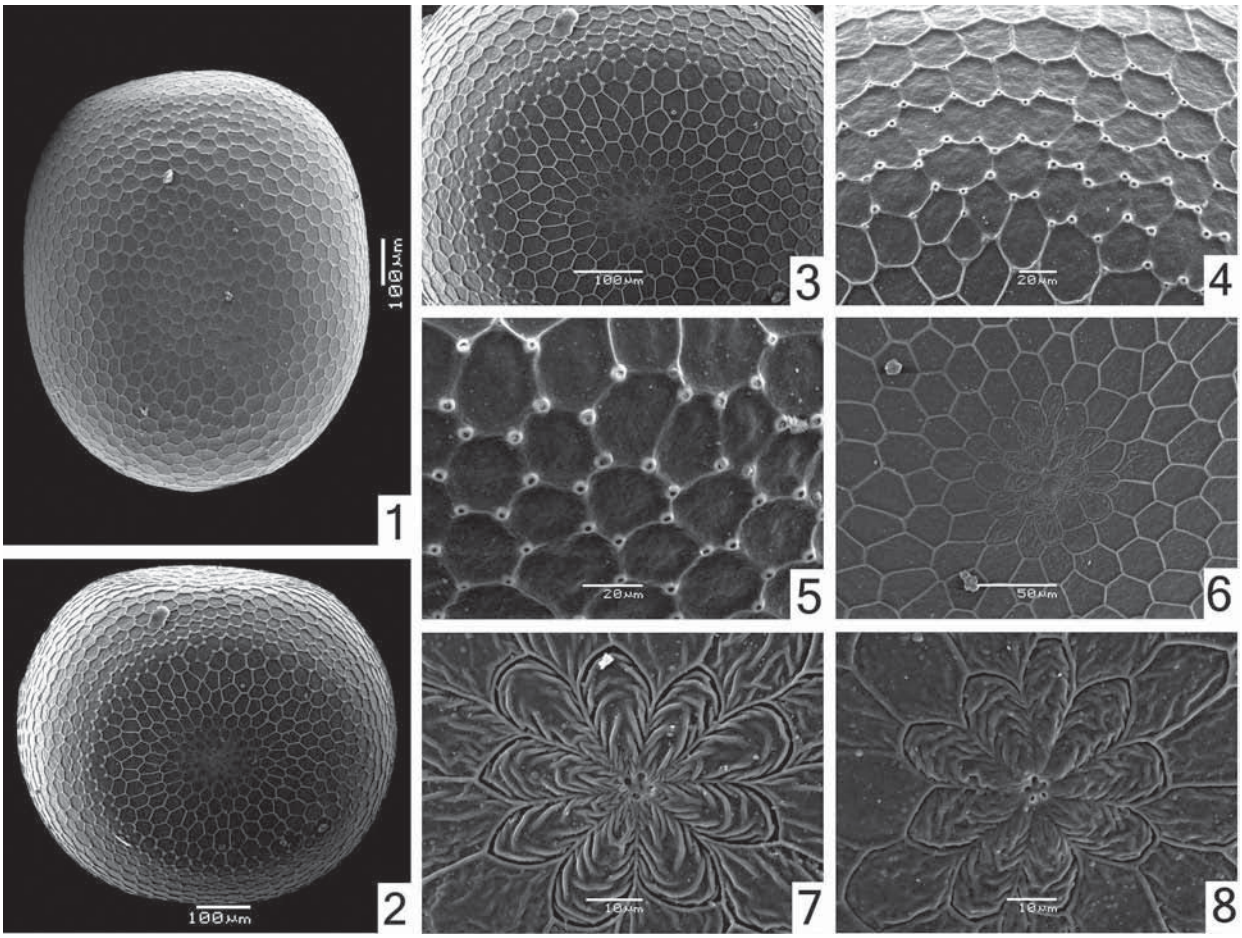
Sampling. One female of *P. obtusaria* was collected at light in the Azapa valley, Arica Province, northern Chile, in November 2006. The individual was transported to the laboratory and kept in a plastic bag until the following day. Twenty-three eggs were deposited by the female, 15 of which were kept in ethanol 70%. The remaining eggs were kept in a plastic vial with paper towel at the bottom and were observed daily until eclosion. The host plant of this species was unknown at the time, so the newly hatched larvae were kept in ethanol 70% for morphological studies.

Morphology. We used the methods described in Vargas et al. (2015) for morphological observations; the nomenclature of Salkeld (1983) for the description of the egg; and the nomenclature of Stehr (1987) for the description of the chaetotaxy of the larva, with the modification proposed by Duarte et al. (2005) for the CD group of the head.

RESULTS

Egg (Fig. 1–8)

Ellipsoid (Fig. 1); micropylar area slightly flattened (Fig. 2); micropylar axis parallel to substrate; surface mostly sculptured by polygonal cells (Fig. 1–6); ridges sometimes poorly differentiated in the cells bearing aeropyles (Fig. 4); 4–5 micropylar openings (Fig. 7–8); micropylar rosette with 7-9 petal-like cells with surface sculptured by transverse grooves (Fig. 7–8); circular



FIGS. 1-8. Egg of Pero obtusaria. **1**) General view. **2**) Micropylar pole. **3**) Detail of the micropylar pole, including micropylar rosette and aeropiles. **4**) Area of aeropyles showing the poorly differentiated ridges of some cells. **5**) Aeropyles. **6**) Micropylar area showing the well-defined polygonal cells surrounding the micropylar rosette. **7**) Micropylar rosette with four openings. **8**) Micropylar rosette with five openings.

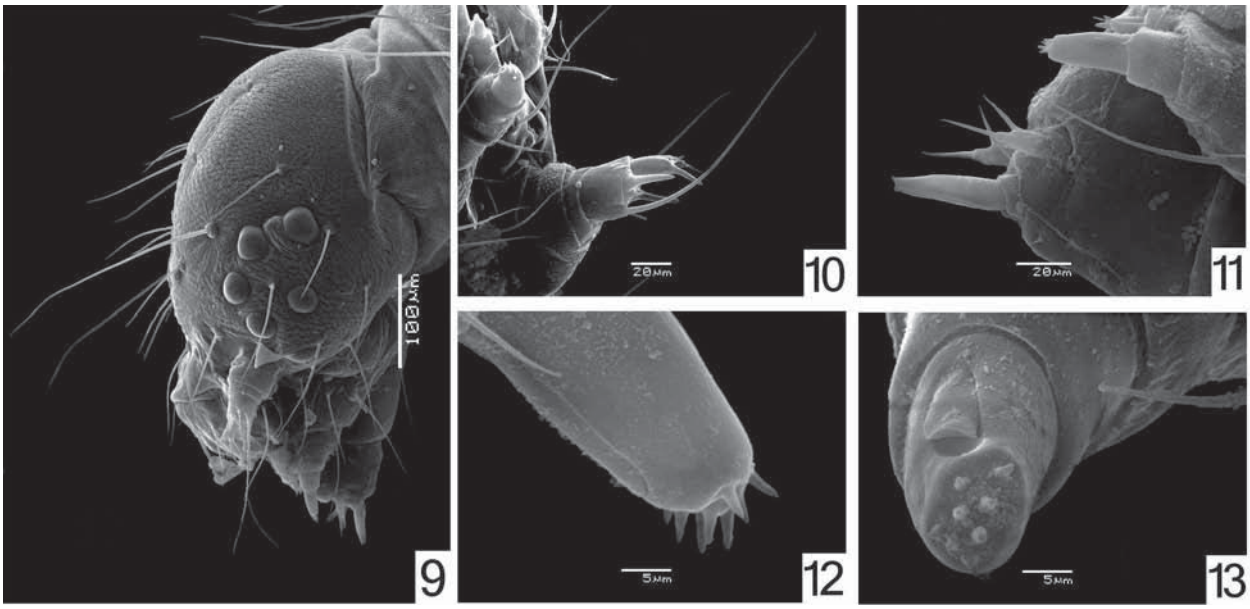
aeropyles at vertex of the polygonal cells forming a ring surrounding the micropylar rosette (Fig. 3-6). Silvery grey immediately after laying, yellowish brown after 3-4 days, greyish brown when the larva is ready to emerge.

Measurements. Length: 1.54–1.60 mm; width: 1.38–1.42 mm.

First instar (Fig. 9-29)

Head (Fig. 9) hypognathous, integument irregularly reticulated, setae hair-like, seta A3 about twice the length of A2; frontoclipeous triangle-like with ventral margin broadly concave. Six circular stemmata laterally; stemmata 1–5 forming a semicircle, stemma 6 at middle between stemmata 1 and 5. Antenna (Fig. 1–2) short, 3-segmented; first segment annular; second segment cylindrical, about twice the length of the first segment, five sensillae on the distal surface; third segment cylindrical, length about the same and diameter about one half of the second segment, sensillae on the distal surface. Anteclipeous membranous, smooth, as a transverse stripe between the frontoclipeous and the labrum. Mouthparts (Fig. 9; 11–13; 22-24) of the chewing type. Labrum (Fig. 22–23) bilobed, distal margin sharply cleft at middle; external surface with six pairs of short hair-like setae and three pairs of pores; internal surface (epipharynx) covered with small spines mostly concentrated on the proximal area, three plain teeth on each lobe. Mandible (Fig. 24) with six teeth on the distal margin; two short hair-like setae on the external surface. Maxilla (Fig. 12–13) with well-differentiated galea and palpus; palpus with eight sensillae on distal surface and one on the medial surface. Labium (Fig. 11) with a cylindrical spinneret at apex; a pair of short setae near the base of the spinneret; two bi-segmented palpi, each segment with a sensillum at apex. Chaetotaxy according to Fig. 20–21.

Thorax and abdomen whitish grey; prothoracic dorsal shield, anal shield and legs dark brown; pinnacles and setae dark brown. Integument irregularly reticulated (Fig. 14, 17). Hair-like setae, either with smooth surface and pointed apex, or with longitudinal carinae and multi-



FIGS. 9–13. Head of the first instar of Pero obtusaria. **9**) Head in lateral view. **10**) Antenna. **11**) Spinneret and labial palpi. **12**) Distal segment of the maxillary palpus. **13**) Tip of the distal segment of the maxillary palpus.

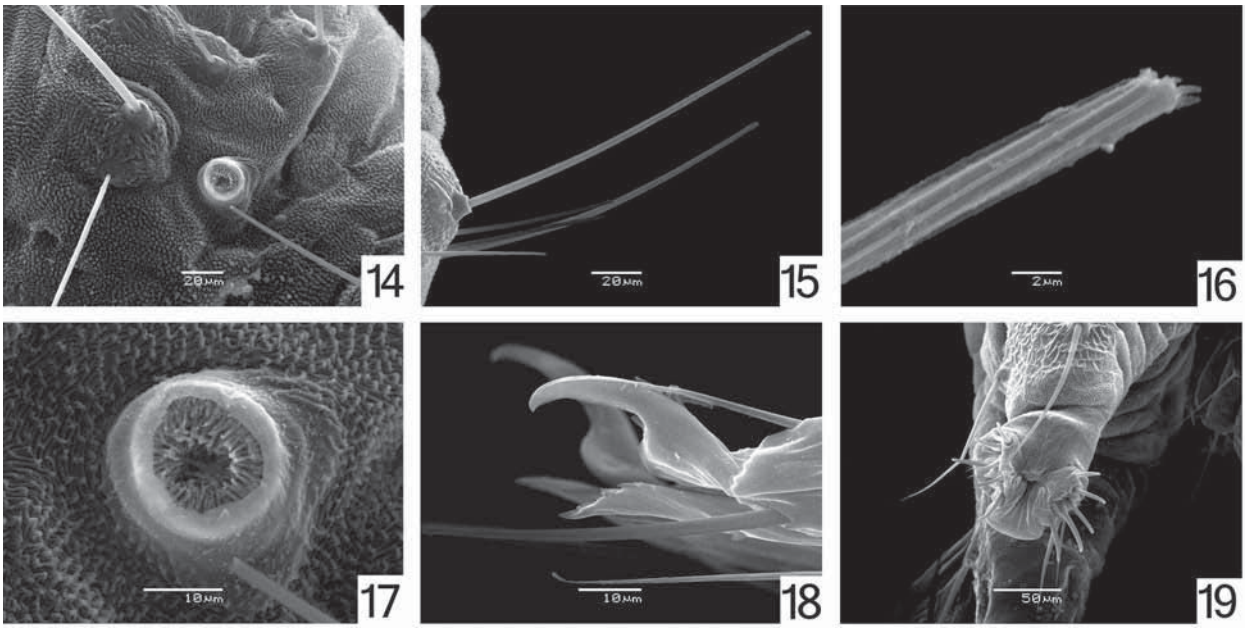
pointed apex (Fig. 15–16). Circular spiracle (Fig. 14, 17) laterally on the prothorax and A1–8, peritrema elevated. Prothoracic dorsal shield (Fig. 26) rectangle-like, margin sinuous, four pairs of hair-like setae (D and XD groups) and three pairs of pores. Anal shield (Fig. 27) semicircular with a sharp anterior cleft almost touching the posterior margin, four pairs of setae (D and SD groups). Thoracic legs well-developed, tarsal claw (Fig. 18) curved, setae mostly hair-like, but TS3 depressed with distal margin saw-toothed. Prolegs (Fig. 19, 28-29) on A6 and A10, provided with hair-like setae, crochets slightly curved. Chaetotaxy according to Fig. 25-29.

Measurements. Length: 5.0–5.5 mm; head width: 0.48–0.5 mm.

DISCUSSION

The Neotropical Region harbors the highest diversity of Geometridae in the world (Scoble et al. 1995, Brehm et al. 2005). However, although species of Geometridae are important in natural and human-altered environments of the Neotropics either as consumers or as prey, the natural history and morphology of their immature stages remain mostly unknown (Marconato et al. 2008, Bodner et al. 2010, Méndez-Abarca et al. 2012, Nelson et al. 2015, Seifert et al. 2015, Sousa-Lopes et al. 2016, Vargas 2016). For instance, although SEM has been widely recognized as a useful tool for detailed studies of the external morphology of immature stages of Lepidoptera (Duarte et al. 2005, Brito et al. 2013, Vargas et al. 2015, Dolinskaya 2016), only a few SEM studies have been performed for eggs, larvae or pupae of Neotropical Geometridae (Beéche et al. 1987, Ibarra-Vidal & Parra 1993, Parra & Ibarra-Vidal 2002, Bocaz & Parra 2005, Vargas et al. 2010, King & Parra 2011, Vargas & Parra 2013). Thus, it is not surprising that this is the first morphological study dealing with immature stages of a Neotropical representative of *Pero*. Indeed, it is also the first to include SEM observations of the first instar for a species of this highly diverse New World moth genus.

Although knowledge of the external morphology of the egg of Geometridae is still incomplete at the global level, the detailed studies currently available suggest that the morphology of this life stage can be useful in the systematics of this moth family (Salkeld 1983, Young 2006). Salkeld (1983) described the egg stage of the Nearctic *P. honestaria* (Walker, 1860) based on specimens collected in Canada, indicating that aeropyles occur on a wide band around the shoulders of the anterior and posterior poles. Furthermore, Salkeld (1983) indicated that the eggs of the also Nearctic *P. morrisonaria* (Edwards, 1881) are not distinguishable from those of *P. honestaria*. Accordingly, the morphological pattern enables differentiating the eggs of the two Nearctic species from those of *P. obtusaria*. In addition, the shape and arrangement of the micropylar rosette also separates *P. obtusaria* from these two Nearctic representatives.



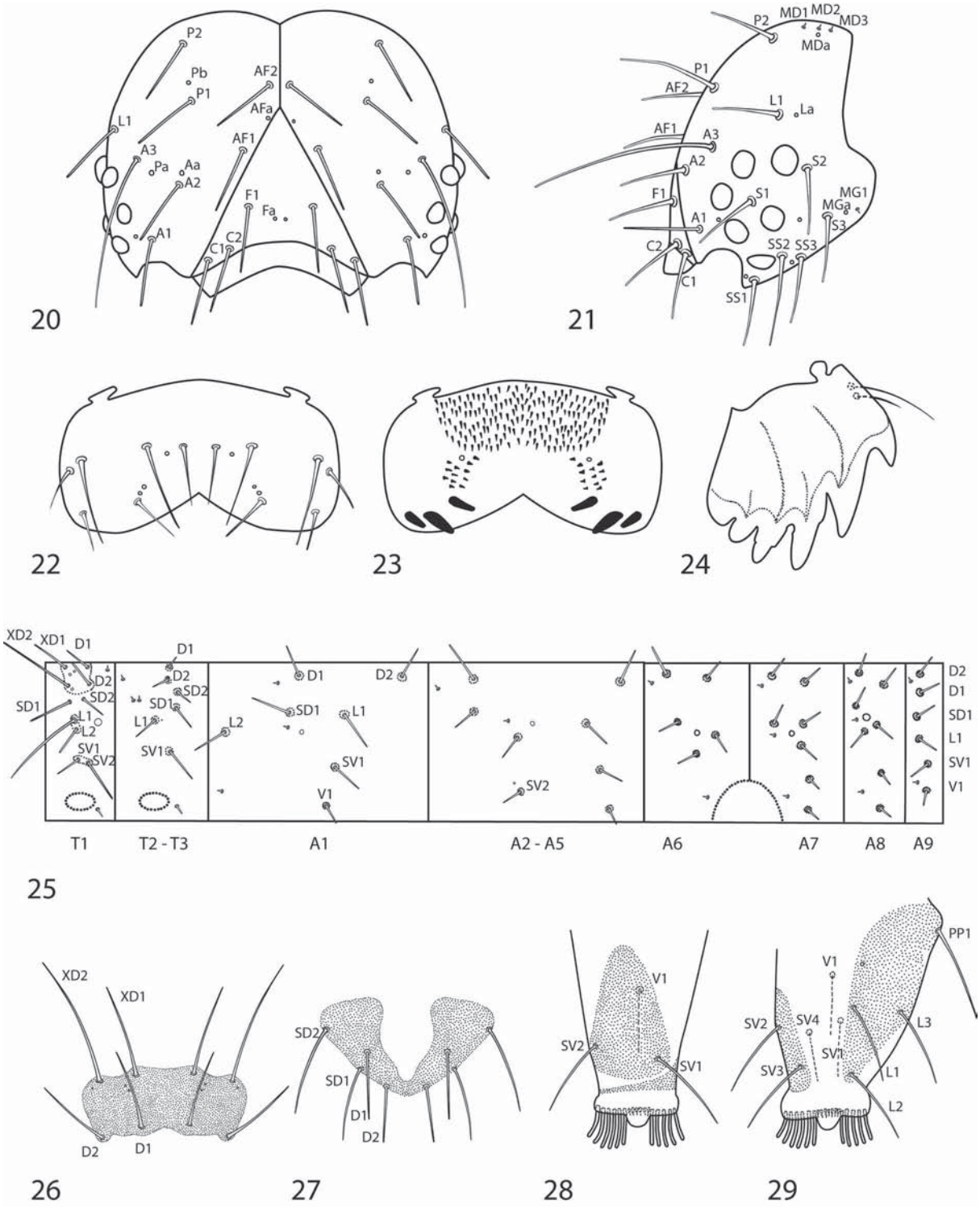
Figs. 14–19. Thorax and abdomen of the first instar of Pero obtusaria. **14**) Prothoracic spiracle and pinnacle of the L group in lateral view. **15**) Mesothoracic D2. **16**) Apex of mesothoracic D2. **17**) Detail of the prothoracic spiracle. **18**) Apex of the metathoracic leg showing the tarsal claw and the depressed and sawed TS3. **19**) Proleg of A6.

Although the external morphology of the first instar of *P. obtusaria* mostly fits the pattern described for the Nearctic *Pero* (Comstock 1963, McGuffin 1963), a few differences were found in the chaetotaxy of the thorax and abdomen. First, there are six setae pairs on the prothoracic dorsal shield of the Nearctic Pero, while four setae pairs are found in *P. obtusaria*. Secondly, the SV group is tri-setose on A2 of the Nearctic Pero, while it is bi-setose in *P. obtusaria*. Unfortunately, further comparisons are not possible at the intra-generic level based on the available morphological descriptions (Comstock 1963, McGuffin 1963). Indeed, external morphology of the first instar has been little studied in the family Geometridae (Grehan et al. 1994, Blaik & Malkiewicz 2003, Vargas et al. 2010, Vargas & Parra 2013). However, there are a few characteristics that would be interesting to explore in further comparative studies, such as the relative length of the cephalic setae (A3, Fig. 9) and the morphology of the setae of the thorax and abdomen (Fig. 15–16), including the TS3 (Fig. 18) on the thoracic leg.

Cephalic A3 setae strikingly longer than A2 (Fig. 9), has not been previously described for the first instar of Geometridae. Thoracic and abdominal setae with longitudinal carinae and multi-pointed apex (Fig. 15–16) have been also described for the first instar of other members of Ennominae (Macariini: Blaik & Malkiewicz 2003; Boarmiini: Vargas & Parra 2013) and Larentiinae (Vargas et al. 2010). Depressed tarsal setae TS3 have been already described for the first instar of Larentiinae (Vargas et al. 2013); however, the distal margin saw-toothed, as found here for *P. obtusaria*, has been not previously reported.

The shape of the prothoracic dorsal shield also appears to be variable in Ennominae. It can be rectangle-like, as in *P. obtusaria* (Fig. 26), or as two plates separated by a membranous longitudinal stripe in the middle in Macariini (Blaik & Malkiewicz 2003) and Boarmiini (Vargas & Parra 2013). Furthermore, the number of setae on the prothoracic dorsal shield also appears to be variable in Ennominae: *P. obtusaria* has four pairs (XD and D groups; Fig. 26), while six setae (XD, D and SD groups) are found in Macariini (Blaik & Malkiewicz 2003), Boarmiini (Vargas & Parra 2013) and the Nearctic Pero (McGuffin 1963).

The shape of the anal shield of *P. obtusaria*, which is semicircular with a sharp anterior cleft that almost touches the posterior margin (Fig. 27), differs from the previously described patterns described for the first instar of other Geometridae. The anal shield is semicircular without a cleft in Macariini (Blaik & Malkiewicz 2003), while this shield is composed of two triangle-like plates separated by a longitudinal membranous stripe in Boarmiini (Vargas & Parra 2013).



FIGS. 20–29. First instar of Pero obtusaria. **20**) Head in frontal view. **21**) Head in lateral view. **22**) Larbum in frontal view. **23**) Internal surface of the labrum. **24**) Mandible. **25**) Chaetotaxy of the thorax and abdomen in lateral view. **26**) Prothoracic dorsal shield in dorsal view. **27**) Anal shield in dorsal view. **28**) Proleg of A6 in lateral view. **29**) Proleg of A10 in lateral view.

An increase in the number of some thoracic and abdominal setae following the first larval molt has been described for several species of Geometridae (e.g.: Parra & Henríquez-Rodríguez 1993, Blaik & Malkiewicz 2003, Vargas et al. 2010, Vargas & Parra 2013). However, this increase is remarkable in the SV group of A6 in the Nearctic species of *Pero*, reaching a few tens of setae on the external surface of the proleg of A6 of the last instars of some species (McGuffin 1963). Unfortunately, as the host plant of *P. obtusaria* was unknown when the specimens of this study were collected, we were unable to assess this character in the subsequent instars of this species. Accordingly, further studies would be required to characterize the external morphology of later instars and pupa of *P. obtusaria*. Meanwhile, we hope the descriptions and illustrations here provided for the egg and first instar of *P. obtusaria* will encourage similar studies involving additional Neotropical representatives of *Pero*, which will help us to reach a better understanding of the systematics of this highly diverse New World moth genus.

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SEASONAL SHORT-TERM DIAPAUSE IN THE FALSE LOCUST SKIPPER, *CHIOIDES MARMOROSA* (LEPIDOPTERA: HESPERIIDAE, EUDAMINAE), FROM CUBA

Additional key words: Eudaminae, *Chioides*, larval diapause, natural history, temperature, West Indies

Virtually unknown for more than 100 years after its description, the Cuban endemic skipper *Chioides marmorosa* (Herrich-Schäffer, 1865) was rediscovered in the 1990s near its historic western range (Roque-Albelo et al. 1995). Ten years later, a new population was discovered at low altitude in the Trinidad mountains at the center of the island (Núñez 2004). More recently the species was also recorded at Viñales, Pinar del Río, expanding its range 100 km to the west (Barro & Núñez 2011).

The species has become one of the most studied Cuban skippers following the discovery of a population in the vicinity of San Antonio de los Baños, about 25 km south-west of Havana, close to its historical location in the eastern coastal limits of Havana city (Núñez and Armas 2015, Armas and Núñez 2015). An interesting aspect of the natural history of this skipper noted by these authors was "the possible existence of at least a short-term larval diapause or dormancy due to lack of food" (Núñez and Armas 2015). Based on their observations between November 2014 and March 2015, the duration of the final instar was, in some cases, longer than three months. However, additional data obtained by Armas and Núñez (2015) between February and September 2015 showed that the duration of the final instar varied from 12 to 48 days only, and larval diapause was not detected during that time.

Based on new observations of the San Antonio de los Baños population conducted between August 2015 and March 2016, we found that the final instar of *C. marmorosa* enters in diapause or dormancy.

In December 2015, we observed in the field, and also in the laboratory, that some final instar larvae, despite having available food, did not eat. The larvae survived for 25 to 60 days as relatively long-term final instar without feeding similar to that previously recorded by Núñez and Armas (2015).

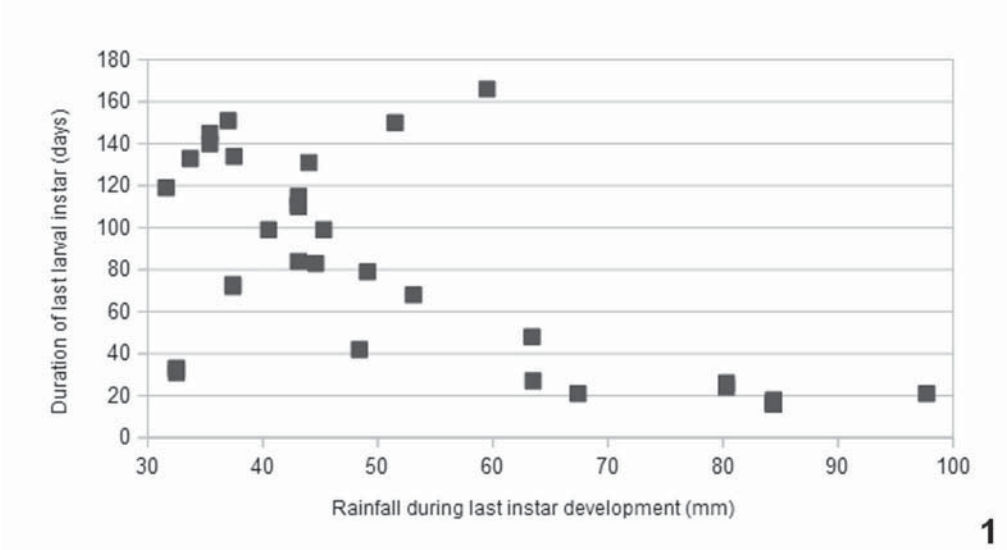
Several final instar larvae that had defoliated their hostplant remained without feeding in their larval shelters for 24 to 100 days between November and March 2015. Armas & Núñez (2015) recorded 16–48 days for the last instar larvae between March and September. Most of these larvae pupated directly from the diapause state, but some larvae fed after the diapause and then pupated.

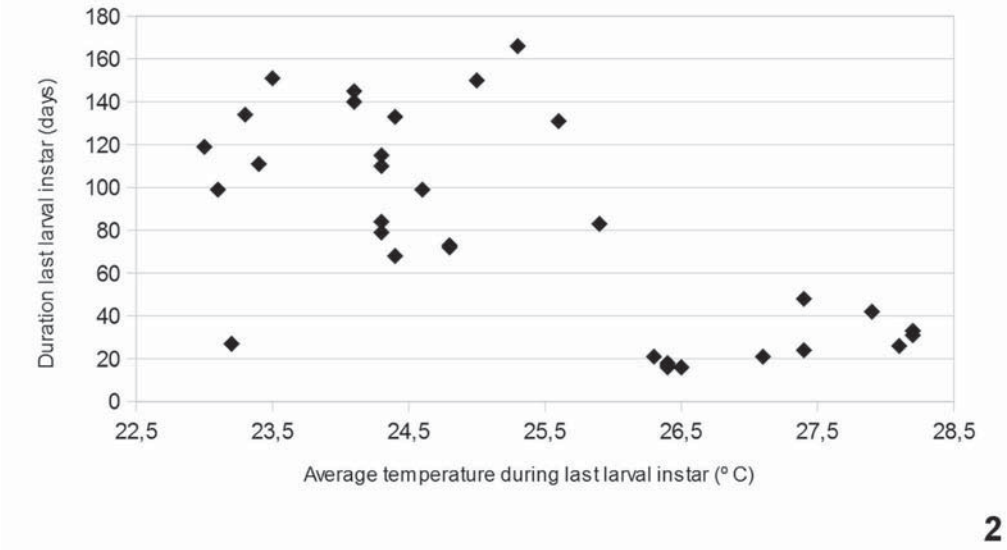
Between May and September 2015, 2 (8.3%) of the 24 pupae observed in the field died. During the period November 2015 to March 2016 the 26 pupae that underwent diapause were kept under observation in the field and, of these, 4 (15.4%) did not hatch. This suggests that short-term diapause might increase mortality of the pupae, but the result is not conclusive and additional observations are needed.

From these observations it is evident that in *C. marmorosa* larval diapause occurs only in the final instar and is restricted to the winter season, associated with the quality of food and its availability. The latter can be affected by defoliation of the host-plant by the larvae and other insects (there are other species that feed on the tree) and the drought during the winter months affects the quality of the foliage; it is much dryer in western Cuba lowlands than the rest of the island (Figure 1). During our study period there was also a noticeable drop of 3.4 °C in average daily temperature during the winter, (average 23.8 °C in winter months November to April, vs. 27.2 °C in summer May to October) which may also have contributed to a slower development (Figure 2). In each figure a change is noted in the duration of the final instar in relation to each abiotic factor, though in both cases exceptions are present. The result is that the span of the immature stage (larvae + pupa) is extended to 5 or 6 months during the winter months. Armas & Núñez (2015) recorded immature periods of only 1.5–3.0 months between February and October.

This is the first time in which seasonal diapause is recorded for a butterfly species in Cuba. Much has been published on larval diapause in Lepidoptera inhabiting temperate regions; however, there is little detailed information on the process in tropical species. Denlinger (1986) reviewed this topic for insects in general noting some Lepidoptera examples but Hesperiidae were not mentioned. Janzen (2004) referred to species of several families, only Papilionidae among butterflies, whose prepupa go through long dormancy periods in dry seasonal forest in Costa Rica.

The few examples we found in the literature on tropical Hesperiidae give only indirect evidence of aestivation in the larval stage due to the absence of adults or other life stages during certain periods of the year (Grund & Hunt 2001, Larsen 2005, Franklin 2011, Palmer & Braby 2012). The only detailed case we found





FIGS. 1–2. Duration in days of the final instar of 36 larvae of *Chioides marmorosa* relative to rainfall and average temperature during final larval instar (Data collected between March 2015 and March 2016 in a population from San Antonio de Los Baños, Artemisa, Cuba). **1**. Rainfall. **2**. Average temperature. Data on temperature and rainfall are from a meteorological station at 4.7 Km south of the studied area.

refers to the larvae of *Hesperia metea* which aestivate during the hot period from late July to early September in Texas (Heitzman & Heitzman 1970). These larvae hibernate during winter as final instars. All of the above cases relate to monocotyledon feeders and members of the subfamilies Trapezitinae and Hesperiinae and this behavior has not been previously reported in the Eudaminae.

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SCAVENGING BEHAVIOR IN LEAF-FEEDING CATERPILLARS

Additional key words: Outbreaks, Erebidae, Geometridae, Noctuidae, predators

More than 99% of lepidopteran species are phytophagous (Strong et al. 1984, Pierce 1995). However, larvae of some species are carnivorous (Pierce 1995). For instance, some lycaenids feed on immature insects such as ants and aphids (Pierce et al. 2002), and some Hawaiian geometrids prey on active insects (Montgomery 1983). Cannibalism has also frequently been reported in the larvae of phytophagous moths and butterflies (Richardson et al. 2010). Furthermore, Wang and Daane (2014) observed larvae of a tortricid species eating dead larvae of conspecifics under laboratory conditions. These observations suggest that phytophagous lepidopterans may scavenge on dead insects in the wild. Although larvae of several moth groups, such as tineids, feed on dead animals (Stehr 1987), scavenging by phytophagous lepidopterans has rarely been reported under field conditions. In this study, we investigated the scavenging behavior of four species of leaf-feeding moth larvae in a forest in central Japan.

In May 2013, we observed an outbreak of the gypsy moth *Lymantria dispar japonica* (Motschulsky) (Erebidae) in a secondary forest in Hiraoka-kouen, Higashiosaka City, Osaka, central Japan (34°40′N, 135°39′E). The larvae defoliated many tall trees, causing leaf-feeding caterpillars to move to the undergrowth. Thus, there were many caterpillars and their predators on the ground and guardrails in the forest; the mean densities of *L. dispar japonica* and other lepidopteran species were 2.1 and 0.8 larvae /m^2, respectively (Sugiura & Yamazaki 2014). Our observations were carried out along a hiking trail (900 m long, 2 m wide; 160–290 m above sea level) on May 4, 9, 13, and 18, 2013. Over 20,000 caterpillars (2.9 larvae/m^2 × 1,800 m^2 × 4 days) were observed on the ground and guardrails, although the number of larvae of each lepidopteran species was not counted. We recorded the species of lepidopteran larvae that were observed feeding on insect carcasses. The species and instars of scavenging larvae were identified based on morphology, color, and size (Sugi 1987).

Nine larvae of four species, namely, *Phigalia verecundaria* (Leech) (Geometridae), *Lemyra imparilis* (Butler) (Erebidae), *Orthosia limbata* (Butler) (Noctuidae), and *O. paromoea* (Hampson), fed on dead lepidopteran larvae (Table 1; Figs. 1–6). One *O. limbata* larva fed on the carcass of a conspecific (Fig. 4; Table 1), while the other caterpillars scavenged on dead larvae of other species (Figs. 1–3, 5, and 6; Table 1). All scavenging larvae were late instars. Larvae of these four species primarily eat tree leaves (Sugi 1987), suggesting that they are facultative scavengers. Because only 9 of

TABLE 1. Field observations on the scavenging behavior of lepidopteran larvae.

Family	Species	Larval code	Food item (dead insects)
Geometridae	*Phigalia verecundaria* (Leech)	1	A geometrid larva (Fig. 1)
		2	An unidentified larva
Erebidae	*Lemyra imparilis* (Butler)	3	A noctuid larva (Fig. 2)
Noctuidae	*Orthosia limbata* (Butler)	4	An unidentified larva
		5	A noctuid larva (Fig. 3)
		6	A conspecific larva (Fig. 4)
	Orthosia paromoea (Hampson)	7	A *Wilemania nitobei* larva (Fig. 5)
		8	A noctuid larva (Fig. 6)
		9	A noctuid larva

>20,000 caterpillars were observed to feed on dead larvae, the frequency of scavenging behavior was low. However, the scavenging behavior of the most abundant species, *L. dispar japonica*, was not observed at this study site, suggesting that the frequency of scavenging may differ among lepidopteran species.

On the same dates, we frequently observed predacious insects attacking caterpillars on the ground and guardrails in the forest. For example, adult predacious beetles of the species *Calosoma maximowiczi* Morawitz (Carabidae) and *Dendroxena sexcarinata* Motschulsky (Silphidae) preyed on various lepidopteran larvae (c.f. Sugiura & Yamazaki 2007, 2014, Sugiura 2016). Larvae of the ladybird beetle *Aiolocaria hexaspilota* (Hope) (Coccinellidae) and an unidentified hoverfly (Syrphidae) attacked and ate larvae of several lepidopteran species. Scars on the dead larvae on which four lepidopteran species fed showed that the larvae had been split by insects other than lepidopterans prior to scavenging by the lepidopterans (Figs. 2, 3, 6). Thus, predacious insects such as *C. maximowiczi* may have killed the caterpillars that were subsequently consumed by four species of lepidopteran larvae.

Scavenging behavior has rarely been documented in leaf-feeding lepidopterans. At this study site, such unusual scavenging behavior may have been driven by the recent gypsy moth outbreak, which reduced food sources for many leaf-feeding larvae and increased predator numbers, which in turn increased numbers of dead caterpillars. We did not observe any lepidopteran larvae feeding on dead caterpillars at the study site in

FIG.1–6. Scavenging behavior in four species of leaf-feeding caterpillars. **1.** A *Phigalia verecundaria* larva feeding on a dead geometrid larva. **2.** A *Lemyra imparilis* larva feeding on a dead noctuid larva. **3.** An *Orthosia limbata* larva feeding on a dead noctuid larva. **4.** An *O. limbata* larva feeding on a dead conspecific larva. **5.** An *O. paromoea* larva feeding on a dead *Wilemania nitobei* larva (Geometridae). **6.** An *O. paromoea* feeding on a dead noctuid larva.

May 2014–2016, years during which there were no outbreaks of gypsy moth.

What is the adaptive significance of scavenging behavior in leaf-feeding lepidopterans? Scavenging behavior may increase longevity and growth rate because of the high levels of protein in dead insects (e.g., Richardson et al. 2010; Polis 1981). However, it may also increase the risk of infection with disease (e.g., Rudolf & Antonovics 2007). Further experiments are needed to clarify the benefits and costs of scavenging behavior.

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TYPE DESIGNATION AND LATE LARVAL STAGES OF *HOLOPHAEA VESTA* (EREBIDAE: ARCTIINAE) IN SURINAME

Additional key words: Arctiini, Euchromiina, *Commelina*, Surinam, Neotropics

Holophaea vesta (Möschler, 1877) (Erebidae: Arctiinae: Arctiini: Euchromiina) is distributed in Guyana, Suriname, French Guiana and Brazil (Hampson 1898, Draudt 1917). In French Guiana, the species is widely distributed and occurs throughout the year (Cerda 2008). We designate and illustrate the type specimens and describe the late larval stages from Suriname.

On 22 July 2015 at Zanderij I, Suriname (5°28'1.52" N, 55°12'23.22" W, 15 m asl, about 42 km S of Paramaribo), the second author noted a dark, setose larva with white markings on its back walking rapidly among low vegetation along a track through savanna forest. The larva was collected and reared to an adult in Paramaribo according to standard methods. Larval length was measured from the anterior end of the head capsule to the end of the anal plate. Genitalia of the imago were prepared according to standard methods. The imago and genital preparation were deposited in the collection of Naturalis Biodiversity Center, Leiden, the Netherlands. Photographs of the early stages were made with a Nikon D300s camera, an AF Micro Nikkor 105 mm 1:2.8 D lens and an SB-800 flash. In September 2015, a search was made for possible types of *H. vesta* in the Museum für Naturkunde, Berlin (MNB), as it houses the Möschler collection. Specimens were photographed with a Nikon D 800 and an AF-S Micro Nikkor 105 mm 1:2.8G lens on a tripod with a standard gray card as background. Photographs were made in NEF-format and without adjustments converted to TIF-files in the same color space.

Type designation. In his description of *Hysia vesta*, Möschler did not designate any type specimens, but stated ".. this species, of which I own two similar specimens, .." (Möschler 1877, p. 637; translation from German by first author). The accompanying drawing of the species (Möschler 1877, plate VIII, figure 7) is shown in Fig. 1i. Kirby (1892) placed it in the genus

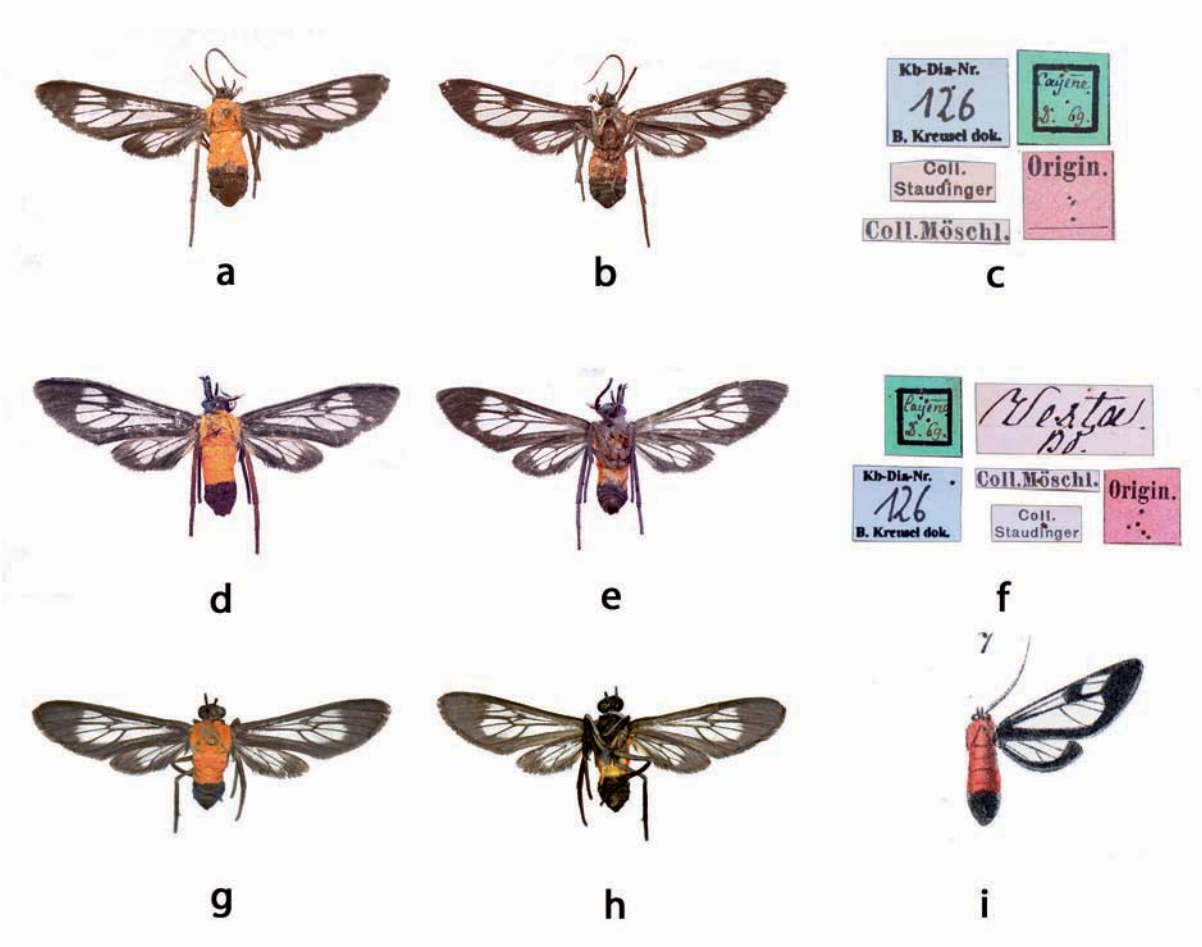

FIG. 1: Specimens and illustration of *Hysia vesta* (now in the genus *Holophaea*) (Erebidae: Arctiinae). **a – c**: designated lectotype (forewing length 12 mm, wingspan 26 mm) in the Museum für Naturkunde, Berlin; **d – f**: designated paralectotype (forewing length 13 mm, wingspan 26 mm) in the Museum für Naturkunde, Berlin; **g - h**: reared specimen (forewing length 11 mm, wingspan 24 mm) from Zanderij I, Suriname; **i**: drawing of *Hysia vesta* (plate VIII, figure 7) in the type description (Möschler 1877). **a, d, g**: dorsal view; **b, e, h**: ventral view; **c, f**: labels on pin of specimen.

Eunomia and Hampson (1898) in the newly erected genus *Holophaea*, which is its current taxonomic status. In the MNB collection, two specimens of *H. vesta* are present that can be attributed to Möschler (Fig. 1a–c and Fig. 1d–f, respectively). Both have a label "coll. Möschl.", a label "coll. Staudinger", a pink label "Origin.", and a rectangular green label " Cayene D. 69" (in all probability Cayenne as French Guiana was called at the time, Datum (date) 1869); one of the specimens has an additional label "Vesta. Bd." (Vesta Boisduval) (Fig. 1c, f). The small, rectangular labels are Möschler's: green, quadrangular or rectangular with an inner square drawn with black or (in other specimens) gold-colored ink. After Möschler's death in 1888, Staudinger bought his collection and incorporated it into his own (Staudinger 1889). The pink labels "Origin" are the characteristic type labels of Staudinger (Wolfram Mey, MNB, pers. comm.). The figure in Möschler's paper (Fig. 1i) has a greater resemblance to the first (Fig. 1a, b) than to the second (Fig. 1d, e) specimen of *Hysia vesta* in the shape of the black patch at the end of the discal cell and the slightly convex orange-black demarcation at the end of the abdomen. In order to stabilize the nomenclature of the taxon and in accordance with recommendation 74B of the ICZN (ICZN 1999), we hereby designate the specimen figured in Fig. 1a–c as the lectotype of *Hysia vesta* (now in the genus *Holophaea*). The specimen illustrated in Fig. 1d–f is the paralectotype. The implication is that the type location is not Suriname, as suggested by the title of Möschler's paper, but French Guiana.

The host plant (Fig. 2a, b). As the larva was not on a plant when found, several plants were offered to it. It accepted *Commelina* cf. *erecta* L. (Commelinaceae),

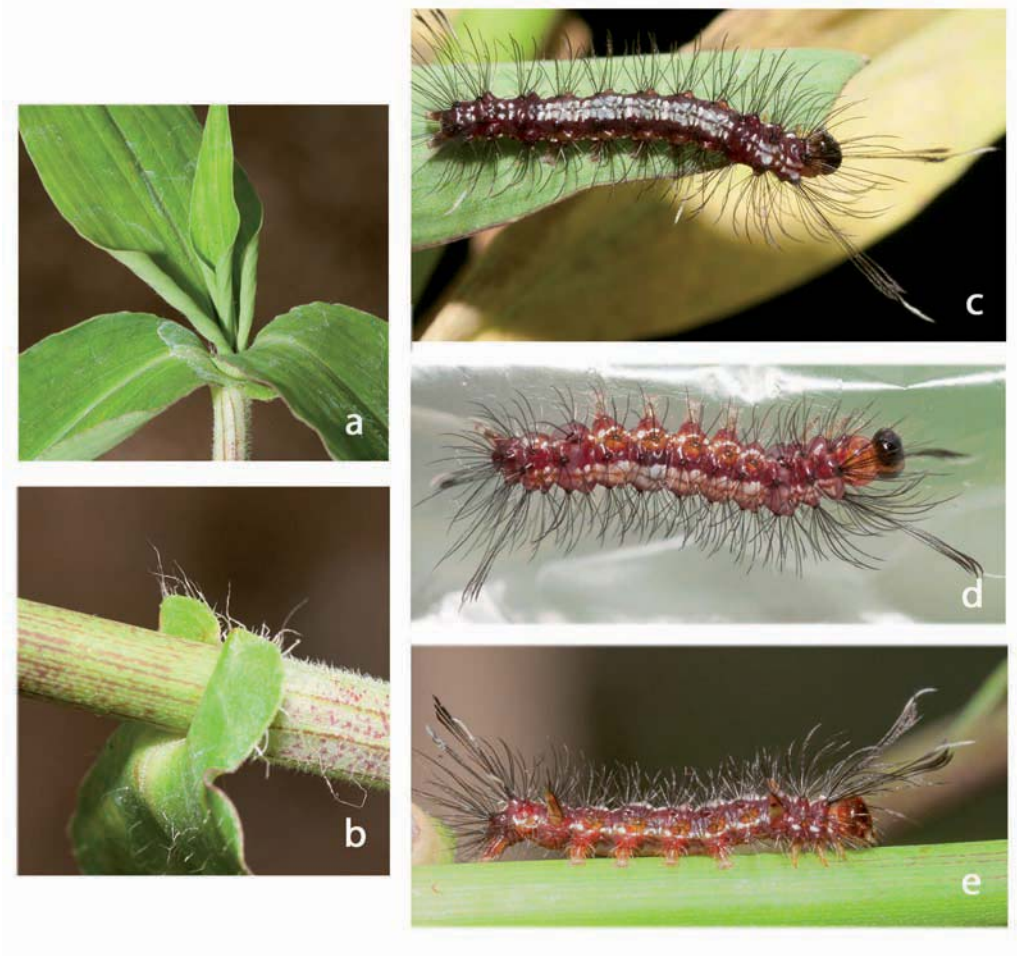

FIG. 2: Host plant and late larval stages of *Holophaea vesta* (Möschler, 1877) in Paramaribo, Suriname. **a**: *Commelina* cf. *erecta* L., upper end of stem with leaves; **b**: *Commelina* cf. *erecta* L., auriculate leaf base with white hairs, clasping the stem; **c**: antepenultimate instar (12 mm), 23 July 2015; **d**: penultimate instar (16 mm), 2 August 2015; **e**: ultimate instar (18 mm), 4 August 2015, note black-tipped brown setal tufts on A1 and A7 (photographs JvdH).

known in Suriname as gado dede, a general name for all Commelinaceae (van Andel & Ruysschaert 2011). Description: Terrestrial, fleshy herb, ca. 40 cm high, stem with red dots, rooting at nodes. Leaf sheaths closed, with short, white hairs. Leaf base auriculate, clasping the stem, long white hairs on margins. Leaves simple, alternate, lanceolate. Flowers or fruits not seen on particular individual.

Antepenultimate instar (Fig. 2c). Overall impression of a dark purple larva with dorsal white markings and multiple verrucae bearing black barbed setae. Head: vertices orange-brown with reticulate black patterning on the dorsal anterior half, and rounded, ill-defined black patches laterally and dorsally nearly bordering intersegmental membrane; medially to stemmata an irregularly defined transverse black patch; epicranial and lateral adfrontal sulci light gray, the color on each side of these gradually changing into orange brown; frons with irregular black patches apically and basally; clypeus yellow-gray; labrum cleft to about 10 % of its length, with vertical irregular black patches laterally, its lower end white-translucent, latero-inferior parts yellow; stemmata black; basal and middle segment of antennae translucent gray, terminal segment yellow with one long and one short white seta; mandibles light orange-brown, tip dark yellow. Cervical membrane light gray.

Ground color of thorax and abdomen dark red-purple, intersegmental membranes white. From T2 to A9 a longitudinal white dorsal band on either side of the middorsal line, consisting of irregularly formed, confluent patches. Also from T2 to A9 a subventral undulating white band, broken up into irregularly formed white spots, with its lower arches ventral to the lateral verrucae and the dorsal arches in the intersegmental area.

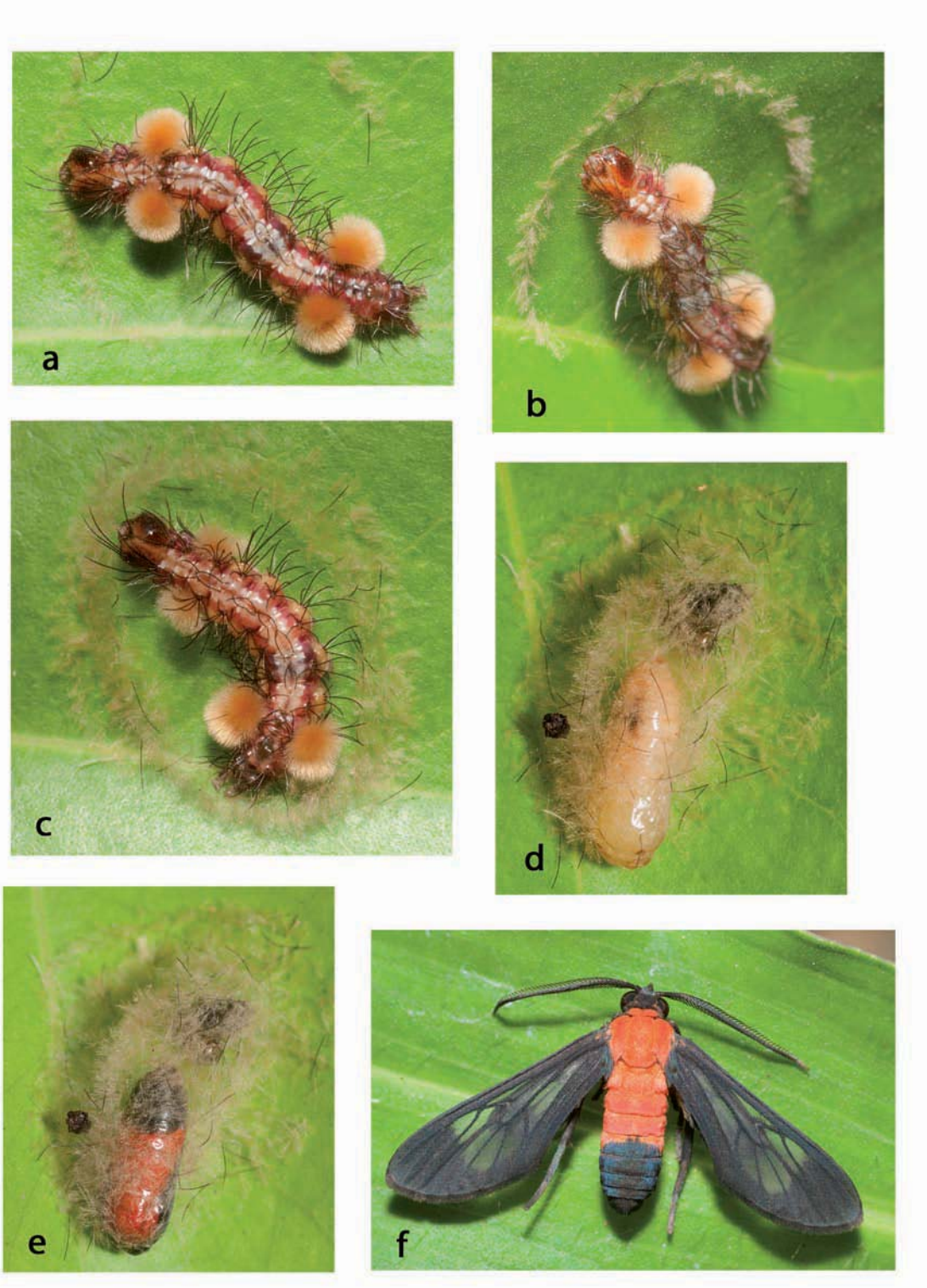

FIG. 3: Pupation of *Holophaea vesta* (Möschler, 1877) in Paramaribo, Suriname. **a**: prepupa constructing cocoon, note expanded A1 and A7 setal tufts (8 August 2015, 17.23 hrs); **b**: same, 17.36 hrs; **c**: same, 19.51 hrs; **d**: cocoon with pupa (11 August); **e**: cocoon with pupa one day before eclosion (16 August); **f**: imago (17 August 2015; forewing length 11 mm, wingspan 24 mm), note fresh orange-red color T1 – A4 (photographs JvdH).

T1 with prothoracic plate light brown to orange-brown with purplish reticulate patterning, length of plate about 80% of the segment. Area behind prothoracic plate with paired dorsal and subventral white spots. T1 also with a lateral small, black verruca and a larger subventral, purple verruca, each with black, non-barbed setae, and caudad to these an oblique white streak with its anterior end about midway between the lateral and subventral verrucae. T2 and T3 with paired white spots in the anterior third and the white dorsal band continuous in the caudal two thirds of the segment; the subventral band is reduced to an oblique white patch in the caudad half of the segments. Middle of T2 with a subdorsal back verruca with barbed black setae adjacent to the dorsal white band; most of these setae have a length of about three body segments, five are about five segments long and one, ending in a white plume, about seven segments. T2 also with a lateral verruca with barbed setae; subventrally and caudad to the lateral verruca, a black verruca with barbed setae. T3 as T2, but with oblique patch of subventral band smaller, the dorsal verruca without the five-to seven-segment-long setae and the lateral verruca with a red base.

Abdomen with prolegs on A3 – A6 and A10. Prolegs with elongate brown base with black or white, nonbarbed setae on pinacula; the planta is gray without setae; crochets heteroideous, arranged in mesoseries. A1 with white dorsal band strongly reduced to five pairs of white dots (one missing on the right-hand side), the caudal ones continuous with the anterior ones of A2; subventral white band reduced to white dots near intersegmental membranes; dorsally, at about 40% of the segment's length adjacent to middorsal purple line, a pair of small, black verrucae with short, unbarbed, black or white setae; subdorsally at about 70% of the length of A1, a larger, black verruca with barbed, black setae; laterally about midway of the segment, a prominent verruca with a purple base and long, black, barbed setae; slightly anterior to this, a subventral black verruca with barbed, black as well as white setae. A2 with prominent dorsal and subventral white bands, position of verrucae as A1, lateral verruca with red base, black barbed setae and one barbed, plumose, white seta. Coloration and verrucae of A3 – A6 as A2, setae on lateral verrucae all black. A7 with dorsal and subventral white bands broken into a series of white spots, position of verrucae as A1 – A6, lateral verruca with purple base and one white seta amidst black ones. A8 as A7, subdorsal verruca with one black, barbed seta ending in a white plume, length about 3.5 body segments, lateral verruca with similar seta about 2.5 segments long. A9 as A7 – A8, dorsal verrucae absent and all setae black. Anal plate purple.

When found on 22 July 2015, the larva measured 12 mm. The next days it fed normally. On 25 July, it was 16 mm. On 26 July, it left the foodplant and became inactive. It molted during the night of 27 – 28 July.

Penultimate instar (Fig. 2d). Similar to previous instar, but with base of T2 lateral verruca red and with oblique, oval black spot ventrocaudad of lateral verrucae of A1 and A7. On 28 July 2015, the newly molted larva was 13 mm, five hours later 15 mm. On 29 July it had resumed feeding, on 30 July it was 18 mm, on 31 July 19 mm. On 1 August, it measured 16 mm and became inactive. It molted in the early morning of 3 August.

Ultimate instar (Fig. 2e). Similar to previous instar, but with modification at the site of the lateral verrucae of A1 and A7: the base has become purple and the previous instar's black, barbed setae and black spots have been replaced by black-tipped tufts of short, orange- brown setae, directed dorsally, laterally and caudad; the distal ends of the tufts are pointed and the setae are adjacent to one another, possibly due to an adhesive secretion. On 3 August, the newly molted larva was 16 mm, ate its exuviae and in the evening resumed feeding. On 4 August, it walked about considerably, measured 18 mm and had returned to the host plant in the evening. During subsequent days, the larva remained on the host plant and fed both during the day and at night. On 5 August, its length was 22 mm.

Pupation and pupa (Fig. 3a-e). On 8 August, the larva was very active and walked about continuously. In the evening, its length was 11 mm, the long, plumose setae had fallen off and the setae of the orange-brown tufts on A1 and A7 had expanded. On the upperside of a leaf, the larva deposited the A1 and A7 setae with the barbs directed preferentially outwards from the cocoon-to-be and loosely secured them with silk during a series of circular movements. The inner layers mainly consisted of white setae arranged parallel to the wall. Thereafter, the roof was spun in a similar fashion. Detached black, barbed setae were loosely interspersed in the walls of the rather flimsy cocoon. Spinning the cocoon took about six hours. The pupa was cream-colored, shiny, rounded anteriorly and measured 8 mm; the exuviae had become detached from its caudad end. On 16 August the pupa had turned red and black.

Imago (Fig. 1g, h and Fig. 3f). In the morning of 17 August, a male *H. vesta* eclosed. Description (after Hampson (1898, p. 265), the types and eclosed specimen): Forewing length 11–13 mm, wingspan 24 – 26 mm. Head black. Antennae bipectinate. Palpi porrect, slightly downcurved, extending well beyond frons, distal two segments covered with short dark gray-brown setae, basal segment with longer dark brown, protruding setae. Proboscis orange-brown, 3 mm.

Thorax dorsally orange-red, laterally black and ventrally light gray covered with short, black setae. Patagia orange-red with laterally long, black setae, forming a laterocaudad plume. Inner side of femora and/or tibiae light gray. Tibial spurs short, tibial spur formula 0-2-2. The rostral four abdominal segments orange-red, the caudad ones black. Wings hyaline with wide black margins and black veins, apex of both wings broadly black. Forewing with vaguely delimited black (post)discoidal spot; there is considerable variation in the extent of black scaling, even between the forewings of the same specimen. The male genitalia of the reared specimen are identical to the ones figured in Cerda (2008) from French Guiana.

Duration of stages.

Antepenultimate instar at least six days, penultimate instar six days, ultimate instar six days, prepupa six hours, pupa eight days.

The larva showed a behavior associated with individual polyphagy: when it was found, it was not on any host plant and in the breeding cage it was regularly on the move and on other plants. In the Arctiinae, individual polyphagy is related to a specialized feeding pattern, called specialized generalism, in which host plant acceptance or rejection is determined by the presence or absence of secondary plant metabolites, notably pyrrolizidine alkaloids (Singer & Bernays 2009). Reluctancy of the larva to feed on other plants as well as the potential presence of antimicrobial secondary metabolites, found in several *Commelina* species that are commonly used as herbal medicine (Ibrahim et al. 2010; Kim et al. 1999), may be indicative of specialist generalist feeding and thus of pharmacophagy. The ultimate instar's barbed setae on the A1 tufts and, to a lesser extent, on the A7 tufts, were deposited in the cocoon wall and likely provided mechanical protection for the pupa. In view of the pupation's long duration, the vulnerable position on top of a leaf and the loss of protective setae in the prepupal stage, the A1 and A7 tufts may also have a role in chemical defence. Additional studies are required to understand more about the early instars, possible larval variation and larval behavior in relation to diet and defense.

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