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Comparative morphological study on pupae of Plusiinae and observations on the vice-like abdominal structures (Lepidoptera, Noctuidae)

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Abstract: External pupal morphology of the following five species of the subfamily Plusiinae (Lepidoptera Noctuidae) is comparatively described and illustrated: Autographa gamma (LINNAEUS, 1758), Chrysodeixis chalcites (ESPER, [1789]), Diachrysia chrysitis (LINNAEUS, 1758), Macdunnoughia confusa (STEPHENS, 1850), Trichoplusia ni (HÜBNER, [1803]). The function of peculiar vice-like structures present on the back of pupa, arranged between the adjacent margins of the first four abdominal segments, found in all examined species usually in number of three, is hypothesized to be the blocking of the pupa to the cocoon silk. Other useful distinctive characters are found in colour, cuticle punctation, semiannular structures present on the cephalic margin of abdominal segments 5-7 as well as spiracles, appendages and cremaster features. A key is also provided for the identification of the species.

Riassunto: Morfologia comparativa in crisalidi di Plusiinae (Lepidoptera Noctuidae) e osservazioni sulle strutture urotergali a morsa. Viene descritta e illustrata in chiave comparativa la morfologia crisalidale esterna di cinque specie della sottofamiglia Plusiinae (Lepidoptera Noctuidae): *Autographa gamma* (LINNAEUS, 1758), *Chrysodeixis chalcites* (ESPER, [1789]), *Diachrysia chrysitis* (LINNAEUS, 1758), *Macdunnoughia confusa* (STEPHENS, 1850), *Trichoplusia ni* (HÜBNER, [1803]). Peculiari strutture urotergali, di cui viene discusso il significato funzionale, sono state riscontrate in tutte le specie esaminate. Queste strutture (da noi denominate "a morsa_n), sono di solito in numero di tre, disposte fra i margini contigui dei primi quattro uriti con lo scopo di bloccare dorsalmente la crisalide ai fili sericei del bozzolo. Altri utili elementi distintivi sono rilevabili nei seguenti caratteri: colore, punteggiatura cuticolare, strutture urotergali semianulari (presenti lungo il margine cefalico degli uriti V-VII), conformazione degli stigmi, delle teche e del cremaster. Viene inoltre fornita una tabella di identificazione delle specie considerate.

Introduction

The large and cosmopolitan subfamily Plusiinae (Lepidoptera Noctuidae), includes temperate and tropical species, often harmful to crops. A comprehensive list of all genera, from a systematic and biological point of view, was done by KITCHING (1987). In Europe 17 genera and 53 species are known (KARSHOLT & RAZOWSKI, 1996), many of which (over 70%) occur in Italy (RAINERI & ZILLI, 1995) including pests of economic importance.

External pupal morphology of Lepidoptera has been studied since the end of the 19th century (POULTON, 1891; CHAPMAN, 1893, 1896; PACKARD, 1895; MOSHER, 1916; SPEYER, 1958); in recent years several subfamilies of Noctuidae of central Europe including the Plusiinae have been described (PATOČKA, 1995a, 1995b, 1996a, 1996b, 1996c).



Fig. 1: – Plusiinae pupa, habitus. Legend: A, antenna; A1–10, abdominal segments 1–10; C, cremaster; E, eye; F, frons; F1, femur of prothoracic leg; H, head; L, labium; L1, prothoracic leg; L2, mesothoracic leg; MS, mesothorax; MT, metathorax; MX, maxilla; P, prothorax; PS, prothoracic spiracle; S, abdominal spiracle; W1, mesothoracic wing; W2, metathoracic wing; WP, wing projection.

Some morphological features typical of the above subfamily do not appear to have received adequate attention. In the Noctuidae group, pupae of Plusiinae are recognisable at a glance because of the swollen rounded prominence of the wing tips and the big, cylindrical and rugose cremaster, with eight hooked spines (SANNINO et al., 1992).

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Fig. 2: *Diachrysia chrysitis* (LINNAEUS): a vice-like structure, dorsal view. Legend: A2–A3, abdominal segment 2 and 3; CAJ, caudal jowl; CEJ, cephalic jowl.



Fig. 3: Pupa, ventral view. 1 Autographa gamma (LINNAEUS). 2 Macdunnoughia confusa (STEPHENS). Legend: F, frons; F1, femur of prothoracic leg; L, labium; L1, prothoracic leg; MX, maxilla.

In the present study 5 species belonging to different genera Autographa gamma (LINNAEUS, 1758), Chrysodeixis chalcites (ESPER, [1789]), Diachrysia chrysitis (LINNAEUS, 1758), Macdunnoughia confusa (STEPHENS, 1850), Trichoplusia ni (HÜBNER, [1803]) have been described, highlighting the common as well as the distinctive characters, and trying to provide a functional explanation for the peculiar vice-like abdominal structures, until now little understood.

Material and methods

Observations were performed at the Tobacco Research Institute (TRI) at Scafati (Salerno, Southern Italy) both on specimens of the TRI collection and on field collected live individuals. Rearing and observation methods have been previously described (SANNINO et al., 1991). All measurements refer to the greatest distance along each axis. Body length is inclusive of cremaster but not of spines. Some measurements are given as the average ± SE (standard error). When necessary, some specimens were sectioned and the parts of interest mounted on slide.

General morphology

The Plusiinae pupal habitus is represented in Figure 1. Pupa typically obtected with the posterior end tapered. Abdominal segments 2–7 (A2–7) movable and the last three joined. Integument smooth or rugose. Cephalic margin of some abdominal tergites possibly punctate. Head with conspicuous eyes. Labial palps exposed. Prothoracic femora exposed. Mesothoracic legs not reaching eyes. Metathoracic legs usually not visible¹. Posterior end of wings and maxillae forming a distinct, rounded, swollen projection which terminates beyond the caudal margin of A4. Prominent forewings largely conceal hindwings. Pairs of spiracles visible, as usual, at the junction of prothorax and mesothorax, and on A1–8 (on A1 they are covered by hindwings, while on A8 they are reduced and non-functional). Abdominal spiracles sometimes elevated.

The dorsal surface of the body has prominent grooves along the caudal and cephalic margin of A1-4 named "vice-like" structures, the shape of which is somewhat typical (figs. 2, 5, 6). These structures, usually in number of three, are arranged between the adjacent margins of the first four abdominal segments and each of them consists of two jowls: the caudal is often provided with particular microreliefs.

On the cephalic margin of abdominal tergites 5–7 is present a particular prominent structure (especially on A5 where it is always well developed), consisting of transversal furrows with ridges between (fig. 7). These structures, named "semiannular", are also in number of three but, unlike the previous, each is arranged on a single segment. Their shape is usually peculiar and thus, as the vice-like, are useful for species identification.

Cremaster conspicuous, pronounced and more or less cylindrical, consisting of a big rugose projecting, often longitudinally canaliculate on the dorsum of the cephalic portion, bearing 8 hooked spiral-shaped spines: two apical longer, and six subapical smaller (fig. 8).

¹ PATOČKA (1996b: 111, fig. 151) mentions to the presence of metathoracic legs on sides of maxillae and also reports this character in the key to genera (PATOČKA, 1995a: 214, fig. 33) as distinctive for Macdonnoughia. It is likely that it be an individual anomaly, very frequent in pupae, as in our specimens no cases of visible metathoracic legs occurred.

Description of species

Autographa gamma (LINNAEUS, 1758)

Кнотко, 1968: 110; Ратоčка, 1996: 113; Sannino et al., 1992: 259.

Pale green when just formed, gradually turning darkish starting from dorsum; black just before adult emergence.

Cuticle generally rugose, granulose on head thorax and appendages, smooth on the rest of body. Dorsal cephalic margin of A1–7 finely punctate by very small papilliform reliefs.

Body cephalic end squat, little prominent and flattened (fig. 3/1).

Lanceolate portion of the labium long a little more than half of the total length. Prothoracic femora length, 8–10 times prothoracic femora width. Caudal end of wings and maxillae extending to caudal margin of A6. Maxillae very long, circling forewing tips. Metathoracic legs not visible (fig. 4/1).

Abdominal spiracles elliptical (ratio length/width ca. 3–3.5/1), rather elevated and, on A3–6, with the cephalic margin prominent with respect to the caudal.

Vice-like structures with the caudal jowl regularly rounded and provided with uniformly distributed papilliform reliefs; cepholic jowl in the middle prominent (fig. 5/1).

Semiannular structures, with 6-8 transversal linear thin ridges, of which the inferior and the superior ones are only sketched. Some papilliform reliefs are present underlying the prominent caudal margin. The area beneath the said structures is little rounded and has some papilliform reliefs (fig. 7/1).

Cremaster as typical in the group, with a ratio length/width ca. 1/1 and the basal portion wide twice the apical. It is dorsally canaliculated at the base and irregularly rugose moving towards the posterior end (particularly on the swelling) (fig. 8/1).

Body length 17.4 ± 0.2 mm (range 16.0-18.8, No. = 34); body width (across the thorax) 5.3 ± 0.1 mm (r. 5-6.2, No. = 34).

Chrysodeixis chalcites (ESPER, [1789])

Ратоčка, 1996: 118; Sannino et al. 1992: 257.

Light green at first, pitch black before adult emergence with some ventral green areas.

Cuticle bright, smooth on abdominal segments, slightly rugulose on head, thorax and appendages. A1–7 not punctate.

Wings and maxillae caudal end, terminating to the cephalic margin of A5. Metathoracic legs not visible (fig. 4/2).

Abdominal spiracles elliptical, rather narrow (ratio length/width ca. 4/1), slightly elevated. Vice-like structures with caudal jowl very arched and thinly rounded, rugose on the contact

surface only; cephalic jowl smooth and very arched (fig. 5/2).

Semiannular structures, with a deep transversal median groove on A5, delimited by acute margins. The area underneath the aforesaid structures is rounded and smooth (fig. 7/2).

Cremaster longer than broad (ratio length/width ca. 2/1) with the basal portion broad as the apical. Dorsally canaliculated in the basal half and irregularly rugose in the posterior half. Apical spines terminating truncate (fig. 8/2).

Body length $16.5 \pm 0.2 \text{ mm}$ (range 15.9-17.5, No. = 39); body width (across the thorax) $4.2 \pm 0.2 \text{ mm}$ (r. 4-4.7, No. = 39).

Diachrysia chrysitis (LINNAEUS, 1758)

Кнотко, 1968: 111; Ратоčка, 1996: 109.

Green-pale brown when newly formed, later becoming black on the only cephalic margin of the abdominal central tergites.

Cuticle rough on head, thorax and appendages, smooth on most of abdominal segments. Dorsal cephalic margin of A1-7 finely punctate by papilliform reliefs as in A. gamma.

Caudal end of wings terminating to the mid of A5. Maxillae extending to wing tips with constant width. Antennae much shorter than mesothoracic legs. Metathoracic legs not visible (fig. 4/3).

Abdominal spiracles elliptical, quite wide (ratio length/width ca. 2-3/1), slightly elevated.

Vice-like structures very noticeable, as usual consisting of two jowls: the anterior one swollen with transversal ridges, while the posterior consists of a noticeable squat prominent tubercle-shaped and rugose protuberance (fig. 2/3, 5/3).

Semiannular structures, with 6–8 transversal linear thin ridges, of which the inferior and the superior ones are only sketched. The prominent caudal margin is beneath barely rugose. The area underneath the said structures presents a deep transversal groove and a narrow rounded, extending surface provided with papilliform reliefs (fig. 7/3)

Cremaster with ratio length/width ca. 1.5/1, dorsally canaliculated on all surface; apical spines very thick, squat, ending with a swelling that breaches up in 2 tips (fig. 8/3).

Body length 18-19 mm; body width 5-5.5 mm.

Macdunnoughia confusa (STEPHENS, 1850)

Кнотко, 1968: 109; Ратоčка, 1996: 111.

Green-white at beginning, brown-black at end stage.

Cuticle surface dull on head, thorax and appendages; almost glossy on abdominal segments. Dorsal cephalic margin of A1–7 punctate by papilliform reliefs, arranged in a dorsomedian position.

Body cephalic end very prominent, cupoliform (fig. 3/2).

Lanceolate portion of the labium long ca. two thirds of the total length. Prothoracic femora length, 5–6 times prothoracic femora width. Caudal end of wings terminating to the cephalic margin of A5. Distance between the tips of mesothoracic legs and forewings about 1–1.5 times longer than the distance existing between the tips of antennae and mesothoracic legs. Metathoracic legs not visible (fig. 4/4).

Abdominal spiracles elliptical (ratio length/width ca. 3/1) and slightly sunken starting from A3–4.

Vice-like structures with caudal jowl flattened on the contact surface and provided with irregular papilliform reliefs (concentrated mainly on the flat margin); cephalic jowl straight in the middle (fig. 4/1)

Semiannular structures, with 6–8 transversal thin ridges of which the top and the bottom ones are only sketched; caudal margin prominent and smooth. The area underneath the aforesaid structures is very rounded and provided with papilliform reliefs (fig. 7/4)

Cremaster with ratio length/width ca. 1.5/1, dorsally caniculated at the basal half and irregularly rugose at the remaining posterior half; apical spines long and thin, terminating truncate (fig. 8/4)

Body length 14-18 mm; body width 4-5.

Trichoplusia ni (Hübner, [1803])

Ратоčка, 1996: 118; Sannino et al., 1992: 259.

Pale green when just formed, rapidly darkens to a uniform brown.

Cuticle smooth or matt, except on head, thorax and appendages rugulose. Dorsal cephalic margin of abdominal segments 1–7 not punctate.

Posterior end of wing and maxillae shaped as usual, but little prominent barely exceeding A4. Metathoracic legs not visible (fig. 4/5).

Abdominal spiracles elliptical (ratio length/width 3-4/1), barely elevated.

Vice-like structures with the caudal jowl sinuous, sinuate in the middle and provided with very small reliefs on the extreme margin only. The cephalic jowl presents a transversal cavity, wave-like on the top and prominent (fig. 6/2).

Semiannular structures provided with 5–6 transversal, rugose and black ridges; on A5 the caudal margin is broad and straight while on A6–7 it is reduced to a simple line. The area underneath the aforesaid structures is roughly straight and provided with rare and small papilliform reliefs (fig. 7/5).

Cremaster squat, laterally angular, long as wide (ratio: 1/1); dorsally canaliculated only in a narrow basal portion and irregularly rugose in the remaining part. Apical spines slightly swollen at the tip (fig. 8/5).

Body length 16.2 ± 0.3 mm (range 15.4~17.1, No. = 26); body width (across the thorax) 4.6 ± 0.2 mm (range 4.3–5.2, No. = 26).

Discussion

Plusiinae pupate on the foliage of the food plant in a flimsy silk cocoon. As a consequence, the pupa is easily exposed to predator attacks and wind shakes. Moreover, being the cocoon almost transparent, its movements make it visible from the outside.

The anchorage provided by cremaster, although enforced by the special spiral-shaped spines (which ensure a secure link), is ineffective against transversal motions, which could be offset by a dorsal blocking device, instead.

Examined pupae were provided with special dorsal anchorage structures, usually in number of three, arranged among the adjacent margins of the first four abdominal tergites. These structures, as can be seen from the illustrations (figs. 2, 5, 6) enable pupa, in its normal lying position, to have a secure grip to the cocoon silk threads, which are squeezed as in a vice. Hence the name "vice-like" structures.

The presence of transversal integumental wrinkles close to the common margins of the said devices, very evident in some species (e.g. *D. chrysitis*), lets believe that these are exposed to constant pressure (because kept tight against each other) already at the pupal formation phase, when integument is not yet hardened (fig. 5/3).

MOSCHER (1916: 115), listing the Plusiinae (sub Phytometrinae) pupal features, mentions "prominent grooves" present on the posterior margin of metathorax and the first four abdominal segments, but does not indicate a possible function. REZBANAYI (1983: 32), comparing the immature stages of *Diachrysia chrysitis* and *Diachrysia nadeja* OBTH., gives only general information on these structures, that are particularly developed in the two species, describing them as "tiefe Falten" (deep wrinkles) on the dorsum.



Fig. 4 (above): Pupa, ventral view. 1 – Autographa gamma (LINNAEUS). 2 – Chrysodeixis chalcites (ESPER). 3 – Diachrysia chrysitis (LINNAEUS). 4 – Macdunnoughia confusa (STEPHENS). 5 – Trichoplusia ni (HÜBNER). Legend: A, antenna; L2, mesothoracic leg; MX, maxilla; A2-A3, abdominal segment 2 and 3; CAJ, caudal jowl; CEJ, cephalic jowl.

Fig. 5 (right): Vice-like structures: dorsal view (left), lateral view (right). 1 – Autographa gamma (LINNAEUS). 2 – Chrysodeixis chalcites (ESPER). 3 – Diachrysia chrysitis (LINNAEUS).





Fig. 6: Vice-like structures: dorsal view (left), lateral view (right). 1 Macdunnoughia confusa (STEPHENS). 2 – Trichoplusia ni (HÜBNER). Legend: CM, caudal margin; R, ridge; SS, semiannular structure.

The similarity of these structures with the "gin-traps" present on the dorsum (and in some instances on the body sides as well) of some Coleoptera families (HINTON, 1945; GRANDI, 1951; HALSTEAD, 1986; DE MARZO, 1994) is undeniable. These mouse-trap devices show a very strong development of the tergites edges, which are often serrated and provided with spines. Their function is clearly of defence, being present only in the fully exposed pupae, not protected by cocoons. For Plusiinae, instead, because of the presence of a silk case and the absence of spines or sharp teeth, one would think that the only function of the vice-like structures be to block the pupa to the cocoon. Indeed, the mature larva, once the construction of the external cocoon is completed, spins inside it a looser web on which the pupa finds an easy grip.

Completely different seems the function of the semiannular structures arranged on the dorsal cephalic margin of the A5, and less prominently on A6–7, already recorded in three Plusiinae (SANNINO et al, 1992) and now found in other two species as well. During the pupal arching,

we observed that the A4-6 caudal margin inserts itself into a deep groove existing on the oforesaid structure (or beneath its prominent margin). The abdominal segments remain thus reciprocally linked, accumulating kinetic energy during the tension phase, which is then suddenly released causing a rapid springing movement sometime along with a distinct sound.

Similar structures have been also described by MOSHER (1916: 28) in various Lepidoptera families (especially in the borers pupae) calling them "flanged plates" yet without indicating their possible function. PATOČKA (1996b: 105) briefly mentions these structures defining them "transversal reliefs" ("hervortretende Querleiste") and pointing out that they are better visible in the exuviae.

Sometimes in the Nymphalidae pupae, there is the presence of an abrupt abdominal restriction, which also has an analogy with the A5~7 structures: AMIET (1998a, 1998b), describing the said morphological particular in genera Bebearia HEMMING and Euptera STAUDINGER, gave it the name "constriction annulaire abdominale" and hypothesized that its function could be to grasp larval exuviae during moulting. However, the structure described by Amiet is substantially different from the Plusiinae's because, besides missing the prominent sclerotized ring, it is only one and made up of two parts, practically symmetrical, obtained at the expense of the A4 caudal margin and A5 cephalic margin. In Plusiinae, instead, the structures are three and each of them is located on the cephalic margin of a single segment.

Finally, be it made clear that both vice-like and semiannular structures are confined to the abdomen because, needing movement for their working, they must be arranged between the abdominal segments, as these are the only movable parts of pupa.

Key to some Plusiinae pupae on tobacco and other cultivated horticultural plants

- 1 Noticeable dorsomedian submarginal protuberances (which are part of the vice-like Diachrysia chrysitis structures) are present between abdominal segments 2-4. Vice-like structures are not shaped as above 2
- 2 Semiannular structures present on the cephalic margin of abdominal tergites 5-7 with a complete development only on A5 where is also present a broad and straight caudal margin. Trichoplusia ni 3

Semiannular structures are evident and well defined on A5-7

- 3 Semiannular structures show a deep transversal groove. Chrysodeixis chalcites Semiannular structures show only thin wave-like ridges . 4
- 4 Body cephalic end little prominent, squat; lanceolate portion of the labium long just a little more than half of the total length; prothoracic femora length 8-10 times prothoracic femora width; vice-like structures with the caudal jowl regularly rounded and provided with uniformly distributed papilliform reliefs. . Autographa gamma Body cephalic end very prominent, cupoliform; lanceolate portion of the labium long ca. 2/3 of the total length; prothoracic femora length 5-6 times prothoracic femora width; vice-like structures with caudal jowl flattened on the contact surface and provided with irregular papilliform reliefs (concentrated mainly on the flat margin).

. Macdunnoughia confusa



Fig. 7: Semiannular structure, lateral view. 1 - Autographa gamma (LINNAEUS). 2 - Chrysodeixis chalcites (ESPER). 3 - Diachrysia chrysitis (LINNAEUS). 4 - Macdunnoughia confusa (STEPHENS). 5 - Trichoplusia ni (HÜBNER), A5.



Fig. 8: Cremaster, dorsal view. 1 – Autographa gamma (LINNAEUS). 2 – Chrysodeixis chalcites (ESPER). 3 – Diachrysia chrysitis (LINNAEUS). 4 – Macdunnoughia confusa (STEPHENS). 5 – Trichoplusia ni (HÜBNER).

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