SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY



Morphological Characterization of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae)

L QUEIROZ-SANTOS¹, MM CASAGRANDE¹, A SPECHT²

¹Lab de Estudos de Lepidoptera Neotropical, Depto de Zoologia, Univ Federal do Paraná, Curitiba, PR, Brasil ²Embrapa Cerrados, Planaltina, Brasil

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Correspondence

L Queiroz-Santos, Depto de Zoologia, Univ Federal do Paraná, Zip Code 81531-980 P.O. Box 19020, Curitiba, PR, Brasil; Iuzianyqueiroz@gmail.com

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Abstract

The cotton bollworm Helicoverpa armigera (Hübner) is a widespread lepidopteran pest found in various crops worldwide. This highly polyphagous species, commonly found both in the Old and New World, has caused significant economic damage as an invasive agricultural pest in Brazil since 2013. The goal of the present study is to provide a detailed morphological assessment of adults and immature stages of H. armigera, as this species is often confused with H. zea (Boddie), a congeneric species that is native to the New World. The biology data were acquired during four full life cycles, and observations on general behavior, nocturnal habits of larvae and adults, and sensitivity of larvae to humidity were recorded. Larval chaetotaxy differs between the first and the remaining instars, which bear L2 on the meso- and metathorax and L3 on A3 through A6, along with conspicuous chalazae and longitudinal bands. Important morphological characters of this species include the following: eggs with four micropylar openings, lined with 12 cells arranged in the shape of a rosette; pupa adecticous and obtect, with prominent spiracles; adults with the distal antennomere striate. Adults exhibit sexual dimorphism in the number of setae on the frenulum and spines on the prothoracic leg. Illustrations of the critical morphological features of this species are provided.

Introduction

Following its introduction to South America, *Helicoverpa* armigera (Hübner, [1808]) (Lepidoptera: Noctuidae: Heliothinae) has become one of the main noctuid pests worldwide (Hardwick 1965; Matthews 1991; Tay *et al* 2013; Sosa-Gómez *et al* 2016, Bentivenha *et al* 2016). Characteristics of this species that likely contributed to its biological success include its dispersal capacity, high reproductive potential, polyphagy, rapid development, and optional diapause at higher latitudes (Hardwick 1965; Fitt 1989; Matthews 1991).

The native distribution of *H. armigera* includes western Europe, central Africa, Australia, Oceania, and parts of Asia (Hardwick 1965). It has been recorded in several localities attacking multiple host crops in Brazil since March 2013 (EMBRAPA 2013; Czepak *et al* 2013; Specht *et al* 2013; Tay *et al* 2013), as well as in other South American countries (SENAVE 2013; Murúa *et al* 2014; Castiglioni *et al* 2016; Arnemann *et al* 2016). *Helicoverpa armigera* is dispersing from South America towards North America and has recently been recorded from Puerto Rico and Florida (CABI 2017).

Hübner described *Noctua armigera* between 1803 and 1808 based on an illustrated plate (Hemming 1937). Subsequently, Hardwick (1965) erected the genus *Helicoverpa*, to which he transferred *N. armigera* and other five taxa, yielding a total of 11 species.

At the moment, several publications have described biological (Hardwick 1965; Zalucki *et al* 1986; Karim 2000; Ali *et al* 2009) and behavioral (Sorensen *et al* 2006; Abbasi *et al* 2007; Feng *et al* 2010; Liu *et al* 2010; Nadda 2013; Jadhav *et al* 2013) aspects of this species, but no previous study offers details on morphological features of *H. armigera*.

Although much has been published concerning nocturnal species considered to be agricultural pests, works on general morphology, especially that of moths, are still scarce. The main goal of this study is to provide morphological characterizations and natural history data on *H. armigera*, a species that feeds on several crops, including soybeans, corn, cotton, green beans, tomatoes, citrus fruits, and pastures (Bueno & Sosa-Gómez 2014). Included here are descriptions of immature stages and adults, emphasizing structures not previously characterized. This information is intended to facilitate the accurate identification of this species as it is often confused with other congeneric taxa, particularly cryptic species.

Materials and Methods

Insects were reared at room temperature under natural light. All immatures were obtained from the second generation of two individualized pairs and kept in PVC cages (80 cm diameter, 60 cm height). The cages were internally coated with a kraft paper as a substrate for oviposition, and the top was covered with voile fabric. This technique was developed by modifying other methods described in the literature (Specht *et al* 2006; Mironidis & Savopoulou-Soultani 2008; Wang *et al* 2008). Adults were fed 10% honey solution in distilled water, which was provided in a hydrophilic cotton ball. Both food and kraft paper coating were changed every 48 h. This rearing procedure produced four batches of 24 male-female pairs.

After eclosion, ten individual larvae of each instar were fixed in Kahle-Dietrich solution and then transferred to ethyl alcohol at 70%. The remaining individuals were kept alive in order to obtain subsequent developmental stages. The larvae were kept in plastic containers (2.5 cm diameter, 7 cm height) coated with absorbent paper. Two groups were fed lettuce leaves with no additional humidity sources, and two groups were given a bean-based artificial diet (Montezano et al 2013). Head capsules were removed from the containers after each molt and stored dry. For pupation, a small amount (3 cm height) of autoclaved expanded vermiculite was added to each container, so that the caterpillars could construct pupal chambers. Ten pupae of each sex were fixed according to the method described above, after its cuticle hardened. Adult specimens were killed in a jar with ethyl acetate, which keeps exoskeletal structures intact for subsequent morphological examination.

Wings were removed and bleached in order to study venation patterns. Head, thorax, abdomen, and appendages were immersed in a 10% potassium hydroxide solution (KOH) and placed in a warm bath until tissues softened and the exoskeleton appeared semitransparent. Structures of interest were then dissected for observation under a light microscope. Voucher specimens are deposited at the Padre Jesus Santiago Moure Collection, Lepidoptera, Universidade Federal do Paraná (DZUP), about numbers DZ 39.223, DZ 39.233, DZ 39.243, DZ 39.253, DZ 39.263, DZ 39.273, DZ 39.283, DZ 39.293, DZ 39.303, DZ 39.313, DZ 39.323, DZ 39.363, DZ 39.373, DZ 39.383, DZ 39.393, DZ 39.403, DZ 39.423, DZ 39.443, DZ 39.453, DZ 39.463, and DZUPIL 0141.

Measurements of immature stages (eggs, larvae, molted head capsules, and pupae) were made using a Wild Heerburg stereomicroscope with a micrometric scale. Head capsule width corresponds to the largest distance between genae in each instar. Body length is the distance from the frons to the last abdominal segment in a pharate larva. Measurements are specified based on means and standard deviation from multiple specimens (*n*).

Illustrations of specimens and structures of interest were prepared with the aid of a camera lucida attached to a Zeiss Stemi SV6 stereomicroscope or a Zeiss Standard 20 microscope. Electron micrographs were produced with a JEOL JSM 6360-LV scanning electron microscope. Ethanol-preserved specimens were dehydrated in a series of 70, 80, 90%, and two 100% ethanol baths for 10 min each. These samples were then moved to a Thornton T14 ultrasonic cleaner for two 30-min baths and dried in a Bal-Tec® CPD-030 critical point dryer. Dehydrated structures were mounted on metallic stubs with 3M[®] copper conductive double-sided tape and gold-coated with a Balzers® SCD030 - Union FL 9496 sputter coater device. Dry samples were mounted directly on metallic stubs with 3M[®] copper double-sided tape and analyzed in low-vacuum mode. Illustrations were prepared using permanent black ink and were subsequently adjusted and formatted using GIMP v. 2.8.2 (www.gimp.org).

Terminology and notation are based on the following contributions: Peterson (1961, 1964) for eggs; Stehr (1987) for chaetotaxy; Peterson (1962) for larval body area; Blaik & Malkiewickz (2003) for labral, maxillary, and thoracic leg setae; Grimes & Neunzing (1986) and Baker *et al* (1986) for maxillary palp sensillae; Mosher (1916) for pupae; Pierce (1909), Comstock (1918), Snodgrass (1935), Casagrande (1979), Scoble (1992), Speidel *et al* (1996), Hallberg *et al* (2003), Kristensen (2003), and Fibiger & Lafontaine (2004) for adult external morphology; and Butt & Cantu (1962) for sex determination. The taxonomic classification follows Hemming (1937).

Results

Biology

Adults (Fig 1A–D) were inactive during most of the day and were active at night, after the evening twilight, during which



Fig 1 *Helicoverpa armigera*. **A** Female in dorsal view. **B** Female in ventral view. **C** Male in dorsal view. **D** Male in ventral view.

mating usually starts. Pairs remain in copula for a few hours up to three days. Oviposition starts one to two days after copulation when females, with their wings spread, lay small egg masses with up to three eggs each. The copula can last up until 15 days, after which females die, presumably from exhaustion. Approximately 10 to 20 eggs were deposited per day during the first days, gradually increasing towards the end of the oviposition period. Based on observations of the four laboratory-reared groups, the first two egg batches did not hatch.

First instar larvae will feed on the chorion and then crawl individually in search of food. If no suitable food is found, they will consume unhatched eggs and other less active larvae. Because of this behavior, larvae were kept isolated during their first instar. In order to test the occurrence of cannibalism, pairs of larvae were placed in containers with food. Cannibalism was not observed under those circumstances; however, one of the larvae would keep the other from reaching the food, leading to its death from starvation. High environmental humidity is poorly tolerated by this instar when compared to subsequent instars.

Before each molt, larvae become motionless, first expelling the head capsule anteriorly, and then the body exuvia posteriorly, through extension and contraction movements. Larvae will not consume the exuviae after molting. The first and second instar larvae wrap themselves in silk prior to molting. No timing pattern was observed for molting or feeding activity—larvae feed or molt regardless of the light period.

After the fourth instar, larvae react to disturbance by raising their head and thoracic segments, and then recurving the head, remaining in this position for a few seconds. Sixth instar larvae stop feeding when they reach prepupal stage. They release a reddish secretion before burrowing into the soil to construct a pupal chamber with silk (Fig 5G–I). The lateral and dorsal parts of the body increase in size and remain motionless for the next six days through the end of pupation. The sixth instar exuvia is abandoned, and the pupa is free inside the pupal chamber, moving only its five posteriormost abdominal segments in a 360° rotation when disturbed. Adults emerge at night.

Description of immature stages

Egg

Subspherical (Fig 2B); cream-white when newly laid (Fig 2A), slightly darker after two to three days, brownish before hatching. Micropylar area (Fig 2C) convex, depressed at center, bearing at least four micropylar openings, surrounded by a group of 12 cells arranged in a rosette pattern: narrower basally, distally elongated with margins broadly round. A single ellipsoid cell, between a pair of petalled cells, tapering towards the distal margin, its apex contiguous to one of the longitudinal striations. Extending from the micropylar area towards egg base, 23 longitudinal striations (Fig 2D), some next to depression formed by the micropylar area, others further apart; transverse grooves arranged at intervals corresponding to approximately half the space between longitudinal striations, forming rectangular cells on the chorion from one pole to the other.

Measurements. Diameter, 0.36 mm \pm 0.02 (*n* = 10); length, 0.45 mm \pm 0.01 (*n* = 10).

First instar larva

Head ellipsoid (Fig 3A), vertex acute, dark brown, covered in unbranched long setae lacking ornations; antenna threesegmented (Fig 3B); spinneret well developed, tubular (Fig 3C), mandibles with five teeth (Fig 3D), four visible externally,



Fig 2 *Helicoverpa armigera*. A Egg. B Egg in dorsal view. C Micropylar area. *Mo* micropylar opening, *Rsc* rosette cell. D Egg ornamentation. *Ls* longitudinal striae, *Ts* transversal striae, *Rs* rosette.

and the fifth located on the upper margin of the internal surface.

Body cylindrical (Fig 4A, B), light brown, with longitudinal light yellow areas: one dorsal, one supraspiracular, and one subspiracular; setae unbranched, inserted in black chalazae, surrounded by spicules (Fig 3E); spiracles round, black, conspicuous in T1 and A1 (Fig 3F) through A8 (Fig 3G), larger in T1 and A8, located above the level of other spiracles. Pronotal plate, anal plate and thoracic legs dark brown, tarsus with well developed, hook-shaped claw (Fig 3H); abdominal legs A3–A6 with crochets in uniserial, uniordinal arrangement.

Measurements. Head capsule, 0.29 mm \pm 0.008 (n = 17); maximum length, 2.1 mm.

Second instar larva

Head as in first instar.

Body light brown with longitudinal brown areas (Fig 4C, D) in dorsal, supraspiracular and subspiracular positions, darker if compared to first instar. Other features as in first instar.

Measurements. Head capsule, 0.46 mm \pm 0.030 (n = 17); maximum length, 5 mm.

Third instar larva

Head light brown with dark spots around the epicranial suture, denser on the frons.

Body dark brown with three light brown areas (Fig 4E, F): the first one narrow, subdorsal, the second one

supraspiracular, as wide as the first one but not continuous, and the third spiracular, twice as wide. Pronotal plate dark brown, with two light brown longitudinal bands extending from the anterior margin to near the posterior margin. Anal plate dark brown, with three light brown spots: one medially in the anterior margin, and one at each end of the posterior margin. Other features as in first instar.

Measurements. Head capsule, 0.72 mm \pm 0.064 (n = 17); maximum length, 9.71 mm.

Fourth instar larva

Head as in third instar.

Body with longitudinal bands of similar width, interspersed by light and dark brown areas (Fig 4G, H), wider on subdorsal and supraspiracular regions, between A1 and A7; light brown band on spiracular area, wide, with dark brown shades below spiracular line. Pronotal plate dark brown, with a pair of parallel and longitudinal light brown areas. Anal plate dark brown, with light brown spots. Other features as in first instar.

Measurements. Head capsule, 1.17 mm \pm 0.108 (n = 17); maximum length, 11.69 mm.

Fifth instar larva

Head as in third instar.

Body (Fig 5A, B) with similar color pattern as in previous instar, marbled aspect more conspicuous in the ventral





region, highlighting the margin of the spiracular band. Subspiracular longitudinal area dark brown, with light brown shades to the base of the thoracic legs. Thoracic legs dark brown; abdominal legs light brown. Chalazae in A1 twice the size of others. Setae D1, D2, SD1 dark brown, decreasing in size on A2; maintaining same pattern through A6; and becoming larger on A7, near chalaza of seta D1. Pronotal and anal plates as in fourth instar. Other features as in first instar.

Measurements. Head capsule, 1.91 mm \pm 0.075 (n = 17); maximum length, 18.71 mm.

Sixth instar larva

Head with six stemmata, arranged in half-moon shape (Fig 6A), the fifth ventrally displaced, all stemmata ovoid, except for the first and the sixth (Fig 6B), with contour poorly defined. Mandibles serrate, bearing six teeth (Fig 6C), the upper tooth shorter, more centrally located. Maxillae well developed (Fig 6D), maxillary palp three-segmented (Fig 6E), median segment with two campaniform sensilla, SC1 and SC2, and a digitiform sensillum, SD; distal segment with eight basiconic sensilla, A1, A2, A3, L1, L2, L3, M1, and M2. Fig 4 Larvae of *Helicoverpa armigera*. **A** First instar in dorsal view. **B** First instar in lateral view. **C** Second instar in dorsal view. **D** Second instar in lateral view. **E** Third instar in dorsal view. **F** Third instar in lateral view. **G** Fourth instar in dorsal view. **H** Fourth instar in lateral view.

Fig 5 Larvae and pupal chamber of *Helicoverpa armigera*. **A** Fifth instar in dorsal view. **B** Fifth instar in lateral view. **C** Sixth instar in dorsal view. **D** Sixth instar in lateral view. **E** Sixth instar in dorsal view. **F** Sixth instar in dorsal view. **G** Pupal chamber in lateral view. **I** Pupal chamber in ventral view.

Fig 6 Scanning electron microscopy micrographs of the sixth instar larva of *Helicoverpa armigera*. A Stemmatal region. B Sixth stemmata. C Inner view of mandible. D Maxilla. E Maxillary palp, sensilla basiconica: A1, A2, A3, L1, L2, L3, M1, and M2; sensilla campaniformia: SC1 and SC2; sensillum digitiformium: SD. F Labium. *Hp* hypopharynx, *Lb* labial palp, *Sn* spinneret, *Spl* spinules.

Labium with spicules on hypopharynx (Fig 6F). Spinneret tubuliform, with a wide distal opening (Fig 7A). Jugular gland ventrally on prothorax (Fig 7B). Other head features as in third instar.

Body may exhibit two distinct color patterns. In the first color pattern, larvae are predominantly brown (Fig 5C, D), with narrow dark brown areas along the dorsal region, interspersed with light yellow areas of equivalent width. Two dark brown areas laterally fused with dark yellow areas, extending from A1 to A8. Supraspiracular area dark brown, light yellow areas between T1 and A8, and light brown with light yellow areas in A8 and A9. Spiracular area light yellow, with brown shading; ventral margin cream-white. Thoracic and abdominal legs light green. Chalazae dark brown, with gray, unbranched setae; setae SD1, D1, and D2 on segments A1, A2, and A8 very distinct. The second body color pattern is predominantly light green with white spicules (Fig 5E, F); dorsally with white narrow area, outlined with mossy green spots anteriorly on each segment. Supraspiracular area dark green, medial area of each segment lighter in color; conspicuous mossy green spots dorsally in A1, A2, and A8. Spiracular area light green, sided dorsally and ventrally by cream-white bands. Chalazae black in T1 and A8 on seta SD1 and in A8 on seta D1; all others white. Thoracic and abdominal legs light green.

Following features identical in both color variants: spiracles elliptical in shape, peritreme light and sponge-like, surrounded by a dark brown ring, the adjacent area lighter in color (Fig 7C–E). Pronotal and anal plates similar to fourth instar. Abdominal legs A3–A6 and A10 with crochets in biordinal uniserial arrangement (Fig 7F), the first ones arranged in internal lateral band, and the second as a semicircle opening distally.

Measurements. Head capsule, 2.87 mm \pm 0.141 (n = 17); maximum length, 25.85 mm.

Head chaetotaxy

Head with 17 pairs of setae from first to sixth instar (Fig 8A, B), primary and tactile, tapering towards apex: A1, A2, A3, F1, Af1, Af2, C1, C2, P1, P2, L1, S1, S2, S3, SS1, SS2, and SS3; labrum with six pairs: Lrl1, Lrl2, Lrl3, Lrm1, Lrm2, and Lrm3; and mandibles with two pairs: M1 and M2 (Fig 9C). Three pairs of

Fig 7 Scanning electron microscopy micrographs of the sixth instar larva of *Helicoverpa armigera*. A Spinneret. B Jugular gland in lateral view. Jg jugular gland. C Thoracic spiracle on T1. D Abdominal spiracle on A8. E Spiracle opening detail. F Abdominal leg crochets A8 in lateral view.

proprioceptor setae dorsally on each epicranium: MD1, MD2, MD3. Ten pairs of pores between epicranium and frons, MDa, La, Pa, Pb, Afa, Aa, MGa, Sb, SSa, and Fa.

Thorax and abdomen chaetotaxy

Setae unbranched, emerging from chalazae, and surrounded by spicules, arranged similarly throughout segments. Pronotal plate with dorsal setae D1, D2, XD1, and XD2, and lateral setae SD1 and SD2 (Fig 9A); setae L1 and L2 anterior to prothoracic spiracle; SV1, SV2, and V1 ventrally located. The same arrangement is observed in all instars. Chaetotaxy of meso- and metathorax similar in first instar, with D1, D2, SD1, L1, SV1, and V1; chaetotaxy in other instars differ from this arrangement due to the presence of L2 laterally, and MV1 and MV2 medioventrally. Prothoracic legs similar in all instars, with 16 setae and a single pore; setae Cx1 located dorsally and Cx2 ventrally; Tr1 and Tr2 dorsally; Fr1 and Fr2 ventrally; Tb1-Tb6 with Tb1 and Tb6 positioned ventrally, Tb3 and Tb4 dorsally, Tb2 and Tb5 laterally.

Ts3, and Ts4 dorsal and Ts2 lateral (Fig 9D). Chaetotaxy of abdominal segments A1 and A2 similar in all instars, with D1, D2, SD1, L1, L2, SV1, SV2, and V1. A3 through A6 more or less equivalent in size in first instar, D1, D2, SD1, L1, and L2 present; other instars also exhibit a lateral L3. Abdominal legs showing the same setal pattern in all instars: SV1, SV2, and V1. A7 and A8 with D1, D2, SD1, L1, L2, SV1, and V1: A9 with D1, D2, SD1, L1, SV1 and V1, and A10 over the anal plate with D1, D2, SD1, and SD2 (Fig 9B). Posterior abdominal legs with L1, L2, SV1, SV2, SV3, and V1 (Figs S1-S2) on the sclerotized portion posterior to the anal plate.

Instar identification

Larval size and color patterns are distinct in the larval instars. In order to distinguish the first and second instar, which have similar color patterns, we used the head capsule growth ratio according to Brooks-Dyar (Daly 1985). That instar can be easily told apart by the linear measurement of the head capsule width (Table 1).

Fig 8 Head capsule chaetotaxy of *Helicoverpa armigera*. **A** First instar in frontal view. **B** Sixth instar in lateral view.

Pupa

Adecticous and obtect, light brown immediately after pupation, dark brown after five days; eyes noticeably dark just before emergence (Fig 10A–C). Elongate, cylindrical, head round, vertex extending towards the posterior region, along the prothorax and antenna; abdomen tapering after A4. Integument dense, smooth, edges of segments well marked (Fig 11(C)).

In ventral aspect (Figs 10A and 11(A)), frontoclypeus goblet-like, between the compound eyes. Hypopharynx visible, shaped like an inverted triangle, with short posterior labial palp. Antenna fine, long, reaching costal margin of forewing, anterior to A5. Legs placed along

the antenna towards the galea in repose; metathoracic leg longest. Galea lanceolate, occupying most part of the anterior half of the ventral side; reaching posterior margin of A4. Hind wing on top of forewing, both ending above anterior margin of A5.

In dorsal aspect (Figs 10B and 11(B)), prothorax small, triangular, apex round, prothoracic spiracle next to basal angle (Fig 12A, B). Mesothorax and metathorax projecting latero-ventrally, forming the pterotheca until the anterior margin of A5.

Abdomen divided in ten segments, spiracles elliptical, conspicuous between A2–A7, reduced in A8 (Fig 12C). Abdomen slightly curved towards cremaster posterior to A5. Cremaster composed of two articulate, parallel projections, tapering distally, approximately as long as A10 (Fig 12D).

Anal opening scar elongate, longitudinal to body axis, located on distal ventral area of A10. Female genital opening scar (Fig 13A) medioventrally located on A8. Male genital opening (Fig 13B) on A9, surrounded by small protuberances.

Measurements (male/female). Maximum width of third abdominal segment, 6.31 mm \pm 0.33 (n = 9) (5.71–7; SE, 0.03); body length, 20.47 mm \pm 1.52 (n = 9) (18.57–22.41; SE, 0.16).

Adult

Head

Hypognathous, entirely covered by scales. Compound eyes semioval, glabrous, prominent, longer than wide. Paraocular area marked in parallel to eye orbit and paraocular suture; laterofacial suture reaching eye orbit slightly above its center. Frontoclypeus subquadrate, prominent, separated from vertex by the transfrontal suture, which is close to, but not fused with, the antennal suture. Subgenal area fused laterally and basally with mandibular rudiment, extending dorsally towards the pilifer. Pilifer setae present. Vertex wide, with chaetosemata throughout, enclosing pair of antennal sockets; antennifer surrounded by antennal suture, and a pair of dark ocelli laterad to the antennal sockets, separated posteriorly from the occiput by an occipital suture (Figs 14A and 15A).

In dorsal and posterior aspects (Fig 14B, C), postoccipitus triangular, separated from occiput by postoccipital suture. Occiput subrectangular, delimited laterally from postgena by temporal sutures. Postgena semicircular, separated from subgena by subgenal suture. Postgena located posteriorly between eye orbit and temporal suture, corresponding to most of hind portion of head. Postoccipitus located dorsally to the foramen, separated from ventral foramen by the tentorial bridge. Posterior tentorial pit at the posterior end of

Fig 9 Chaetotaxy of *Helicoverpa armigera* larvae. **A** Pronotal plate, first instar larvae. **B** Anal plate, first instar larva. **C** Clypeus, labrum and mandible, sixth instar larva. **D** Thoracic leg, fifth instar larva.

tentorial bridge, next to ventral foramen. Anterior tentorial arm shaped as a semi-lozenge. Hypostomal bridge distal.

In ventral aspect (Fig 14D), labrum covered by frontoclypeus. Pilifer lateral and ventral to labrum, semielliptical, with several setae at base. Hypopharynx (Fig 16E), medial, between galeae, shorter than pilifer. Maxillary palp reduced, round, frontal to galea. Stipes elongate. Cardo 1/10 length of stipes. Labium extending

Table 1 Mean and standard deviation of maximum head capsule length (mm) observed in specimens of *Helicoverpa armigera*. $\overline{X} \pm Cl$ average and consistency index, *n* number of samples, *GR* growth ratio between larval instars.

Instar	\overline{X} ± Cl 95%	n	GR
 I	0.29 ± 0.004	17	1.5912
11	0.46 ± 0.014	17	1.5660
Ш	0.72 ± 0.029	18	1.6255
IV	1.17 ± 0.051	17	1.6280
V	1.91 ± 0.036	17	1.5
VI	2.87 ± 0.067	17	
Mean growth rate			1.5821

ventrally and medially, located posteriorly and below the hypostomal bridge. Postgena along compound eyes, separated from subgena by the subgenal suture.

Head appendages

Antenna

Filiform, not sexually dimorphic, with 83 flagellomeres tapering towards apex (Fig 15B), the last flagellomere distinct from others, striate and reduced, cone-like (Fig 16B, C). Microsetae throughout internal surface of flagellomeres. Length approximately 11× eye width. Scape globular, robust, flattened at extremities. Pedicel ring-like, shorter than scape, about as long as the width of first flagellomere (Fig 16A).

Mouthparts

Labium

Subtriangular, weakly sclerotized, medial in proboscidial fossa, bordered laterally by the stipes and ventrally by the hypostomal bridge.

Fig 10 Pupa of *Helicoverpa armigera*. **A** Ventral view. **B** Dorsal view. **C** Lateral view.

Labial palp tri-segmented, approximately twice as long as head in lateral view. Proximal segment shorter than mid segment. Distal segment reduced in size compared to other two. Reuter's sensitive spot present on internal surface of basal segment. Vom Rath's organ present on distal segment as an invagination of integument, longer than half of the length of the segment (Fig 15C, D).

Maxilla

Located in the anterior portion of proboscidial fossa, constituted by cardo, stipes, reduced maxillary palp, and galea. Galea cylindrical, with styloconica sensilla on anterior portion, approximately $6.3 \times$ eye width (Fig 16F).

Fig 12 Pupa of *Helicoverpa armigera*. **A** Thoracic spiracle in anterior view. **B** Thoracic spiracle in lateral view. **C** Abdominal spiracle. **D** Cremaster.

Cervix

Reduced, almost entirely membranous, except for a pair of Yshaped lateral cervical sclerites, composed of an anterior arm articulating with the head and ventrally enclosing the "cushion"-like cervical organ, covered by numerous setae; dorsal arm 1/3 length of anterior arm, articulating distally with propleuron; ventral arm approximately as long as anterior arm, projected distally (Fig 17A).

Thorax

Prothorax

The smallest thoracic segment, mostly membranous.

In dorso-lateral aspect: pronotal dorsal plate subtriangular, articulating posteriorly with prescutum II, and laterally with a membranous area and parapatagium. Patagium oval, conspicuous if compared with other structures in this segment. Parapatagium semielliptical, connected to dorsal plate of pronotum (Fig 18). On propleuron, first spiracle next to the distal portion of parapatagium. Pre-episternum commashaped, projecting anteriorly to episternum. Coxa projecting ventrally to episternum.

In ventral aspect: episternum wide, mostly occupying the coxal articulation; connected anteriorly to the ventral arm of the cervical sclerite; medially constituting the discrimen; posteriorly projecting towards the triangular espinasternum through the furca.

Mesothorax

The largest thoracic segment. In dorsal aspect: prescutum oval, located antero-medially in relation to scutum. Scutum wide (the largest sclerity in the body), smooth, slightly convex, scutal suture delimiting the suralare, adnotale and

Fig 13 Posterior region of pupa of *Helicoverpa armigera*. **A** Female in ventral view. **B** Male in ventral view.

Fig 14 Head of *Helicoverpa armigera*. **A** Frontal view. **B** Dorsal view. **C** Posterior view. **D** Ventral view.

posterior notal wing process; scutum separated from scutellum by the scuto-scutellar suture. Scutellum half the length of scutum, distally ending at the postnotum. Postnotum sickle-shaped.

Fig 15 Head and cephalic appendages of *Helicoverpa armigera*. A Head in lateral view. B Antenna. C Labial palp in external lateral view. D Labial palp in inner lateral view.

In lateral aspect: tegula (Fig 17B, C) large compared to other structures, forming an anterior lobe, distally curved, subtegula reaching the anterior margin of the basalare. Basalare round. Folds of pleural process of forewings and lateral margin of tergopleural apodeme projected and then fused distally. Subalare ellipsoid, reaching adnotal sclerite and posterior notal wing process. Axillary cord connecting to postalar plate.

On membranous area: anepisternum and katepisternum delimited by anepisternum suture, pre-episternal suture separating pre-episternum from katepisternum. Epimeron V-shaped, separated anteriorly from anepisternum and katepisternum by the pleural suture, from the meron by the basicostal suture; the upper portion of the distal arm surrounding the second spiracle anteriorly.

In ventral aspect: discrimen $5 \times$ longer than discrimen I, forming the base of the basisternum triangle, which extends to the furcal pit. Katepisternum projecting ventrally, reaching the basisternum, separated by the sternumpleural suture towards the medial line and laterally around the coxa (Fig 19).

Metathorax

Larger than the prothorax and smaller than the mesothorax. In dorsal aspect: scutellum subtrapezoidal, located between scutellar plates, which project antero-laterally around the scutellum and postnotum II. Scutum reaching distally to the scutellum, separated by the scuto-scutellar suture. Lateral medial portion and anterior portion of scutellum delimited by membranous area from postnotum II (Fig 20).

In latero-ventral aspect: scutum projected distally, supporting the ventral end of the axillary cord ventrally, and anteriorly separated from suralare by the scutal suture. Pleural process of wing arched, located between suralare and anepisternum. Basalare slightly above, also round, but much smaller than anepisternum, surrounding the distal portion of second spiracle. A short anepisternum suture between anepisternum and katepisternum. Katepisternum rectangular, delimited anteriorly by a membranous area that separates the segments, distally separated from the epimeron by the pleural suture, ventroanteriorly separated from the basisternum, partially covered by the katepisternum and eucoxa, the distal angle separated from eucoxa by the marginopleural suture.

Fig 16 Head and cephalic appendages of *Helicoverpa armigera*. A Scape, pedicel, and proximal flagellomeres. B Distal portion of the antenna. C Detail of the last flagellomere. D Ocelli in frontal view. E Hypopharynx in frontal view. F Distal portion of the galea.

Antero-posterior axis of coxa positioned obliquely to the discrimen.

Subalare isolated, on membranous area, posterior to the dorsal portion of katepisternum and below the axillary cord. Epimeron U-shaped, the tympanic membrane at the end of its distal arm (Fig 25A), and bearing a tympanal sclerite.

In ventral aspect: discrimen approximately the same size as discrimen I, extending medio-longitudinally from the anterior margin of basisternum to furcal pit. Sternumpleural suture limiting the basisternum, which consists of a narrow, long sclerotized plate along the katepisternum.

Thoracic appendages

Axillary sclerites

Wings articulate with thorax through three irregular-shaped sclerites. On the anterior wing, first axillary sclerite located posterior to the adnotal and anterior to the second axillary sclerite. The second axillary sclerite articulating with the first axillary and with the proximal median plate. The third axillary sclerite connected anteriorly with the proximal median plate and latero-posteriorly with anal veins. The costal sclerite articulates with the costal (C), subcostal (Sc), and radial (R) veins, latero-anteriorly to the humeral plate and distal to the proximal median plate. Dorsally, the distal median plate meets the median (M) vein, located anterior to the proximal medial plate (Fig 21A).

On the hind wing, the costal sclerite articulates with C, Sc, and M, and is the base of the frenulum. The first axillary sclerite is projected into three arms: the anterior, basal, connected to the scutum, the second to the axillary cord, and the third to the second axillary sclerite and to the lateral margin of one of the median plates. The third axillary sclerite articulates with the anal (A) veins posteriorly and anteriorly to the medial plate (Fig 21B).

Wings

Forewing subrectangular (Fig 22A), with 14 longitudinal veins (C, Sc, R1-R5, M1-M3, Cua1 e Cua2, 2A e 3A) and 4 crossveins (dcs, dcm, dci, m-cu). Males and females with identical venation pattern except for having slightly different outlines. Costal margin: slightly convex distally from base to R_4 ;

Fig 17 Cervical region and tegula of *Helicoverpa armigera*. A Cervical sclerite in lateral view. B External lateral view. C Inner lateral view.

external margin: weakly sinuous from R_4 to CuA_2 ; internal margin: convex from base to 2A. Costal vein (C) dilated until slightly distad to costal margin. At the wing base, between the costal sclerite and proximal median plate, a single vein bifurcates, the first branch forming Sc, which remains unbranched through the distal 1/4 of the costal margin. R parallel to Sc, separating into R_1 and Rs at the end of discal cell. Rs divided into the following branches: R_2 , R_3 , R_4 , and R_5 ; R_2 , R_3 , R_4 reaching the costal margin, R_3 adjacent to R_4 , reaching the apex of forewing; R_5 ending at the external margin.

Areola, on apical part of discal cell, delimited by the common branch from R_2 and by dcs. Discal cell elongate, extending slightly beyond half of the wing, delimited by the upper discocellular (dcs), median discocellular (dcm), lower discocellular (dci), and medio-cubital (m-cu); dcs forming an oblique angle, dcm sinuous, dci, and m-cu slightly convex. Median veins (M) independent after discal cell, M_2 much closer to M3 than to M1. Cubital veins (CuA) starting on the distal upper portion of median plates and bifurcating on the second half of the discal cell; branch CuA₁ forming the distal

Fig 18 Helicoverpa armigera, thorax in dorsal view.

margin of discal cell, reaching m-cu and extending through the external margin; branch CuA₂ continuing along the anal angle of the wing. Vein 2A starting at the distal, lower end of the median plates and ending on the internal margin, close to the anal angle. Vein 3A starting at the proximal part of the lower median plate, projecting tenuously through the end of the first 1/3 of 2A, and then merging with 2A. Retinaculum on ventral surface consisting of wing coupling mechanism; located close to and above Sc in males (Fig 23A), and below CuA in females (Fig 23B).

Hind wings with 8 longitudinal veins (C, Sc+R₁ Rs, M, CuA, A) and 2 crossveins (dcm and dci), outline convex, slightly sinuous on external margin. Costal margin from the base through Sc+R₁; external margin from Sc+R₁ through 2A; internal margin from base through 3A. Frenulum at the base of costal margin, a single thick seta in males, two setae in females (Fig 23A, B). Vein C dilated, short, half of the size of frenulum, part of the base of costal sclerite. Subcostal+R₁ (Sc+R₁) starting at the distal end of costal sclerite and partially from median plates, dilated, curving towards costal margin, ending on wing apex. Radial sector (Rs) basally very close to $Sc+R_1$, diverging after the second 1/3 of the discal cell, ending next to Sc in the external margin. Vein M1 starting at upper part of cell, while M2 is vestigial in males and absent in females; M3 starting at lower part of cell. Discal cell closed by dcm and dci in males, and by the fusion of these two crossveins in females. CuA emerging from median plates, running through the end of the discal cell, then bifurcating into CuA1 and CuA2. Veins 2A and 3A articulating with median plates and third axillary sclerite, the former ending at the external margin and the latter ending on the anal angle.

Fig 19 *Helicoverpa armigera,* thorax in ventral view.

Legs

Prothoracic leg sexually dimorphic, with a spiny area on the internal surface in males (Fig 25B) which is absent in females.

Coxa elongate, cylindrical, articulating basally with the propleuron and distally with the trochanter, which is triangular, 1/5 coxa length. Femur cylindrical, the largest leg segment. Tibia slightly shorter than 1/2 femur length, with few spines and an apical tibial spur; epiphises on upper 1/2 approximately 1/2 length of tibia. Tarsi with five tarsomeres, the proximal one as long as the tibia, all others about the same size, the sum of all four slightly longer than length of the basal tarsomeres. All tarsomeres with spines present on internal and lateral surfaces. Tarsal claw bifid, with long, curved projections, the internal projection slightly shorter; unguitractor plate with cushionlike arolium distally (Fig 24A).

Meso- and metathoracic legs similar. Coxa longitudinally divided by the coxal suture into an anterior eucoxa and

Fig 20 Helicoverpa armigera, thorax in lateral view.

Fig 21 Wing base sclerites of *Helicoverpa armigera*. **A** Forewing in dorsal view. **B** Hind wing in dorsal view.

Fig 22 Wings of Helicoverpa armigera. A Forewing. B Female hind wing. C Male hind wing.

Fig 23 Frenulum and retinaculum of *Helicoverpa armigera*. **A** Female. **B** Male.

Fig 24 Legs of Helicoverpa armigera. A Prothoracic leg. B Mesothoracic leg of female. C Mesothoracic leg of male. D Metathoracic leg.

Fig 25 *Helicoverpa armigera*. **A** Tympanum. **B** Spines on the male prothoracic femur.

posterior meron. Trochanter as in prothoracic, but longer. Mesothoracic femur longer than metathoracic femur, both elongate. Mesothoracic tibia in females with two distal spines and in males with a row of spines and a pair of spurs (Fig 24B, C). Metathoracic tibia in both sexes with lateral spines and two pairs of spurs, the proximal pair inserted slightly posterior to middle of tibiae and the distal pair next to the articulation with the tarsus. Mesothoracic tarsus with

Fig 26 Abdomen of Helicoverpa armigera. A Male, lateral view. B Female, lateral view. C Male, ventral view. D Female, ventral view.

numerous spines laterally and internally, with five tarsomeres, proximal tarsomeres larger, distal tarsomeres decreasing in size. Metathoracic tarsus longer than mesothracic (Fig 24B–D).

Abdomen

Pregenital segments

In both males and females, pregenital segments constituted by tergum and sternum, both sclerotized, and membranous pleura. Intersegmental membranes and pleura more developed and sclerites smaller in females. Male with eight apparent segments, the ninth and tenth modified into genitalia. Females with seven apparent segments, the eighth, ninth, and tenth modified into genitalia. Spiracles along the abdomen are elliptical from segments 1 through 7, the first covered by the tympanic plate, which is located between the tergopleural plate and sternum I+II. Sternum I is reduced and fused with sternum II, changing the shape of the abdominal base (Fig 26A–D).

Male genitalia

Tegumen narrow, articulating posteriorly with the uncus and anteriorly with the arm of tegumen and arm of saccus. Uncus long, hook-shaped, its apical portion covered with setae. Gnathos linked to tegumen by a tenuous membrane, projected posteriorly towards uncus apex. Saccus round, with dorsal arms fusing with the ventral arms of tegumen (Figs 27A, B and 28A). Valve approximately 6× longer than wide, flattened medial-laterally; external margin of distal region projecting dorsally with two rows of small spines forming a corona. Costa internally narrow, not reaching margin of corona; sacculus wide anteriorly (Fig 28B, C). Upper and lower fulturas slightly sclerotized, associated with valves, supporting the aedeagus. Aedeagus cylindrical, enclosed in the membranous manica. Bulbus ejaculatorius opening dorsal, elongated, proximal. Vesica helicoidal, distal, with 11 sclerotized tack-like cornutti, the distal larger (Fig 28D-G).

Female genitalia

Anterolateral margin of eighth tergum slightly curving anteriorly, forming an anterior apophysis. Same region in ninth tergum forming the posterior apophysis. Both apophyses narrow, the anterior one slightly curved, the posterior one straight, slightly shorter. Analis papilla weakly sclerotized, covered by elongated scales (Fig 29B). In ventral aspect, antivaginalis lamella laterally fused to postvaginalis lamella. Postvaginalis lamella twice as long as antevaginalis. Ostium opening between lamellae, at opening of bursa copulatrix (Fig 29A). Bursae copulatrix membranous, with four signa: the dorsal one smaller, suboval; the others elongate, twice as large; all with microspines present ventrally. Spermatheca helical, twice as long as bursae. A sclerotized plate links the spermatheca to the bursae duct in its distal half (Fig 29A, B).

Discussion

Biology

Several heliothine moths are considered economically important agricultural pests due to the damage they can cause to crops worldwide (Matthews 1991; Scoble 1992; Kitching & Rawlins 1998). Adult emergence, feeding, and oviposition occur at night. Larvae can feed during the day or night, regardless of instar, as previously described in other *Helicoverpa* species (Callahan 1958; Hardwick 1965).

Noctuid species share certain oviposition behaviors, such as ovipositing singly or in clusters, or in egg masses that are multiple-layered. Eggs are covered with secretions, scales, or simply devoid of coating. Eggs vary in size, shape, and structure (Peterson 1961, 1964; Gomez Rolim *et al* 2013). Egg morphology and oviposition behavior described herein for *H. armigera* are consistent with what has previously been observed in other species of Noctuidae.

Larval cannibalism has been described in species of *Schinia* (Hübner, 1818) and in the Brazilian native species *H. zea* (Boddie, 1850) after the fourth instar (Hardwick 1965; Jones 1880; Body *et al* 2008). This behavior, however, was not observed in *H. armigera* beyond the first instar. Instead, whenever pairs of larvae from any instar were placed in a shared container, one would prevent the other from feeding, starving it to death.

Helicoverpa armigera larvae are considered polyphagous as they can feed on several different host plants, demonstrating high levels of nutritional adaptability. This study corroborates that no significant difference in eggs, larvae, pupae, or adults are observed in laboratory-reared individuals that were fed natural compared to artificial diets (Hardwick 1965; Fitt 1989; Hamed & Nadeem 2008; Ali *et al* 2009; Assemi *et al* 2012).

Lepidopteran species exhibit remarkable variation in pupation strategies, which may relate to different survival mechanisms during this stage. The last instar digs into the soil to pupate, a behavior described for all *Helicoverpa* species and could be a style to increase survival. Pupation in the soil was observed in both field crop and reared specimens (Hardwick 1965; Karim 2000; Ali *et al* 2009). A similar behavior has also been reported in species of *Schinia* (Hardwick 1958). The presence of conspicuous spiracles facilitates breathing while underground and is associated with burrowing habits (Angulo *et al* 2006).

Fig 27 Male genitalia of *Helicoverpa armigera*. **A** Anterior view. **B** Posterior view.

Immature stages

Characters in the immature stages, such as the presence of spicules in the larvae and the position of L2 and L2 in T1, are major synapomorphies of Heliothinae (Hardwick 1958; Kitching & Rawlins 1998). These features were also observed in *H. armigera*, whose larvae exhibit a larger number of spicules in early instars that decrease as the larva develops, as well as a similar location of setae L1 and L2 on T1 in agreement with the description provided by Mitter *et al* (1993) for all instars. Immatures share additional morphological features, such as the presence of well-defined bands and spicules along the body, conspicuous chalazae, and unbranched setae lacking ornation. This tends to hinder the rapid and accurate differentiation of *Helicoverpa* species in the field.

The brown color pattern of third instar larvae is also observed in other congeneric species such as *H. punctigera* (Wallengren, 1860), *H. gelotopoeon* (Dyar, 1921), and *H. zea.* Other species, such as *H. hawaiiensis* (Quaintance & Brues, 1905) and *H. assulta* (Guenée, 1852), are mostly green (Hardwick 1965). The two-color pattern observed in

Fig 28 Male genitalia of *Helicoverpa armigera*. **A** Lateral view. **B** Valvae in external view. **C** Valvae in inner view. **D** Aedeagus in dorsal view. **E** Aedeagus in lateral view. **F** Aedeagus in ventral view. **G** Vesica in dorsal view.

the sixth instar of *H. armigera* had not been reported for other species in this genus to date. This merits careful investigation, as it might be associated with sexual dimorphism.

The presence of an exocrine jugular gland in the last instar larvae of *H. armigera* agrees with previous contributions concerning other species of Noctuidae, as well as other Lepidoptera families, such as Hesperiidae, Notodontidae, Nymphalidae, and Pieridae. These glands act as a defense mechanism (Peterson 1962, Borges *et al* 2010, James *et al* 2012, Vegliante & Hasenfuss 2012).

Fig 29 Female genitalia of Helicoverpa armigera. A Ventral view. B Lateral view.

The absence of abdominal legs on A3 in the trifine subfamilies is associated with foraging, as it facilitates crawling long distances, and does not match the lepidopteran ground plan, in which abdominal legs are present on A3 through A6 in all larval instars (Kitching & Rawlins 1998). Even though *H. armigera* has great movement potential within the host plant search scenario, the presence of abdominal legs on A3 contrasts with previous morphological characterizations of some trifine subfamilies.

Adults

There are a variety of abiotic factors in Brazil that can favor the dispersal of species with high reproductive capacity, which directly affects economically important crops. *Helicoverpa armigera* adults are notable due to their robust morphological structures, such as a wide thorax, which enables them to fly long distances (Feng *et al* 2004, Ali *et al* 2009), an ability that can certainly help them colonize new environments.

Most noctuids have filiform antenna that are similar in general structure. These structures have several kinds of sensory setae, which can capture information on temperature, humidity, olfactory, and sexual pheromones (Jefferson *et al* 1970; Hardwick 1970; Castrejón-Gómez *et al* 1999; Seada 2015). No morphological differences were found between male and female antennae, an aspect also observed by Diongue *et al* (2013) in their study of types and functions of sensory setae of antennomeres. However, electron microscopy analyses revealed structures such as the ones on the

last flagellomere (Fig 18), which lacks setae, indicating a possible sensory function.

Certain insects have tympanic organs located in various parts of their exoskeleton which can perceive acoustic information. The tympanic membranes of some moth species are fused to the metathorax and are located adjacent to the anterior margin of the abdomen, with an aperture that is sometimes partially covered by a "lid"-like membrane (Forbes 1916; Kiriakoff 1963; Hoy & Robert 1996; Mhatre *et al* 2009). A tympanic sclerite covers the tympanic orifice in *H. armigera*, and a tympanic plate also covers the first abdominal spiracle.

Differences in the number of setae on the frenulum in males and females have also been described in Tortricidae (Yang & Brown 2009; Rota *et al* 2009; Monsalve *et al* 2011) and *Pseudobiston pinratanai* Inoue, 1994 (Geometridae) (Rajaei *et al* 2015). In *H. armigera*, only one seta is present in males, while females have a pair of setae. Besides the number of setae on frenulum, their position on the retinaculum also differs, a feature that is helpful in sex determination.

The sclerotized cornutti in male *H. armigera* vesica are also observed in other species of the same genus, as well as a few other species of Heliothinae (Hardwick 1965, 1970).

The position and presence of four signa in the bursa copulatrix of female *H. armigera* have also been observed in *Helicoverpa atacamae* Hardwick, 1965, *H. hawaiiensis*, and *H. punctigera*. However, the pattern is distinct from *H. gelotopoeon* (Hardwick 1965).

This contribution offers the first detailed morphological study of the economically important noctuid species, *H. armigera*. Several morphological structures described herein will contribute to the taxonomic knowledge of this

species and will also be useful for distinguishing males and females. Tools for the accurate identification of this economically significant species are fundamental for field work and crop management. Future comparative studies may help differentiate this species from *H. zea*, whose representatives also occur in Brazil and are often confused with *H. armigera* due to close morphological resemblance. A similar case of misidentification with *H. armigera* was shown by Gillian & Passoa (2014) for *H. gelotopoeon*, a species found in southern South America, which is also considered an important economic pest.

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