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MORPHOLOGY OF IMMATURE STAGES AND ADULTS OF *HELICOVERPA ARMIGERA*

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ABSTRACT

***Helicoverpa armigera* (Hübner) is a highly polyphagous insect pest of worldwide occurrence, including India. In the present study, a detailed morphological assessment of *H. armigera* showed the following: typically dome-shaped egg with ribbed surface; larva having coriaceous skin, biordinal crochets and 11 primary setae on the prothoracic segment; pupa adecticous and obtect with prominent posterior tip cremaster; and adult forewings characterized by the presence of 7-8 black spots along the apical margin and a reniform shaped brown marking, more prominent on the underside. Adults exhibit sexual dimorphism in the colour of vestiture and forewings. The genitalic traits like the presence of usually 12 or less sets of cornuti in aedeagus, apically broadened harpe having length ranging between 4.5 to 4.9 mm and a single lobe at the base of everted vesica in males, and four distinct signa on bursa copulatrix in females, also distinguishes it from other congeneric species.**

Key words: *Helicoverpa armigera*, morphology, egg, larva, chaetotaxy, pupa, wings, sexual dimorphism, genitalia

Heliothinae is a small subfamily of noctuid moths that includes some of the major agricultural pests of worldwide importance (Mitchell and Gopurenko, 2016), of which an important one is *Helicoverpa armigera* (Hübner). This pest species has an exceptionally wide geographical distribution and host range (Gomes et al., 2018). In India, it is known to attack around 96 crops, including major cereals, legumes, oilseeds, cotton, and a wide range of horticultural crops (Srivastava and Joshi, 2011). Depending on the crop, *H. armigera* induced damage can lead to 50 to 90% yield loss, inflicting huge monetary loss (Chakravarty et al., 2019). Correct identification of the pest is an essential requisite for devising effective IPM strategies (Chakravarty et al., 2018). The external morphological characters of life stages (Ranjith and Chellappan, 2015), wing colour patterns (Ethier and Despland, 2015), and genitalia morphology (Pogue, 2004; Dias et al., 2010), have been found to be highly informative and useful for species recognition in lepidopteran insects. Though much has been published concerning general morphology, biology and behavioural aspects of *H. armigera* (King, 1994; Ali et al., 2009; Kingsolver et al., 2011; Tang et al., 2016; Queiroz-Santos et al., 2018), studies offering details on key identification features of this species from India are still limited and scattered. Recently, doubts have also been raised regarding existence of unidentified cryptic species (Gill et al., 2015) or different subspecies (Chakravarty et al., 2020) of *H. armigera* in the country, as it exhibits differential responses to various selection pressures. Thus, the present study was undertaken to provide a revised and detailed morphological characterization of all the immature stages and adults of *H. armigera* from India. The information offered here is meant to expedite the accurate identification of this pest species, as it is often confused with other congeneric species. This study will also form a basis to understand the effects of genetic and environmental factors on morphological features, and to capture its intraspecific morphological variations among geographically isolated populations.

MATERIALS AND METHODS

The larvae of *H. armigera* were collected from chickpea fields of the Agricultural Research Farm, Banaras Hindu University during February-March, 2017-2018 and were reared at the Biocontrol Laboratory, Department of Entomology and Agricultural Zoology, Banaras Hindu University, for two consecutive generations, in a digitally controlled insect rearing chamber (Instech Environmental Chamber, Instech Systems, India; 25± 1°C, 65± 5% RH and 12 hr photophase), following standard procedures as described by Chakravarty et al. (2018). All the morphological characters were analyzed from the second filial generation, and illustrations of these, along

with measurements of all stages (egg, larva, moulted head capsule, and pupa), and genitalia were made in a stereozoom microscope with an image analyzer (Leica DM1000). The larvae and adult specimens were killed with ethyl acetate before examination (Queiroz-Santos et al., 2018). The setal arrangement on the prothoracic segment of larvae was studied following Ranjith and Chellappan (2015), and compared with the illustrations of setal arrangements of *H. armigera* by Passoa (2014). The method suggested by Padwal et al. (2018) was followed with minor modifications for dissection of genitalia from both male and female moths and preparation of their slides. All the morphometric measurements are specified based on mean and standard deviation values from 50 randomly selected specimens, using a statistical package (SPSS, Version 16).

RESULTS AND DISCUSSION

Egg: The eggs were typically dome-shaped, circular in cross-section, and with the micropyle being borne on a small mound at the apex of the dome. The surface of the eggs was sculptured in the form of longitudinal ribs. These were gleaming yellowish white when freshly deposited (Fig. 1a). The colour gradually changed during the next 14 to 36 hr to a rather muddy yellow, at which time a brown subequatorial band appeared on the egg (Fig. 1b). Concurrently, or somewhat later, this band darkened. Between 48 and 72 hr after deposition, the whole egg became dark grey (Fig. 1c) as the larva matured within the chorion. The infertile eggs became dark yellow and shriveled after three to four days. Oviposition occurred singly, with female moths preferably selecting soft surfaces for laying eggs. The size varied from 0.51± 0.04 x 0.49± 0.05 mm. Egg morphology and oviposition behaviour described herein for *H. armigera* are consistent with what has previously been observed by King (1994), Deepa and Srivastava (2010), Gomez-Rolim et al. (2013) and Queiroz-Santos et al. (2018).

Larva: The larval stage had six instars, of which the first was translucent creamish white and lacked prominent markings; their head, prothoracic and supra-anal shields, thoracic legs, spiracles and setal bases were black and thereby giving them a spotted appearance (Fig. 1d), as also observed by Bhatt and Patel (2001). The larva became segmentally marked with the end of the stadium. The second instar resembled the first in appearance, but the dermal spinulation was more pronounced (Fig. 1e). In the third instar (Fig. 1f), the abdominal prolegs became fully developed on third to

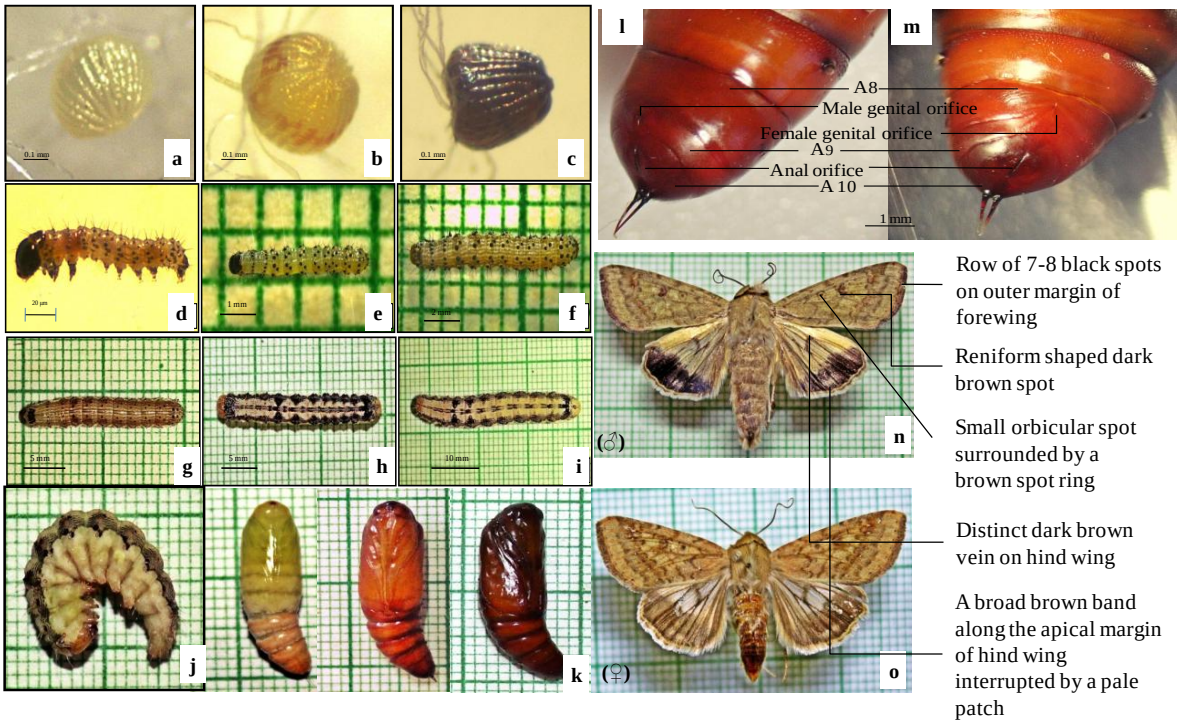

Fig. 1. Life stages of *H. armigera*, a-c: Egg; d: First instar; e: Second instar; f: Third instar; g: Fourth instar; h: Fifth instar; i: Sixth instar; j: Pre-pupa; k: Colour change in pupa; l: Posterior region of male pupa in ventral view; m: Posterior region of female pupa in ventral view; n: Male moth; o: Female moth

sixth and tenth abdominal segments, and these remained until last larval instar. In the fourth instar (Fig. 1g), the dorsal tubercles on the first abdominal segment formed a distinct "saddle-like" structure. In the penultimate (fifth) larval stadium (Fig. 1h), the two colour phases (green and brown) became distinctly evident and the general macular pattern became better defined or complex. These were also found responding to external disturbances by raising their head and thoracic segments (Chakravarty et al., 2018). The two colour-patterns observed in *H. armigera* larvae had not been reported for other species in this genus to date (Queiroz-Santos et al., 2018). In the final (sixth) instar (Fig. 1i), an abrupt change in body colour occurred. Head was pale green or orange, often with white reticules. The trunk showed a variety of shades of brown, yellow, green, and even black colouration (Chakravarty et al., 2020). The larval skin was coriaceous, and the crochets were typically biordinal. The chalazae of the first abdominal segment and spiracular rim of the eighth abdominal segment were twice the size of others (Fig. 2). The body length and width of first, second, third, fourth, fifth and sixth instar larvae was recorded as 1.68± 0.07 and 0.46± 0.04 mm, 3.94± 0.05 and 0.72± 0.04 mm, 8.65± 0.13 and 1.61± 0.06 mm, 15.40± 0.24 and 2.95± 0.05 mm, 24.52± 0.21 and 3.36± 0.07 mm, and 30.76± 0.33 and 4.72± 0.08 mm, respectively. The head capsule width of corresponding stages were 0.32± 0.03 mm, 0.48± 0.04 mm, 0.71± 0.06 mm, 1.22± 0.07 mm, 1.87± 0.11 mm, and 2.70± 0.10 mm. The present findings are in accordance with earlier reports of Deepa and Srivastava (2010), Gill et al. (2015), and Chakravarty and Srivastava (2020).

Chaetotaxy: Most of the formal keys for the identification of lepidopterans at larval stage rely heavily on chaetotaxy, particularly primary setae (Ranjith and Chellappan, 2015). In the present study,

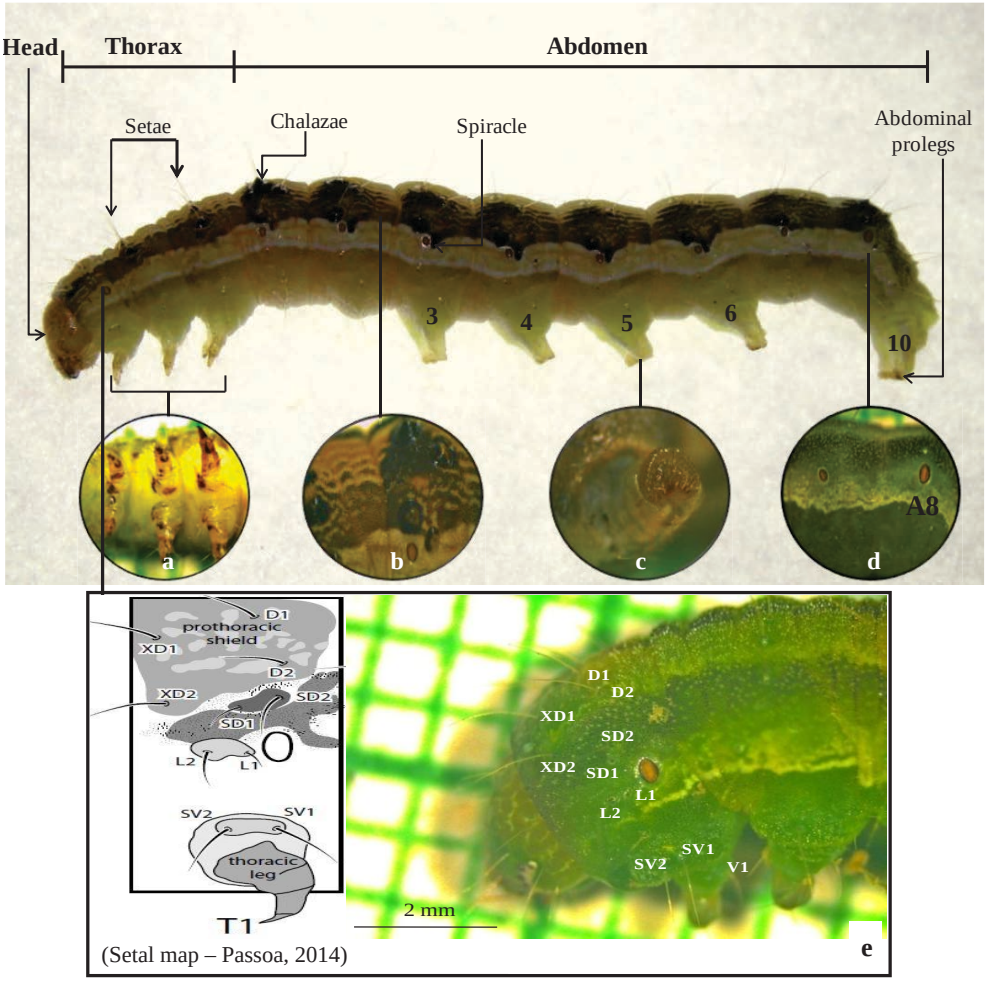

Fig. 2. Lateral view of *H. armigera* larva (sixth instar), a: Thoracic legs, b: Coriaceous skin, c: Biordinal crochets, d: Spiracular rim of the eighth abdominal segment (A8) twice the size of others; e: Setal arrangement on the prothoracic segment

11 unbranched primary setae were observed on the prothoracic segment that included two each of dorsal (D1, D2), subdorsal (SD1, SD2), lateral (L1, L2), subventral (SV1, SV2) and additional (XD1 and XD2) setae, along with one ventral seta (V1) (Fig. 2). These setae have specific names based on their positions. The additional setae exist near to the anterior margin of the pronotal plate. The dorsal setae were situated posterolateral to their respective additional setae. Both the subdorsal setae lied near the lateral margin of the pronotal plate, and the lateral setae are anterior and horizontally aligned to the prothoracic spiracle. The two subventral setae lied above coxa while, the single ventral seta was post coxal in position and most ventrally located. The same arrangement was observed in all instars. These observations are in accordance with Passoa (2014) and Ranjith and Chellappan (2015), who prepared diagnostic keys based on the above characters to distinguish *H. armigera* from related species. The position of seta on the prothoracic segment observed in the present study was also in consonance with Singh and Goel (1987) and Sri et al. (2010) who described the taxonomy of Noctuidae with special reference to immature stages.

Prepupa: The last instar larva after its complete development, did not moult but was contracted and shortened into a grub-like prepupa (Fig. 1j). Its mean length and width were 22.78± 0.25 mm and 5.50± 0.09 mm, respectively, and it had pale yellowish green or yellowish brown body, as also observed by Deepa and Srivastava (2010) and Chaudhary et al. (2016). This stage digs into the soil to pupate; a behavior described for all *Helicoverpa* spp., and could be a style to increase survival (Queiroz-Santos et al., 2018).

Pupa: The pupa was adecticous, obtect, rounded at ends, smooth textured and edges of segments well marked. Newly formed ones were yellowish green, which became mahogany brown within few hours of its formation and further darkened prior to adult emergence (Fig. 1k). The pupa measured 18.56± 0.19 x 6.42± 0.15 mm, corroborating with Ali et al. (2009) and Chaudhary et al. (2016). In ventral view, a goblet like frontoclypeus, triangular shaped hypopharynx, and lance-shaped galea, covering a significant portion of the anterior half were easily noticeable. Legs were placed along the antenna towards the galea in repose. Hind wings were placed over forewings, both ending above the anterior margin of the fifth abdominal segment. Dorsally prothorax was small and triangular; mesothorax and metathorax projecting latero-ventrally, forming the pterotheca. The abdomen had ten segments with elliptical and conspicuous spiracles, between the second and seventh segment, and reduced in the eighth one. A posterior-tip cremaster in the form of two tapering parallel spines was borne on the tenth abdominal segment terminus. An elongate anal opening scar was also located on the distal ventral area of the tenth abdominal segment. Female genital pore was located medioventrally on the eighth abdominal segment while, the male genital opening was situated on the ninth segment, surrounded by small protuberances (Fig. 1 l,m), as also observed by Gill et al. (2015) and Queiroz-Santos et al. (2018).

Adult: The adults were stout bodied moths with broad thorax. Antennae were of the filiform type, not sexually dimorphic and covered with fine hairs, an aspect also observed by Diongue et al. (2013). Male and female moths were readily distinguishable based on colouration of vestiture and forewings. In males, they were greenish brown while, female moths had orange brown to dark brown vestiture and forewings (Fig. 1n,o). The forewings of both sexes were also characterized by the presence of brown, broad, and irregular transverse bands and seven to eight black spots along the apical margin. A dark brown, reniform shaped marking was also present on the forewing of each sex, more prominent on the underside. The hind wings of both sexes were dull creamish with a broad dark brown border at the apical end, interrupted by a pale patch. Males and females also had identical venation patterns. Each of the forewings had 14 longitudinal veins (C, Sc, R_1-R_5, M_1-M_3, CuA_1, CuA_2, 2A, 3A) and four cross veins (dcs, dcm, dci, m-cu) (Fig. 3a). Hind wings with eight longitudinal veins (C, Sc + R_1, Rs, M, CuA, A) and two cross veins (dcm, dci) (Fig. 3b). Wingspan was 39.82± 0.28 mm, and the males were usually smaller than females, as also reported by Nylin and Gotthard (1998) and Brambila (2009).

Male genitalia: The various parts of male genitalia observed were uncus, harpe, corona, vinciculum, saccus, corpus genitalis, aedeagus, and cornuti (Fig. 4). Uncus was small, cylindrical and hook-like towards its tip. Vinculum was V-shaped and the paired clasping organ (harpes or valvae) articulated from its caudal margin. The length of apically broadened harpe ranged between 4.5 to 4.9 mm, which was consistent with what has previously been observed by Pogue (2004). It also had numerous rows of closely set spines anteriorly (corona). Saccus was short (0.57± 0.04 mm), bell-shaped with curved apical portion. The length of corpus genitalis was 3.28± 0.07 mm. Aedeagus was 4.46± 0.06

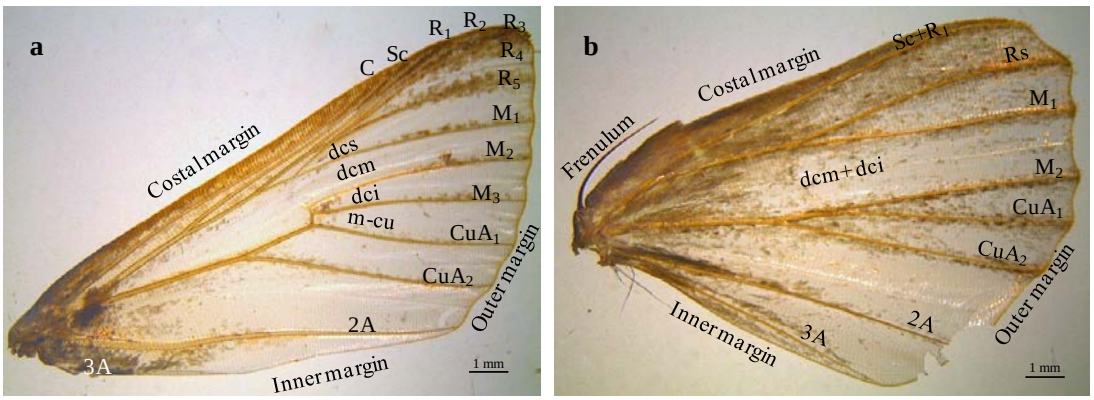

Fig. 3. Wing venation of *H. armigera*, a: Fore wing; b: Hind wing; Costa (C), Subcosta (Sc), Radius (R), Media (M), Cubitus (Cu), Anal (A), Upper discocellular (dcs), Median discocellular (dcm), Lower discocellular (dci), Medio-cubital (m-cu)

mm long, weakly sclerotized structure with usually 12 to 14, or less sets of cornuti (sclerotized spines) inside it, in accordance with Krinski and Godoy (2015). However, Brambila (2009) reported that presence of 12 or fewer sets indicating that the specimen "could be" *H. armigera*, while more than 15 sets indicates it to be "probably" *H. zea*. The long spiral tube called vesica was also partially everted out from the aedeagus, and only a single lobe or diverticula was found at its base.

Female genitalia: The various parts of female genitalia observed were ovipositor, anterior and posterior apophysis, ductus bursae, bursa copulatrix, and bursa seminalis (Fig. 4). The ovipositor consisted of two curved hairy lobes, hairs probably having sensory function. Anterolateral margin of the eighth tergum extended and formed the anterior apophysis, and the same region in ninth tergum formed the posterior apophysis. Both apophyses were narrow; the anterior one slightly curved while the posterior one was straight and slightly shorter. Bursa copulatrix and bursa seminalis were two distinct diverticula of ductus bursae, and 3.95± 0.02 mm and 6.54± 0.05 mm long, respectively. Bursa copulatrix was membranous with four distinct heavily sclerotized areas (signa) readily visible on its surface, of which, three were long and one short, structurally apposed. Bursa seminalis or spermatheca was helical, sclerotized, twice as long as bursa copulatrix, and it was attached to the bursae duct in its distal half by a sclerotized plate. The observations made herein are in agreement with Hardwick (1965), and Ranjith and Chellappan (2015).

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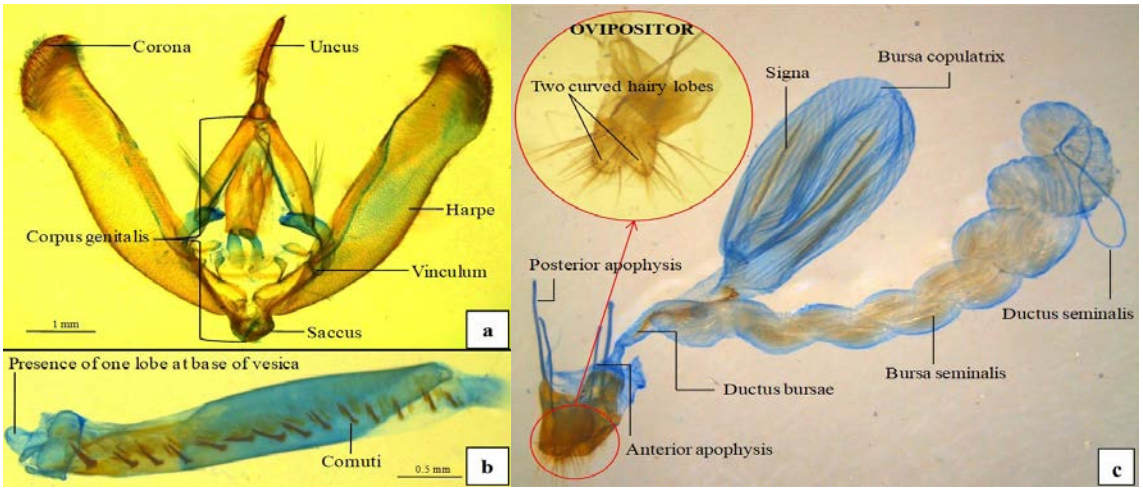

Fig. 4. Genitalia of *H. armigera*, a: Entire male genital structure excluding aedeagus; b: Aedeagus with uninflated vesica; c: Female genitalia

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REFERENCES

Ali A, Choudhury R A, Ahmad Z, Rahman F, Khan F R, Ahmad S K. 2009. Some biological characteristics of *Helicoverpa armigera* on chickpea. Tunisian Journal of Plant Protection 4: 99-106.

Bhatt N J, Patel R K. 2001. Biology of chickpea pod borer, *Helicoverpa armigera*. Indian Journal of Entomology 63: 255-259.

Brambila J. 2009. Instructions for dissecting male genitalia of *Helicoverpa* (Lepidoptera: Noctuidae) to separate *Helicoverpa zea* from *Helicoverpa armigera.* USDA-APHIS-PPQ. Accessed July 5, 2017.

Chakravarty S, Padwal K G, Srivastava C P. 2020. Larval body colour polymorphism in *Helicoverpa armigera* (Hübner) populations across India. Indian Journal of Entomology 82(3): 415-422.

Chakravarty S, Srivastava C P, Keval R. 2018. Biology of *Helicoverpa armigera* (Hübner) on chick pea-based artificial diet under laboratory conditions. Annals of Plant Protection Sciences 26(2): 265-269.

Chakravarty S, Srivastava C P, Keval R. 2019. Interbreeding status of *Helicoverpa armigera* (Hübner) populations across India. Journal of Experimental Zoology, India 22(1): 315-320.

Chakravarty S, Srivastava C P. 2020. Age specific variability in the larval head morphometry of *Helicoverpa armigera* (Hübner) populations across India. Journal of Experimental Zoology, India 23(1): 329-333.

Chaudhary M G, Chaudhary H K, Chaudhary R J. 2016. Biology of *Helicoverpa armigera* (Hübner) Hardwick on okra in the laboratory condition. Advances in Life Sciences 5(12): 5032-5037.

Deepa M, Srivastava C P. 2010. Biological characteristics of *Helicoverpa armigera*. Annals of Plant Protection Sciences 18: 370-372.

Dias F M S, Casagrande M M, Mielke O H H. 2010. Alternative techniques to study characters of the genitalia in Lepidoptera. Neotropical Entomology 39(6): 1044-1045.

Diongue A, Yang J, Lai P. 2013. Biomorphometric characteristics of different types of sensilla detected on the antenna of *Helicoverpa armigera* by scanning electron microscopy. Journal of Asia-Pacific Entomology 16(1): 23-28.

Gill A K, Arora R, Pathania P C. 2015. Morphological characterization of *Helicoverpa armigera* (Hübner) populations from various hosts in Punjab. Agricultural Research Journal 52: 73-78.

Gomes E S, Santos V, Ávila C J. 2018. Biology and fertility life table of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in different hosts. Entomological Science 20: 419-426.

Gomez-Rolim A A S G, Yano S A C, Specht A, Andrade C G T J, Sosa-Gómez D R. 2013. Morphological and molecular characterization of the eggs of some noctuids associated with soybean in Brazil. Annals of Entomological Society of America 106(5): 643-651.

Hardwick D F. 1965. The Corn Earworm Complex. Memoirs of the Entomological Society of Canada 97(40): 1-247.

King A B S. 1994. *Heliothis/ Helicoverpa* (Lepidoptera: Noctuidae). Mathews G A, Turnstall J P. Insect pests of cotton. CAB International, Wallingford, U.K. 39-106 pp.

Kingsolver J G, Woods H A, Buckley L B, Potter K A, Maclean H J, Higgins J K. 2011. Complex life cycles and the responses of insects to climate change. Integrative and Comparative Biology 51(5): 719-732.

Krinski D, Godoy A F. 2015. First record of *Helicoverpa armigera* (Lepidoptera: Noctuidae) feeding on *Plectranthus neochilus* (Lamiales: Lamiaceae) in Brazil. Florida Entomologist 98(4): 1238-1240.

Mitchell A, Gopurenko D. 2016. DNA Barcoding the Heliothinae (Lepidoptera: Noctuidae) of Australia and utility of DNA Barcodes for pest identification in *Helicoverpa* and relatives. Plos One 11(8): e0160895.

Nylin S, Gotthard K. 1998. Plasticity in life history traits. Annual Review of Entomology 43: 63-68.

Padwal K G, Sharma S K, Singh S K. 2018. Dissection and slide mounting technique for male and female genitalia of *Leucinodes orbonalis*. Journal of Entomological Research 42(2): 259-262.

Passoa S C. 2014. Identification guide to larval Heliothinae (Lepidoptera: Noctuidae) of quarantine significance. Gilligan T M, Passoa S C. LepIntercept, An identification resource for intercepted Lepidoptera larvae. Identification Technology Program (ITP), USDA/APHIS/ PPQ/S&T, Fort Collins, CO. 19 pp. Accessed at www.lepintercept.org.

Pogue M G. 2004. A new synonym of *Helicoverpa zea* (Boddie) and differentiation of adult males of *Helicoverpa zea* and *Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae: Heliothinae. Annals of the Entomological Society of America 97(6): 1222-1226.

Queiroz-Santos L, Casagrande M M, Specht A. 2018. Morphological charcaterization of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae). Neotropical Entomology 47(4): 517-542.

Ranjith M T, Chellappan M. 2015. DNA barcoding compliments morphological identification in tomato fruit borer, *Helicoverpa armigera* (Hübner). Entomon 40(3): 125-138.

Singh G P, Goel S C. 1987. Body chatotaxy of three noctuid caterpillars (Lepidoptera). Deutsche Entomologische Zeitschrift 34(4-5): 373-381.

Sri I A, Rao V R, Sekhar P R, Chalam M S V. 2010. Taxonomic studies on different lepidopteran caterpillars on cotton, chilli and pulses. Annals of Plant Protection Sciences 18: 104-107.

Srivastava C P, Joshi N. 2011. Insect pest management in pigeonpea in Indian scenario: A critical review. Indian Journal of Entomology 73(1): 63-75.

Tang T, Zhao C, Xu L, Qiu L. 2016. Factors affecting larval cannibalism in the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Oriental Insects 50(1): 23-33.

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