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Zahid Mahmood Sarwar
Department of Entomology,
Bahauddin Zakariya University,
Multan, Pakistan

Muhammad Razaq
Department of Entomology,
Bahauddin Zakariya University,
Multan, Pakistan

Sarfraz Ali Shad
Department of Entomology,
Bahauddin Zakariya University,
Multan, Pakistan

Waqar Jaleel
College of Agriculture, South
China Agricultural University,
Guangzhou 510642, China

Syed Arif Hussain Rizvi
College of Agriculture, South
China Agricultural University,
Guangzhou 510642, China

Muhammad Faheem Khalid
Department of Entomology,
Bahauddin Zakariya University,
Multan, Pakistan

Taxonomic studies of Genus *Helicoverpa* Hardwick with new record of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) from Pakistan

Zahid Mahmood Sarwar, Muhammad Razaq, Sarfraz Ali Shad, Waqar Jaleel, Syed Arif Hussain Rizvi and Muhammad Faheem Khalid

Abstract

Helicoverpa armigera (Hubner) and *Helicoverpa zea* (Boddie) are very important pests of many crops. *H. zea* was formerly known from western regions. To study the Taxonomy of both species, the specimens of genus *Helicoverpa* were collected from Multan, Pakistan using light traps. These species are identified on the basis of male genitalia and other morphological characters. This is the first species i.e. *H. zea* of the genus *Helicoverpa* recorded from Pakistan. Diagnostic characters and dichotomous identification key to both species are provided.

Keywords: *Helicoverpa armigera*, *Helicoverpa zea*, male Genitalia, taxonomy and first record

Introduction

The polyphagia's nature of the Old World cotton bollworm *Helicoverpa armigera* (Hubner) originated from Oriental region however now it is one the most devastating pest in all subcontinents [5] and [7]. Other bollworm *Helicoverpa zea* (Boddie) [3] is commonly known as European corn worm. *H. armigera* is a very close relative of the corn earworm; Both *H. zea* and *H. armigera* are physiological variable and cannot be identified consistently without genital dissection. [11]. In the comprehensive monograph of [7] on the genus *Helicoverpa*, the key to adults is based on male and female genitalic characters. Therefore, study on the base of dissection is necessary to accurately identify adult males of both species. The purpose of this paper is to provide an efficient procedure for the differentiation of *H. zea* and *H. armigera* males using several diagnostic genitalic characters.

Helicoverpa Hardwick [7] was originated by [7]. Later on [15] spread *Helicoverpa* as synonym of *Heliothis* Oshsenheimer. In the beginning *H. zea* and *H. armigera* has been treated as single wide spread species and included under genus *Heliothis* [9] [6]. This synonymy was due to very similar morphological appearance of larvae and adult of both species. After this, for reliable identification and confirmation of this synonymy [4] and [12] described genital structures of *H. zea* and *H. armigera*. They identified that the genitalic structures have significance deference in various groups of family Noctuidae. Classification, identification and construction of keys are possible only after differentiate between these two similar species [11] restricted only to new world.

Though, many studies has been conducted only on the base of morphology and damage of bollworms of genus *Helicoverpa*, but there are no taxonomic studies conducted on the *Helicoverpa* species of the region, so, in this region like pakistan, the name *H. armigera* have been used as synonymous for *H. zea*. [13] [1] [14] [2]

To overcome this issue and gap, the present study has been carried out from Punjab, Pakistan for first time on taxonomy of the genus *Helicoverpa*. Here we report presence of *H. zea* in Punjab, Pakistan; we also provide diagnostic characters and dichotomous key for further identification.

Materials and Method

The specimens were collected from District Multan, the southern region of Punjab, Pakistan, during 2015-2016 with the help of light traps. During the study (2015-2016) 198 samples of the genus *Helicoverpa* were examined and identified. Different sites of Multan district were selected for specimen's collection. After collection by light trap, the specimens were killed by

Correspondence

Zahid Mahmood Sarwar
Department of Entomology,
Bahauddin Zakariya University,
Multan, Pakistan

potassium cyanide poison. Then these specimens were pinned, stretched and preserved properly in insect wooden boxes. Forewings and hind wings of each species were separated from the body of an adult moth by giving upward jerk. After the separation both wings were dipped into 70% alcohol for 2-3 minutes, then placed in sodium hypochlorite or bleaching agent for 15-20 minutes depending upon the magnitude of the moths for descaling, later on transferred both wings into glacial acetic acid for 15 minutes and finally mounted these wings on a glass slides with Hoyer's medium. For the study of male genitalia the abdomen of specimen was detached from the body and dipped overnight in 10% KOH solution to get the musculature sufficiently relaxed. Then KOH was removed from genitalia by washing in distilled water for 2 or 3 times. For appropriate dryness and clearance, the male genitalia was dipped in different concentrations of alcohol, 50%, 60%, and 70% for 15 minutes respectively. After this, the genitalia were also cleared in clove oil. The dissection was done with the help of needles and fine forceps under an Olympus SZ61 binocular stereoscope microscope in 75% alcohol. For appropriate identification and classification the labeling of male genitalia was done by following the terminology of [10]. The photography of male genitalia was prepared with the help of Olympus digital camera (DPX 200). Dichotomous key was also provided. All the research material was deposited in to the department of entomology Bahauddin Zakariya University Multan, Pakistan.

Result and Discussion

Discussion

The present research work deals with the taxonomic studies of *Helicoverpa* moths from Multan, Pakistan. Morphological details of the genitalia were considered as a reliable tool for species identification.

Laboratory procedure

The precise morphology of genitalia depends upon particular techniques; details of the techniques adopted are given below.

1. Firstly the abdomen was detached by holding the specimen stiff, by breaking the joint between thorax and abdomen with forceps to giving up ward jerk. It was examined practically to use the complete abdomen of specimen because some of the genitalia extend anteriorly almost up to the thorax, specifically in the female genitalia.
2. KOH was used to soften the abdomen and other body parts. Abdomen was dipped in a 10%, KOH solution for 24 hours or boiled for few minutes which was more satisfactory. For the removal of KOH, abdomen was dipped in 70% of alcohol and preservative was washed by distal water.
3. The sample was shifted to a cavity dish and the genitalia was removed in alcohol, using dissects forceps and needles under stereo microscope.
4. The genitalia was stained for 10 minutes by immersion i.e. 50%, 60% and 70% alcohol respectively. These stains were used for the clearance of genitalia.
5. The male genitalia was spreaded. Both valvae were spread with the help of needles, and dissection-pins. These were left in a spread position for 20-25 minutes.
6. The genitalia were also cleared in clove oil for 5 minutes.
7. The permanents mounts were necessary for specific study and permanent record. A thin solution of Hoyer medium was used. The specimen was mounted on the glass slides, arranged in the desired position, and covered with cover

slips to preventing from air bubbles in the mount with the help of stereo microscope and micro-pins.

8. The glass slides of specimens were dried in horizontal position, and consequently labeled with details as to the source of the specimens.

Result

Diagnostic Characters

Frons mostly smoothed with a corneous plate below; head and thorax roughly haired; mid and hind tibiae spine in some species; fore tibiae tiny and wide, spines at sides, with one or two long claw on outer side and a short claw or three claws on inner side; the discal cell is more than half as long as fore wing, but in hind wing much shorter than fore wing, veins 3, 4, 6 and 7 on long stalks; forewing long and narrow with prominent apex but hind wing broader and shorter.

Key to the studied species of genus *Helicoverpa* Hardwick

1. Male genitalia valva long with four rows of spines, aedeagus with 10-11 cornuti; Uncus short and simple ... *armigera* [8]
2. Male genitalia with valva longer than above; aedeagus with more than 11 cornuti; Uncus long and arched apically ... *zea* [3]

Helicoverpa armigera (Hubner) [8]

Hubner, 1805 [8], Samml. Eur. Schmett.

In female forewing dark-brown but males have lighter, gray color wings. A dark band present between exterior transversal and sub-marginal outlines transversal lines. A black color spot expressing the reni form. A borderline series of dark spots is present. Hind wings lighter in color, pale-yellow with brown band before outer edge. Black round spot placed in the mid of wing.

Male Genitalia: Uncus small and simple, extensive sclerotized, apex with shrill bristles. Tegumen elongate, pointed distal tops, proximal portion is v-shaped. Vinculum u-shaped. Saccus shorter. Sacculus well developed, elongated, compressed and symmetrical. Valva elongated, highly sclerotized and fringed with thick hairs. Rows of spines are present on corona anteriorly. Juxta is square shaped but divided medially. Aedeagus elongated and consistently sclerotized. Vesica is an eversible sac like. Cornuti usually 10-11 in number which are visible on the swollen vesica within the aedeagus (Figure a-d).

Distribution: Universally distributed.

Helicoverpa zea (Boddie) [3]

Boddie, 1850 [3], Sout. Cultiv.

A sub basal black line present in forewing from costa to vein 1. Black and whitish patches are present in orbicular and Reni form stigmata outlined. A dark dentate mark present below the cell. Hind medial line forked from costa. Hind wing covered with magenta ochreous with long black and whitish patch outside it. A wide black marginal band mostly with whitish spots in the center of margin. On underside the paler, the orbicular and Reni form stigmata are dark black.

Male Genitalia: Uncus elongated and curved apically, tapering, ending with a sharp spine-like bristle. Tegumen long wide; valva elongated. Juxta arched anteriorly and broad posteriorly. Corona fringed with dense row of spines. Vinculum shorter and v-shaped. Saccus small. Aedeagus consistently sclerotized, small and thick. Vesica with more than 11 cornuti (Figure-h).

Remarks: The specimens of genus *H. zea* were collected from District Multan, the southern region of Punjab, Pakistan; during 2015-2016 this species has been described for the first time from Multan, Pakistan.

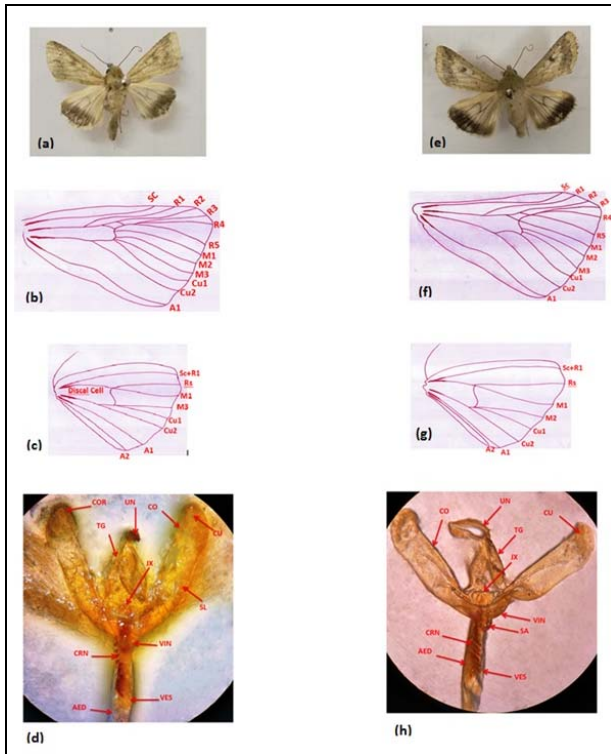


Fig (A-d): *Helicoverpa armigera*: (a) Adult; (b) Fore wing; (c) Hind wing; (d) Male genitalia (E-h) *Helicoverpa zea*: (e) Adult ;(f) Fore wing; (g) Hind wing; (h) Male genitalia

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