



## DIGAMA HEARSEYANA (LEPIDOPTERA: NOCTUIDAE), A NEW THREAT TO KARONDA (*CARISSA CARANDUS*) IN RAJASTHAN

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### ABSTRACT

An infestation of *Digama hearseyana* Moore, 1859 (Lepidoptera: Noctuidae) on karonda, *Carissa carandus*, was first noticed in 2014 as sporadic occurrence at the experimental farm of the ICAR-Central Institute for Arid Horticulture at Bikaner in Rajasthan. The maximum incidence was observed in second fortnight of August and minimum in second fortnight of April during 2014 and 2015. The number of larvae ranged between 1.07 to 5.77 and 1.23 to 5.97 larvae / plant during second fortnight of April and second fortnight of October 2014 and 2015. This species is characterized by the male forewing pale greyish-brown; hind wing testaceous- yellow with a small brown mark on middle margin; abdomen testaceous with a dorsal row of black dots and legs yellowish with dark bands. The mean body length up to genitalia of the male and female adult vary (11.28 mm and 11.28 mm, respectively). Since *D. hearseyana* belongs to a species complex of *Digama* where reliable identification of individual species is very daunting task, DNA barcode based on standard barcoding gene cytochrome oxidase I (COI) of the mitochondrial DNA was developed and subsequently molecular identity established.

**Key words:** *Digama hearseyana*, *Carissa carandus*, incidence, diagnostics, morphometrics, mtCOI, first report. seasonal incidence, damage

In the species complex of *Digama* moths (Noctuidae: Aganainae), the morphological identification of the individual species is very difficult. One of its species, *Digama hearseyana* Moore (1859) is herein reported for the first time as a new threat to the cultivated plant karonda. *Digama hearseyana* is distributed in South Africa, China, throughout India, Sri Lanka, Myanmar and Australia (Smetacek, 2008; Hampson, 1892). Karonda grows naturally in the Himalaya at elevations of 30 to 1,800 m, in the Siwalik Hills, the Western Ghats and in Nepal and Afghanistan. It is cultivated in India on a limited scale in Rajasthan, Gujarat, Bihar, West Bengal and Uttar Pradesh, as well as in the Sri Lanka lowland rain forests. It is a hardy, drought tolerant plant that thrives well in a wide range of soils (Khare, 2007; Lim 2012). The pest status of *D. hearseyana* is unknown and hence this study explores its diagnostics, morphology and mtCOI DNA barcode.

### MATERIALS AND METHODS

The adult specimens were sent to the Division of Entomology, Indian Agricultural Research Institute, New Delhi for identification. The measurements of various body parts of male and female adults were made

under a stereozoom microscope (Radical Instruments, Ambala, Haryana) using Jenoptic Pro 2.8.0 software. The larvae were reared under laboratory conditions ( $28 \pm 2$  °C and  $60 \pm 5$  % RH). The terminology followed is after Haldhar (2012), Haldhar and Singh (2014) and Haldhar et al. (2015). The illustrations were scanned at 600 dpi black and white, and mounted using Adobe Photoshop c8.0. Three years old karonda trees were randomly selected with three replications at the Experimental Farm of the ICAR-Central Institute for Arid Horticulture ( $28^{\circ}06'E$   $73^{\circ}21'$ ) with replications being at different locations. Incidence of *D. hearseyana* was observed on each tree and symptoms of damage (in comparison with uninfested plants) were observed from April to October, 2014 and 2015, respectively. The sampling was done by visual observation and manual counting. Mean incidence was calculated as % of trees infested with and number/ trees calculated.

For DNA extraction larvae were collected from infected karonda plants and reared to adults in the laboratory. Four adults (2 from each location) were used for DNA extraction. Genomic DNA was extracted from whole adults using a DNeasy® Blood and Tissue Kit (Qiagen, Cat. # 6950), following the

supplier's instructions. PCR amplifications were performed using standard barcoding primers (LepF1 and LepR1) in a 50 µL reaction volume consisting of 25.0 µL of 2X Fermentas PCR Master Mix (Fermentas, Cat. # K0171); 2.5 µM of each of LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and characterization performed as per Firake et al. (2013). For accuracy purpose, both DNA strands were sequenced for all the samples. The DNA sequences obtained were analyzed using Pregap4 and Gap4 programs within the Staden Molecular Biology analysis software (Staden et al., 1998). Nucleotide sequences were aligned using the sequence alignment program Clustal X and were checked manually. Sequence identity was determined by BLASTN search (Altschul et al., 1997) against the nr DNA database deposited in GenBank. Transformations were used as necessary before analysis and data on incidence and population of *D. hearseyana* were analyzed through one-way ANOVA using SPSS software (O' Connor, 2000). The means were compared using Tuskey honestly significant difference (HSD) tests ( $p=0.05$ ). The standard error of the mean was calculated with formula  $\frac{s}{\sqrt{n}}$  where, 's' is the sample standard deviation and 'n' is the size (number of observations) of the sample.

## RESULTS AND DISCUSSION

### Diagnostics and morphometrics

Tables 1 provides the details of morphometrics and life stages depicted in Figs. 1 and 2. Observations reveal that the female is distinctly large and laid eggs singly on leaves. Eggs were pale blackish brown measuring 0.57 mm x 0.36 mm. The 1<sup>st</sup> larval instar was brownish black with 1.91 mm x 0.35 mm. The lengths of 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> instar larvae were 4.59, 8.15, and 12.99 mm, respectively. The 5<sup>th</sup> instar larvae were 15.70 mm long, 2.19 mm wide, and light brownish in color with hairs on dorsal surface of the body. Pupae were 13.18 mm long, 3.96 mm wide and reddish color with laterally compressed. Head, thorax is brownish fuscous; collar with pair of black spot, abdomen orange yellow, with dorsal and lateral series of black spots. Adults females had a body length up to genitalia 12.07 mm x 11.28 mm. The female length of fore wing, hind wing, head, thorax, abdomen, proboscis, fore, middle and hind legs were 14.04, 12.06, 2.57, 2.98, 6.96, 7.13, 13.51, 14.80 and 15.62 mm, respectively. The male width of fore wing, hind wing, head, thorax and abdomen were 5.01, 5.75, 2.04, 2.95 and 2.58 mm, respectively. The lengths

Table 1. Morphometrics of life stages of *D. hearseyana* (n=10)

| Life stage                | Length (mm)<br>Mean ± SE | Width (mm)<br>Mean ± SE |
|---------------------------|--------------------------|-------------------------|
| Egg                       | 0.57 ± 0.010             | 0.36 ± 0.007            |
| 1st instar larva          | 1.91 ± 0.025             | 0.35 ± 0.009            |
| 2nd instar larva          | 4.59 ± 0.106             | 0.98 ± 0.007            |
| 3rd instar larva          | 8.15 ± 0.119             | 1.80 ± 0.016            |
| 4th instar larva          | 12.99 ± 0.098            | 2.19 ± 0.015            |
| 5th instar larva          | 15.70 ± 0.100            | 2.19 ± 0.013            |
| Pupa                      | 13.18 ± 0.024            | 3.96 ± 0.020            |
| Female<br>up to genitalia | 12.07 ± 0.070            | 2.98 ± 0.047            |
| Male<br>up to genitalia   | 11.28 ± 0.420            | 2.66 ± 0.027            |
| Adult body parts          |                          |                         |
| Parts measured<br>(mm)    | Male<br>Mean ± SE        | Female<br>Mean ± SE     |
| Length of fore wing       | 12.03 ± 0.075            | 14.04 ± 0.077           |
| Width of fore wing        | 5.01 ± 0.029             | 6.88 ± 0.074            |
| Length of hind wing       | 9.67 ± 0.041             | 12.06 ± 0.100           |
| Width of hind wing        | 5.75 ± 0.033             | 6.94 ± 0.042            |
| Length of head            | 2.18 ± 0.005             | 2.57 ± 1.237            |
| Width of head             | 2.04 ± 0.008             | 2.40 ± 0.006            |
| Length of thorax          | 2.65 ± 0.007             | 2.98 ± 0.013            |
| Width of thorax           | 2.95 ± 0.010             | 3.21 ± 0.010            |
| Length of abdomen         | 6.53 ± 0.020             | 6.96 ± 0.020            |
| Width of abdomen          | 2.58 ± 0.020             | 2.81 ± 0.012            |
| Length of proboscis       | 6.62 ± 0.035             | 7.13 ± 0.035            |
| Length of antenna         | 7.60 ± 0.020             | 9.03 ± 0.030            |
| Length of fore leg        | 11.48 ± 0.018            | 13.51 ± 0.022           |
| Length of middle leg      | 12.21 ± 0.040            | 14.80 ± 0.022           |
| Length of hind leg        | 13.28 ± 0.022            | 15.62 ± 0.028           |

of male and female antennae were 7.60 mm and 9.03 mm, respectively.

Moor and Thomas (1958-59) reported that palpi upturned, the second joint reaching vertex of head, the third long; with a black spot at end of each joints. Hind wing is orange yellow with obsolete fuscous marginal patches at apex and vein 2 in the form similis; the underside of some specimens with a black spot at end of cell. Cilia are orange yellow.

This is the first report of *D. hearseyana* as its damage, incidence, morphometrics, and DNA barcodes (on karonda) are not available.

### Incidence and symptoms

*D. hearseyana* was observed on karonda tree in



Fig. 1. Aggregation of *D. hearseyana* on *C. carandus*



Fig. 2. Developmental stages of *D. hearseyana*. A, eggs; B, young larva; C, late instar larva; D, pupae; E, adult male; F, adult female.

the hot arid region of north-western India, (i.e., Thar Desert) and identified as *D. hearseyana*. During the present study, the average incidence of *D. hearseyana* on trees ranged between 10.00 to 60.00 and 11.67 to 63.33 percent during 2014 and 2015, respectively (Table 2; Figs. 1,2). The incidence and numbers of *D. hearseyana* were higher in July-I to September-I and

the maximum incidence of 60.00 and 63.33 % were recorded in August-II and the minimum in October-II (16.67 and 18.33%) during 2014 and 2015, respectively. Thus the highest mean number of this *D. hearseyana* per tree was recorded in August-II (5.77 and 5.97/ plant) followed by August-I (4.8 and 4.83/ plant) and the lowest was in October-II (16.67 and 18.33/ plant)

Table 2. Seasonal incidence of *D. hearseyana* on *C. carandus* (ICAR-CIAH, Bikaner)

| Months       | Mean incidence (%) <sup>a,b</sup> |                             | Mean no. of larvae/plant <sup>b</sup> |                    |
|--------------|-----------------------------------|-----------------------------|---------------------------------------|--------------------|
|              | 2014                              | 2015                        | 2014                                  | 2015               |
| April-II     | 10.00 (18.04) <sup>a</sup>        | 11.67 (19.88) <sup>a</sup>  | 1.07 <sup>a</sup>                     | 1.23 <sup>a</sup>  |
| May-I        | 13.33 (21.33) <sup>ab</sup>       | 15.00 (22.59) <sup>ab</sup> | 1.47 <sup>ab</sup>                    | 1.70 <sup>ab</sup> |
| May-II       | 16.67 (24.04) <sup>bc</sup>       | 20.00 (26.55) <sup>b</sup>  | 1.53 <sup>ab</sup>                    | 1.73 <sup>ab</sup> |
| June-I       | 20.00 (26.44) <sup>cd</sup>       | 23.33 (28.77) <sup>bc</sup> | 2.87 <sup>cd</sup>                    | 3.03 <sup>bc</sup> |
| June-II      | 23.33 (28.77) <sup>d</sup>        | 26.67 (30.98) <sup>cd</sup> | 3.20 <sup>de</sup>                    | 3.47 <sup>c</sup>  |
| July-I       | 36.67 (37.24) <sup>ef</sup>       | 38.33 (38.23) <sup>e</sup>  | 3.63 <sup>de</sup>                    | 3.87 <sup>c</sup>  |
| July-II      | 46.67 (43.07) <sup>fg</sup>       | 48.33 (44.03) <sup>f</sup>  | 4.00 <sup>efg</sup>                   | 4.23 <sup>cd</sup> |
| August-I     | 53.33 (46.89) <sup>g</sup>        | 55.00 (47.86) <sup>fg</sup> | 4.80 <sup>fg</sup>                    | 5.00 <sup>d</sup>  |
| August-II    | 60.00 (50.77) <sup>gh</sup>       | 63.33 (52.75) <sup>gh</sup> | 5.77 <sup>h</sup>                     | 5.97 <sup>e</sup>  |
| September-I  | 43.33 (41.14) <sup>f</sup>        | 45.00 (42.10) <sup>ef</sup> | 4.63 <sup>fg</sup>                    | 4.83 <sup>d</sup>  |
| September-II | 33.33 (35.20) <sup>e</sup>        | 35.00 (36.22) <sup>de</sup> | 3.93 <sup>ef</sup>                    | 4.20 <sup>cd</sup> |
| October-I    | 26.67 (31.06) <sup>de</sup>       | 28.33 (32.13) <sup>cd</sup> | 2.20 <sup>bc</sup>                    | 2.40 <sup>b</sup>  |
| October-II   | 16.67 (24.04) <sup>bc</sup>       | 18.33 (25.30) <sup>b</sup>  | 1.27 <sup>a</sup>                     | 1.40 <sup>a</sup>  |

<sup>a</sup>Values in parentheses angular transformed values; <sup>b</sup> Values followed by different letters significantly different (Turkey's HSD test,  $p < 0.05$ ).

during 2014 and 2015, respectively. During a survey of the natural vegetation, the population of this pest was found to be higher on young trees than the old trees. The larvae damage the fresh emerging leaves and flowers which led to the suppression of growth of the karonda tree through scraping of leaves and flowers. Gurule and Nikam (2013) reported that the moth fauna of northern Maharashtra is characterized by larger proportions of Erebidae, Noctuidae, Crambidae, Geometridae and Sphingidae, which are also among the most diverse families of moths in this region, other families being relatively rare (or at least under-collected, especially Microlepidoptera).

### MtCOI DNA barcoding

The standard insect DNA barcoding primers (LCO and HCO) used in this study amplified the target fragment of the mt COI gene of mtDNA and direct sequencing gave good quality sequences. A final 658 bp sequence were used for alignment after trimming the ambiguous ends (5' and 3') of the sequences. No genetic variation was observed among the four individuals sequenced. BLASTN search of 658 bp sequence in the NCBI GenBank yielded many possible targets corresponding to the 5' end of COI gene and showed over 99% identity with *D. hearseyana* sequences (KX863151, KX862543, KX862440, KX862080, KX862000, KX861976 and KX862075). These sequences originate from different countries. The present study confirms the identification and the mtCOI sequence submitted to NCBI (accession number KT827247). During survey

of natural vegetation, the population of *D. hearseyana* was found to more on younger plants. Besides BLASTN search the complete Open Reading Frame (ORF) was also successfully obtained for the *D. hearseyana* sequence. The species level identity at molecular was also successfully established in other insects including *Arge xanthogaster* (Firake et al., 2013).

This pest is damaging economically important parts of plant, such as leaves and branches, therefore, management practices needs to be developed.

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