

Article

## Thoracic morphology of the pomegranate fruit borer (Lepidoptera: Lycaenidae) larva

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### Abstract

The pomegranate fruit borer, *Deudorix isocrates* (Fab.) is a serious pest of pomegranate causing remarkable economic loss and reported from most of the pomegranate growing countries. The research work on this pest has been undertaken on its bio ecological aspects and management. There are the least reports available on its morphological studies. This is the first study has been done on morphological details of the thoracic region of the sequential larval instars. The description is based on detailed light microscopic study of cuticular features, spiracles and appendages. This study is important to understand identification marks of larval instars, larval metamorphosis and taxonomic features. With increase in size the larval instars show peculiar morphological changes in the prothoracic shield and prothoracic spiracles as identification mark of each larval instar and found important to understand development of respiratory organs. The microscopic studies on thoracic appendages revealed general lepidopterist type. Results add to our knowledge of thoracic metamorphosis during larval period and provide a standard for further research on the morphological characters of *Deudorix isocrates* (Fab.).

**Keywords** *D. isocrates*; pest; thoracic metamorphosis; prothoracic shield; prothoracic spiracles.

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### 1 Introduction

The study on larval morphological characteristics is critical for a better understanding of their development and taxonomy. Although the larval stages of Lepidoptera have less structure than adults do, their morphological study is significant, particularly in the field of taxonomy and phylogenetic relationships (Hasenfuss and Kristensen, 2012). Most cultivated plants are damaged and attacked by larva of lepidopteran pest, which is the second most diverse insect pest after coleoptera (Rose and Singh, 2010). *Deudorix isocrates* (Fab.) (Lepidoptera: Lycaenidae) is a severe economic pest on pomegranate and guava (Kumar et al., 2017; Devi et al., 2021). *D. isocrates* has been studied for a long time in terms of ecological and economic aspects, its larva has received very little attention. *D. isocrates* larva have had very few morphological and biological

studies. Lepidopteran classification mainly relied on morphological observations of adults, despite the fact that recent studies have repeatedly helped to resolve classifications based on immature morphology (Kitching, 1984; Scoble, 1995; Freitas and Brown, 2004; Duarte et al., 2005; Willmott and Freitas, 2006). The purpose of this study is to offer thorough thoracic morphology which will be helpful in taxonomic study of lycaenids and to find morphological features for perfect identification of the pest.

## 2 Materials and Methods

Eggs of *Deudorix isocrates* were collected from the field during the field survey and reared in glass Petri plates. After hatching the larvae were reared in the laboratory to obtain sequential instars. The larval instars were determined by observing casting of the skin during each consecutive moult. From each instar stage some larvae were used for permanent mounting preparation while remaining larvae were reared to obtain later instar stages. The live specimens were used to record general morphological features of thoracic region. To study microscopic details randomly selected larvae from each instar were killed by insect killing jar and preserved in 10% formaldehyde. The preserved specimens were used for permanent mounting preparations. The preserved specimens were rinsed in distilled water, dissected to separate thoracic region and appendages and boiled in 10% KOH to make it translucent. The material was again rinsed in distilled water, dehydrated by ascending grades of alcohol, cleared in xylene and mounted in DPX. The morphological details were studied using a compound microscope with ocular micrometer to record measurements and illustrations were prepared by Camera lucida.

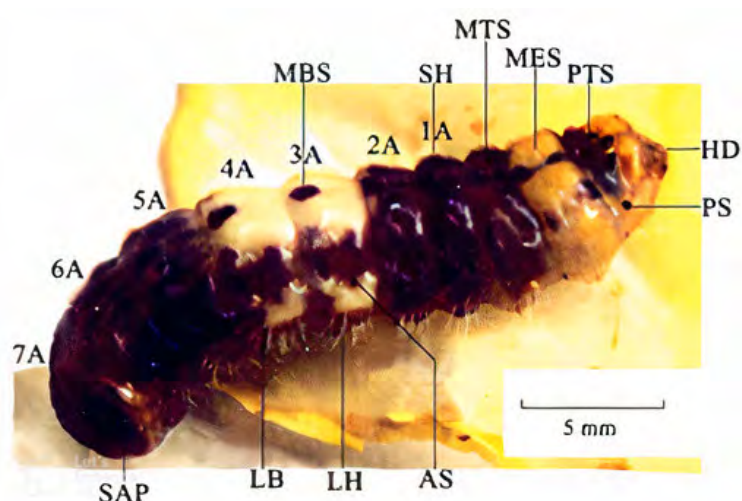
## 3 Results

Thorax of the larva consists of three segments. General surface of these segments including terga, pleura and sterna is characteristically pigmented as a species-specific feature. The pro and mesothoracic terga up to 2nd instar are whitish which becomes pale brown in 3rd and yellow with brown tinge in 4th instar stage (Fig. 1) of larva. The brown pigmentation of metathoracic tergum becomes darker from 1st to last instar. The prothoracic pleuron shows brown band just above the prothoracic spiracle and continues in meso and metathoracic pleura. The general thoracic surface is very poorly sclerotized except central portion of prothoracic tergum and clothed with various types of setae. The tergal region of thoracic segments is extended laterally, which conceals the thoracic appendages. Prothoracic tergum is extended a bit anteriorly. The extended portion covers the head capsule. There is only one pair of spiracles in thoracic region of the larva. The pair is present in prothorax. It is placed in a shallow depression, which is formed on lateral sides of the prothoracic tergum. Each thoracic segment bears a pair of "walking legs".

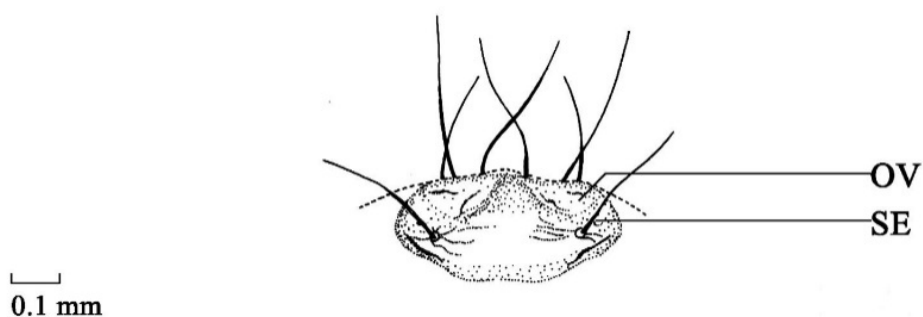
The central sclerotized portion of prothoracic tergum is peculiarly subjected to changes in pattern of sclerotization and pigmentation from instar to instar (Figs. 2 to 5). The central sclerotized part of prothoracic tergum is referred here as a prothoracic shield. Morphologically, these features of prothoracic shield are significant and helpful for identification of a specific instars in field.

In 1st instars stage, the prothoracic shield is localized to a small single, oval plate (Fig. 2). The sclerotization is weak and colour is pale. The microscopic observations reveal relatively dark brown pigmentation at anteriomesal portion of the oval plate. There is a pair of long, translucent setae like hair, distributed on the plate. In 2nd instar (Fig. 3) the sclerotization is enhanced and a single plate becomes relatively hard. The anterior half of the plate extends laterally whereas posterior region becomes roughly semicircular. The laterally extended portion of both the sides and the anteriomesal portion are very much dark brown thus the prothoracic shield becomes easily visible. Moderately long hair are distributed in bilateral symmetry in relation to the median axis. In 3rd instars stage (Fig. 4), sclerotization further enhances and the

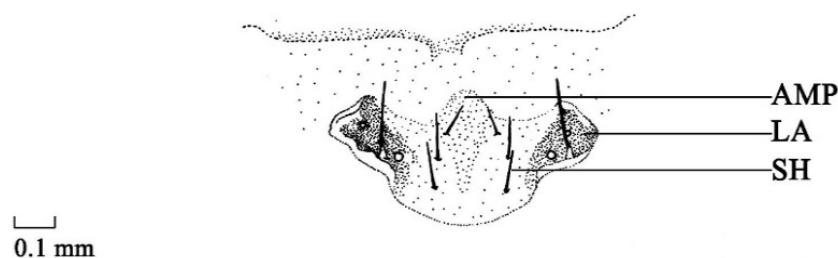
prothoracic shield becomes relatively much harder than that of previous instars. The dark, reddish brown pigment is distributed roughly throughout the surface hence, it becomes remarkably distinct. Though it appears a single plate, the microscopic observations suggest that the plate tends to split in to two halves due to an unsclerotized narrow strip that is formed along the median axis. There are numerous well sclerotized, dark brown, short hairs distributed throughout the surface of plate. These hairs are distributed in bilateral symmetry in relation to the median axis. In full-grown larva, the prothoracic shield is highly sclerotized in such a way that the sclerotized region shows two horizontally placed, triangular halves by the side of unpigmented median strip along central axis of the tergum (Fig. 5). Each half is roughly triangular with dark black colouration at each corner and along central axis while rest part of the triangle is gray in colour.



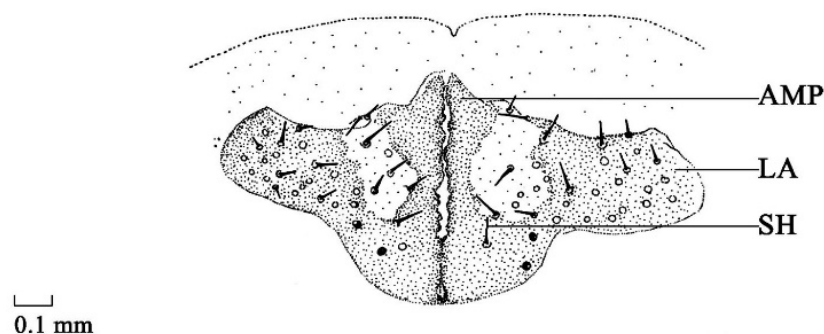
**Fig. 1** Last (4th) instar larva of *D. isocrates* (Fab.). 1A-7A: Abdominal segments; AS: Abdominal spiracles; HD: Head; LB: Lateral colour band; LH: Lateral hair; MBS: Median brown spot; MES: Mesothoracic segment; MTS: Metathoracic segment; PS: Prothoracic spiracle; PTS: Prothoracic segment; SAP: Suranal plate; SH: Short brown hairs.



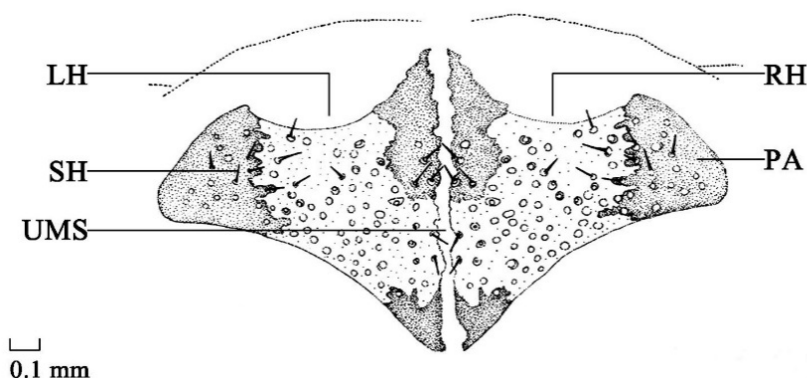
**Fig. 2** Prothoracic shield of 1<sup>st</sup> instar larva of *D. isocrates*. OV: Oval Plate; SE: Seta.



**Fig. 3** Prothoracic shield of 2<sup>nd</sup> instar larva of *D. isocrates*. AMP: Anterior median pigmented area; LA: Lateral pigmented area; SH: Sclerotized hair.



**Fig. 4** Prothoracic shield of 3<sup>rd</sup> instar larva of *D. isocrates*. **AMP**: Anterior median pigmented area; **LA**: Lateral pigmented area; **SH**: Sclerotized hair.



**Fig. 5** Prothoracic shield of 4<sup>th</sup> instar larva of *D. isocrates*. **LH**: Left half; **PA**: Pigmented area; **RH**: Right half; **SH**: Sclerotized hair; **UMS**: Unsclerotized median streak.

The microscopic observations of prothoracic shield (Fig. 5) reveal that each half defines four angles. However, in gross it appears triangular. The sclerotized halves are clothed with numerous short, stout, sclerotized hairs, which are pigmented with brown.

There is a pair of spiracles situated on lateral aspect of the prothoracic segment hence called as the prothoracic spiracle. There is no any report on detailed morphology of prothoracic spiracles in *D. Isocrates*.

In general, the prothoracic spiracle consists of peritreme, the atrium in which the actual spiracular orifice is enclosed and the closing apparatus which is situated at the inner end of atrium. Out of these structures, the surface view of prothoracic spiracle shows all parts except the closing apparatus. It is visible in the KOH treated material. The structural elements of closing apparatus are assembled on outer wall of the tracheal opening in to the atrium.

With increase in size (Table 1) morphologically the prothoracic spiracle changes from instar to instar. In 1st instar, it is circular (Fig. 6). In 2nd instar it becomes oval (Fig. 7) In both the instars it remains wide open. It is surrounded by sclerotized peritreme. The wall of atrium shows crosswise striations, which run from peritreme to the spiracular opening. In 3rd instar the spiracular opening is occluded due to formation of trabeculae (Fig. 8). The trabeculae run towards the center and most of them are branched, terminally. The terminal branches form network, which is more or less limited towards the central part of the spiracle. The

trabecular branches join with each other. Thus, the sclerotized sheet is formed. This formation occupies the spiracular orifice leaving a narrow vertical spiracular aperture. The sclerotized sheet is fringed with sharply pointed bristles. These are the phases of development of spiracular structure from 1st instars to 3rd instars.

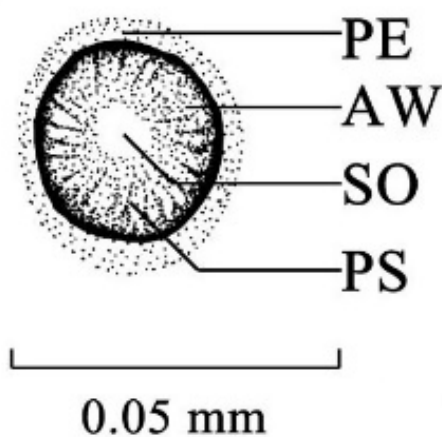
**Table 1** Morphometric observations of thoracic structures of *D. isocrates* larva.

Larval Instar	Average Bodylength in mm (n=10)	Average breadth at thoracic segments in mm (n=10)		Size of prothoracic spiracles in mm (n=10)	
		Prothoracic segment	Meso and Metathoracic segment	Average height	Average Width / diameter
<b>1<sup>st</sup> instar</b>	2.38 (1.50-4.50)	*	*		0.029** (0.026 - 0.032)
<b>2<sup>nd</sup> instar</b>	6.9 (5.00-9.50)	1.76 (1.25 - 2.50)	1.95 (1.25 - 2.80)	0.066 (0.064 – 0.070)	0.053 (0.051 - 0.058)
<b>3<sup>rd</sup> instar</b>	11.51 (10.0 - 14.00)	2.61 (2.00 - 3.00)	3.34 (2.00 - 4.00)	0.120 (0.102 – 0.140)	0.080 (0.077 - 0.09)
<b>4<sup>th</sup> instar</b>	23.01 (15.5 - 31.00)	4.23 (4.00 - 5.00)	5.23 (4.00 - 7.00)	0.333 (0.205 – 0.384)	0.211 (0.192 - 0.243)

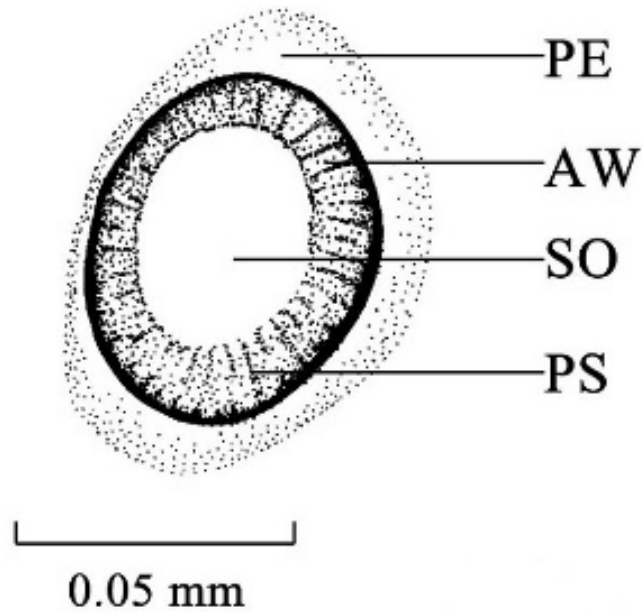
Figures in parentheses are range values.

\* Not measured being small size.

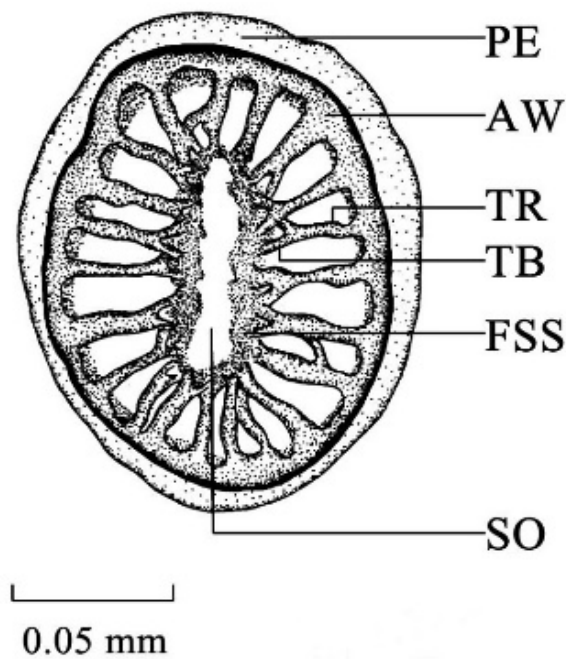
\*\* Diameter is measured since the spiracle is circular.



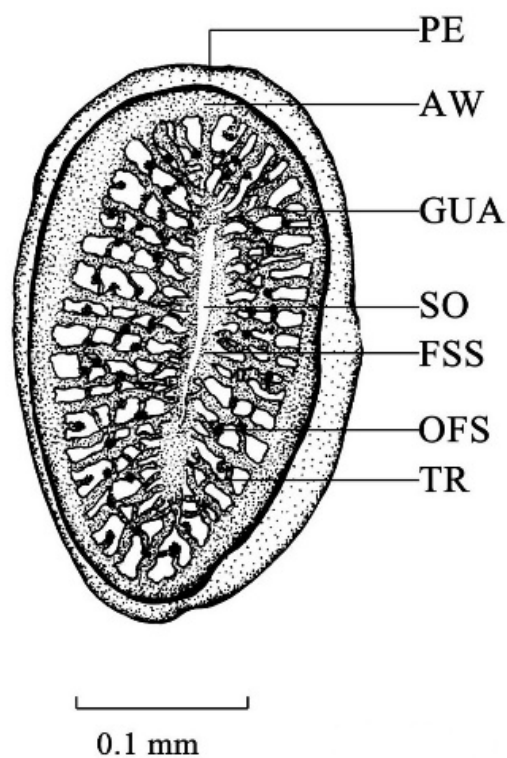
**Fig. 6** External surface view of Prothoracic spiracle of 1<sup>st</sup> instar larva of *D. isocrates*. **AW**: Atrial wall; **PE**: Peritreme; **PS**: Pigmented weakly sclerotized streaks; **SO**: Spiracular opening.



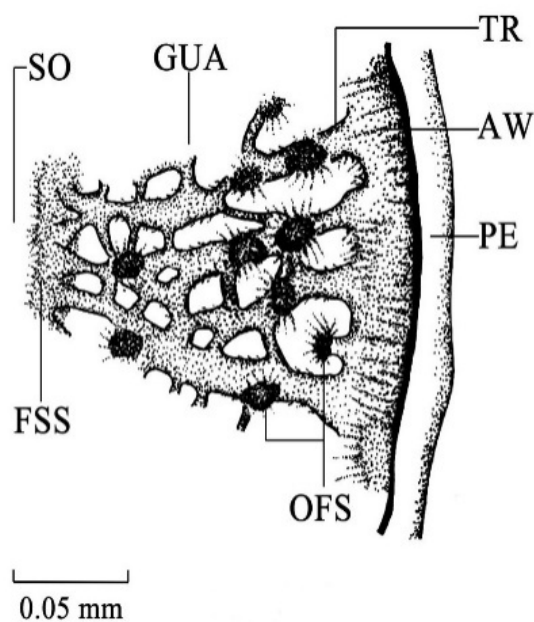
**Fig. 7** External surface view of prothoracic spiracle of 2<sup>nd</sup> instar larva of *D. isocrates*. **AW**: Atrial wall; **PE**: Peritreme; **PS**: Pigmented weakly sclerotized streaks; **SO**: Spiracular opening.



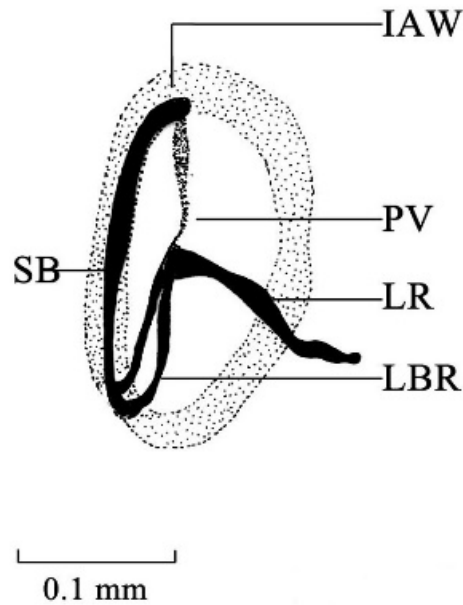
**Fig. 8** External surface view of Prothoracic spiracle of 3<sup>rd</sup> instar larva of *D. isocrates*. **AW**: Atrial wall; **FSS**: Fringed sclerotized sheet; **PE**: Peritreme; **SO**: Spiracular opening; **TR**: Trabeculae; **TB**: Trabecular branch.



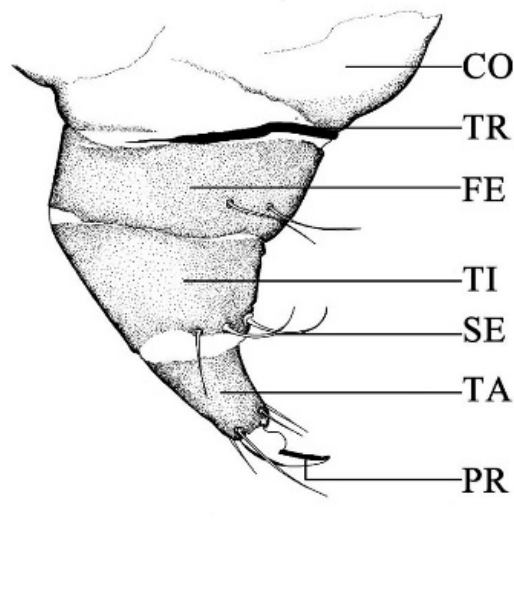
**Fig. 9** External surface view of Prothoracic spiracle of 4<sup>th</sup> instar larva of *D. isocrates*. **AW**: Atrial wall; **FSS**: Fringed sclerotized sheet; **GUA**: Guarding apparatus; **OFS**: Oval fringed sclerotized spot; **PE**: Peritreme; **TR**: Trabeculae; **SO**: Spiracular opening.



**Fig. 10** Magnified view of Prothoracic spiracle of 4<sup>th</sup> instar larva of *D. isocrates*. **AW**: Atrial wall; **FSS**: Fringed sclerotized sheet; **GUA**: Guarding apparatus; **OFS**: Oval fringed sclerotized spot; **PE**: Peritreme; **SO**: Spiracular opening; **TR**: Trabeculae.



**Fig. 11** Internal view of Prothoracic spiracle showing closing apparatus of 4<sup>th</sup> instar larva of *D. isocrates*. **IAW**: Inner atrial wall; **LBR**: Looped bar; **LR**: Lever; **PV**: Posterior valve; **SB**: Sclerotized bow.



**Fig. 12** Thoracic appendage of 4<sup>th</sup> instar larva of *D. isocrates*. **CO**: Coxa; **FE**: Femur; **PR**: Pretarsus; **SE**: Seta; **TA**: Tarsus; **TI**: Tibia; **TR**: Trochanter.

In full-grown larva, the thoracic spiracle appears as dark black, oval spot by naked eyes but, microscopic observations reveal that it is not that simple. The oval spiracle (Fig. 9) is peripherally marked by the peritreme. The peritreme is present in the form of narrow sclerotized rim around the atrial wall. The atrial wall is



projected outwardly and forms an elevated sclerotized ring on the general body surface. The atrial wall is produced into rigid, sclerotized processes. These rigid processes are fringed with minute, sclerotized and sharply pointed bristles. Such fringed processes are called trabeculae. The trabeculae are numerous branched while projecting towards the center of spiracle. The branched trabeculae form the network, which appears as the 'guarding apparatus'. Some of the branches of trabeculae remain free and directing outwards. Thick endings of these branches appear as fringed oval or circular dark spots (Fig. 10) which are evenly distributed on the guarding apparatus. The ends of terminal branches of trabeculae merge with each other and form a fringed flattened sclerotized sheet around the spiracular orifice. The surrounding flattened sheet also fringed with short, sclerotized and sharply pointed bristle. In this manner the guarding apparatus encloses the spiracular orifice, which is present in the form of vertical slit like opening.

The closing apparatus (Fig. 11) consists of different sclerotized elements. These elements are situated at the inner end of an atrium and assembled on outer wall of tracheal opening in to the atrium. There is a sclerotized bow. It lies anteriorly and encircles the anterior half of the tracheal opening. The posterior valve is provided with outwardly projected well sclerotized lever. The lever hinges on the looped sclerotized bar. Other ends of looped bar hinge with the ventral end of sclerotized bow.

There are three pairs of cone shaped ventrally directed thoracic appendages. Each thoracic segment bears one pair. Each appendage articulated to the pleural region of each thoracic segment. Each appendage (Fig. 12) is formed of five parts; the coxa, the trochanter, the femur, the tibia and the tarsus. All appendages are short and stumpy. They are articulated with thoracic pleura by conspicuous, oval lobe like coxa. Both the proximal and distal ends of coxa are wider than the length. The wall of coxa is sclerotized at inner angle. This restricted region appears brown. There are a few setae and sclerotized hairs along its proximal region. Along its distal border there is partially sclerotized incomplete ring, the trochanter. It lies along inner angle between coxa and femur. Distal to the incomplete ring of trochanter lies the femur. It is small, uniformly sclerotized with light brown colour. It is also wider than its length. The part is roughly cylindrical and bears a few setae towards distal end. Distally femur articulates with tibia. Tibia is slightly elongated than the femur. It is wider at its proximal end but tapers proportionately towards the distal end. It is also uniformly sclerotized and looks brown. It bears a few long setae. Some setae are straight while some are curved. The tibia articulates with the tarsus distally. It is terminal tapering part of "cone". Its sclerotization and colour are similar to that of other parts. Distally the tarsus bears a pretarsus. It is a single highly sclerotized, curved, pointed claw. At the proximal inner surface, the claw bears a round, lobe like protuberance. It seems it is characteristic of lepidopterous claw. The features of thoracic appendages of 1st to 4th instars are similar in all respects. However, there is gradual increase in size from 1st to 4th instar.

#### 4 Discussion

The pigmentation of thoracic terga and pleura in each instar stage of *D. isocrates* larva is appeared as species specific character and helpful to distinguish them from each other and to identify the pest species during early stage of its development. In lycaenids comparatively larger prothoracic segment than meso and metathoracic segment is reported earlier (Eaton, 1982; Ballmer and Wright, 2008) but in *D. isocrates* the prothoracic segment is smaller than meso and metathorax. Laterally extended tergal plates of thoracic segments (Richards and Davies, 1977) are the specialized lycaenids characters found in *D. isocrates*. Presence of prothoracic shield, prothoracic spiracle and three pairs of appendages are the general characteristics of lepidopteran larvae (Todd, 2013; Stehr, 1987; Graca and Solis, 2018; Marquis et al., 2019; Greeny et al., 2009; Kaminski, 2008; Kaminski and Carvalho-filho, 2012) present in *D. isocrates* as a general lepidopterous characteristics but their microscopic features are specialized. The thoracic terga are unsclerotized except the central region of

prothoracic tergum. Hence it is termed as a prothoracic shield (MacKay, 1962). Sometimes it has also been termed as prothoracic tergal plate (Eaton, 1982) and prothoracic plate (Barao and Moreira, 2010). In the present study the term 'prothoracic shield' is used according to its heavy sclerotization. Including Lycaenidae the studies on prothoracic shield of lepidopteran larvae (Scoble, 1995; Duarte and Robins, 2009) reported its significance in identification and classification of caterpillars. In *D. isocrates* larvae the microscopic details appeared as diagnostic features to identify this pest at larval stage as well as to distinguish sequential larval instar stages from each other. The gradual changes in prothoracic shield during larval development are also reported in other groups of Lepidoptera (Moraes et al., 2012). In 1st instar larva the prothoracic spiracles are circular and become elliptical in later instar stages. The similar observation has been reported in *H. armigera* (Queiroz-Santos et al., 2018). On external surface the spiracle exhibits peritreme and filter apparatus (Schmitz and Wasserthal, 1999; Wasserthal, 2003) which is also labelled as guarding apparatus (Snodgrass, 1935; Richards and Davies, 1977) or sieve plate (Forster, 2010) in different species of lepidopteran larva. The present study is the first report on instarwise gradual development of filter apparatus and its microscopic details. The filter apparatus is composed with finest divisions of repeatedly branched processes. The repeatedly branched processes have been called as trabeculae (Tonapi, 1994; Forster, 2010). In *D. isocrates* larva the trabeculae and their terminal branches develop in 3rd instar. The network of branching trabeculae, fringed terminal spots and fringed sheet which occludes the spiracular orifice develop during the 4th instar. The closing apparatus of spiracle composed with a sclerotized bow, looped bar and a lever (Tonapi, 1959; Srivastava, 1975; Richards and Davies, 1977; Eaton, 1982; Nikam and Khole, 1989; Chapman, 1998; Schmitz and Wasserthal, 1999; Wasserthal, 2003) is produced from the inner wall of atrium and the closing mechanism is operated by muscles. These lepidopterist elements of closing apparatus are present in the prothoracic spiracle of *D. isocrates* larva. The cone shaped thoracic locomotory appendages in *D. isocrates* are five segmented terminally bear hook shaped tarsal claw (Barao and Moreira, 2010; Queiroz-Santos et al., 2018). In present study it is referred as a pretarsus. The present study is helpful for the understanding of detailed morphology of larval thorax, metamorphosis during larval period, diagnostic features of larvae for perfect identification of the pest during early stages of *D. isocrates*.

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