Article

Cranial morphology of *Deudorix isocrates* (Fab.) (Lepidoptera: Lycaenidae) larvae

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Abstract
Deudorix isocrates (Fab.) is a globally important agricultural pest. The study regarding this pest is mostly concentrated on its management. Several morphologically significant features have been overlooked. In the present study we examined the structure and function of the larval cranial morphology and its appendages. The description is based on the detailed morphological study of cranial sutures, cranial areas, antennae and mouthparts of the fourth instar larva. The cranial structures and appendages are found significant to understand pest activity and taxonomic features. Supporting to the pest activities, the larva has usual the lepidopterous pattern of biting and cutting mouthparts consisting of labrum, mandibles and maxillolabial-hypopharyngeal complex. This type of mouthparts helps to cut fruit rind and make an entrance hole while infesting the fruit. The results add to our knowledge of larval cranial capsule, antennae and mouthparts morphology and provide a standard for further research on the morphological characters of *Deudorix isocrates* (Fab.)

Keywords *D. isocrates*; pest; cranial areas; cranial sutures; mouthparts; morphology.

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1 Introduction

Studies on larval morphological characteristics are critical for better understanding of development and taxonomy. Although the larval stages of Lepidoptera have less structure than adults, their morphological study is significant, particularly to study taxonomy, development and phylogenetic relationships (Hasenfuss et al., 2012). The morphological characteristics of the cranium, such as the shape of the cranial capsule, sutures, cranial areas, antenna and the mouthparts of the larva can all be employed as taxonomic criteria. (Mutuura, 1980). An in-depth understanding of the structure and function of morphological features is essential for understanding evolution, ecomorphology, taxonomy, and phylogenetic studies (White et al., 2021). The cranial capsule and its appendages such as antenna and mouthparts are important in taxonomic studies. The antenna is sensory structure and antenna specific studies are significant for pest control (Rana and Mohankumar, 2017). The mouthparts of an insect are crucial to understand its feeding mechanism. Mouthpart morphology can reflect previously unknown insect eating habits (Koçakoğlu et al., 2020).

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* (Fab.) is a key stumbling block to produce adequate quantities and quality pomegranate fruits. The pomegranate fruit borer is the most common, polyphagous, and damaging pest, with a wide range of host plants including pomegranate, citrus, guava, litchi, apple, peach, pear, plum, and sapota (Khandare et al., 2018). Although, *D. isocrates* has been studied in terms of ecological and economic aspects, its larvae have received very little attention. *D. isocrates* larvae have had very few morphological and biological studies. Most of the phylogenetic and morphological studies are done using larvae of Coleoptera and Diptera (Lawrence et al., 2011; Oca-Aguilar et al., 2014).

The purpose of this study is to offer thorough cranial morphology, which includes cranial suture, cranial areas and head appendages such as antennae and mouthparts of *D.isocrates* larvae, which will aid in the taxonomic and morphology of lepidopteran insect.

2 Material and Methods

The larval phase *D. isocrates* consists of four instar stages. The fourth instar stages of the larvae were obtained from field by collecting infested fruit (Fig. 1 and Fig. 2) while some early instar stages were reared in a laboratory up to fourth instar. The collected specimens were preserved in 10% formaldehyde. The cranial capsules were dissected out and rinsed in distilled water during slide preparation. Cranial capsules were heated in 10% KOH to make them translucent and washed in distilled water. Dehydration was carried out by ascending grades (30%, 50%, 70%, 90% and 100%) of ethanol and cleared in xylene. Mounting was done in DPX. The mouthparts were separated from cranial capsule and the same procedure was repeated to make their permanent slide preparations. These preparations were used to analyze the microscopic features of the mouthparts. Camera Lucida and Micrometry were used to create illustrations

Fig. 1 Pomegranate fruit infested by larva of *D.isocrates* (Fab.).

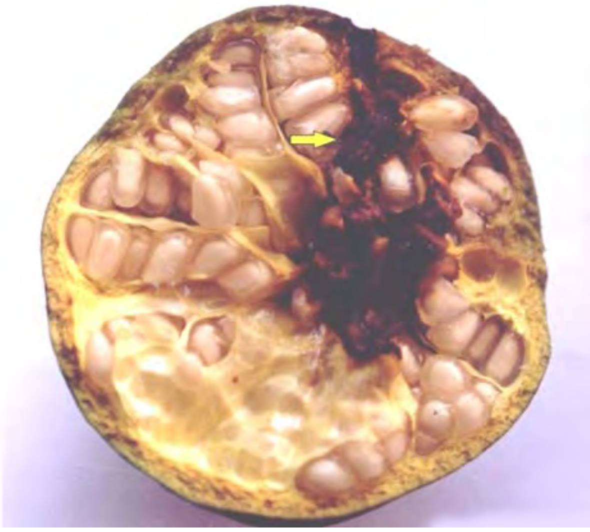

Fig.2

Fig. 2 Internal damage of pomegranate fruit due to larva of *D.isocrates* (Fab.).

3 Results

The fourth instar larva of *D. isocrates* (Fig. 3) is on an average 23.01 mm long and 6.80 mm wide at the broadest body region (i.e. 4th abdominal segment). The sclerotized cranial capsule is spherical if viewed from the front and more or less oval if viewed laterally. Average width of the cranial capsule is measured 1.74 mm. The size of cranial capsule gradually increases towards the last instars. The cranial capsule is dark brown with black pigmentation on the parietal areas. Hence, central portion of cranial capsule appears to be relatively paler.

3.1 Cranial areas and sutures

From the frontal view (Fig. 4), the vertex of the cranial capsule is notched. The median groove is present in this notch. It runs posterioventral and anterioventral sides of the cranial capsule. The cranial area consisting of this groove is the postfrons. The postfrontal area appears very narrow, stripe like, which is delimited laterally by the frontal lines. The frontal lines from both the sides of median groove run ventral wards up to the mid region of the cranial capsule and diverge from each other. The diverged branches run towards the anterior mandibular articulation of respective sides. The cranial surface that lies by the left and right sides of postfrons is unpigmented. It appears as a pale vertical band. The dorsal most lateral surface beyond the band is also unpigmented. Hence, it appears whitish while the area ventral to the postfrons and parietals is dark reddish brown. In the cranium of the larva, the frons has become invaginated along the median-longitudinal groove of the head. The median longitudinal groove runs longitudinally from the posterior edge of cranial capsule up to apex of the clypeus, on the anterior face.

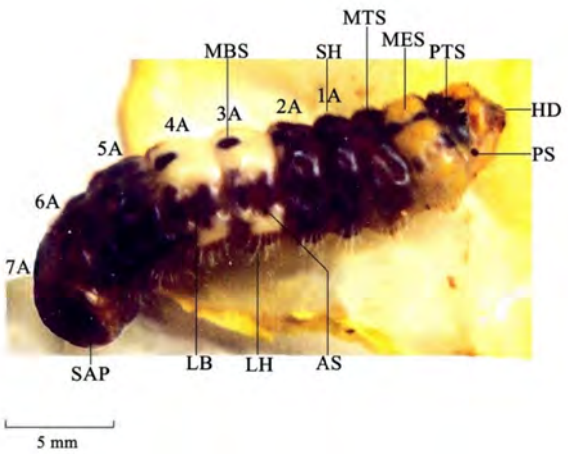

Fig. 3 Last 4th instar larva of *D.isocrates* (Fab.). 1A-7A: abdominal segments, AS: abdominal spiracles, HD: head, LB: lateral colour band, LH: lateral hair, MBS: median brown spot, MES: mesothoracic segment, MTS: metathoracic segment, PS: prothoracic spiracle, PTS: prothoracic segment, SAP: suranal plate, SH: short brown hairs.

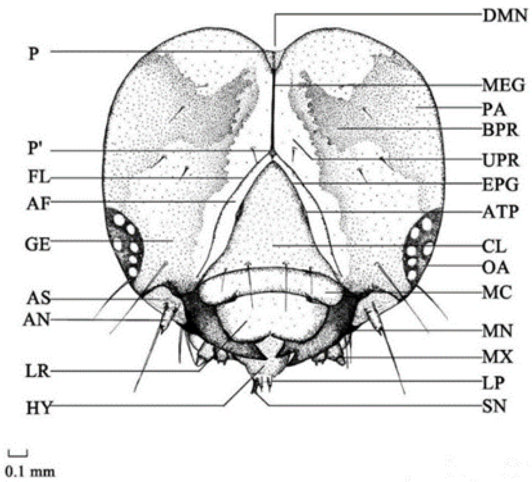

Fig. 4 Frontal view of cranial capsule of 4th instar larva of *D. isocrates.* AF: antefrons, AN: antenna, AS: antennal socket, ATP: anterior tentorial pit, BPR: black pigmentation, CL: clypeus, DMN: dorsal median notch, EPG: epistomal groove, FL: frontal line, GE: gena, HY: hypophargyx, LP: labial palp, LR: labrum, MC: membranous conjuctiva, MEG: median groove, MN: mandible, MX:maxilla, OA: ocellar area, P-P': postfrons along with median groove, PA: parietals, SN: spinneret, UPR: unpigmented region.

The clypeus lies ventral to the postfrons. It is laterally lined by upwardly arched epistomal groove and ventrally by a membranous conjuctiva. The epistomal groove is dark reddish brown having microscopic, colourless, vertical depressions of the anterior tentorial pits. A number of setae are distributed on the clypeal surface. The setae situated on the ventral margin are relatively longer. Lateral to the clypeal plate lies the unpigmented strip like antefrons. The antefrons lies between the epistomal groove and the frontal line. Both the frontal line and the epistomal grove come close to each other near the region of anterior mandibular articulation. The unpigmented area along the postfrons and the frontal line is quite distinct against the pigmented area of the parietal region and the ocellar region. Thus, the unpigmented area of the cranial capsule of fourth instar appears inverted 'Y'. It is not always seen in other instars.

The ventral to the clypeus is conjuctiva. It is roughly rectangular membranous lobe. It articulates the labrum to the clypeus. The labrum is a slightly sclerotized, pigmented plate. It bears a notch on the ventral margin. The notch is placed at the median axis. The two diverging arms of this sulcus enclose the central clypeus, whereas the area between the Y-shaped suture and the bifurcations of the echdysial cleavage line is the frons.

From the lateral View (Fig. 5), the dorsal half of the cranial capsule is contributed by the parietal, which extends posterior up to the postoccipital suture. The sparsely distributed black granules cover the parietal area. The ventral portion of the cranial capsule is formed by gena. Predominantly it consists of ocellar area. Dark black pigment is distributed in the anterior half of the ocellar area while posterior half appears pale. The ocellar area consists of all the six stemmata. The postgena is situated posterior to the ocellar area. The socket of the antenna is situated ventral to the pleurostomal ridge and is located between the mandible and ocellar area of each side. In the lateral view, the mouthparts such as labrum, mandibles, and maxillae with cardo, stipes, maxillary palpi, and galea are visible. The labium shows its postmentum, prementum, labialpalpi and spinneret. The hypopharynx takes anterior position to the maxillae. On the posteriolateral side the reddish brown postoccipital suture and a narrow band like sclerite of postocciput and occipital suture show their presence.

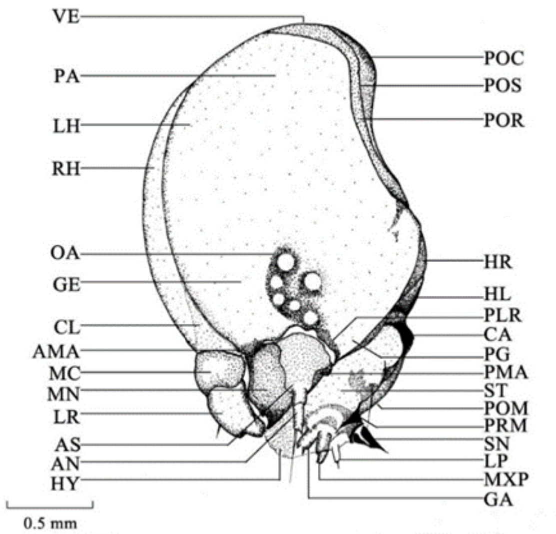

Fig. 5 Lateral view of cranial capsule of 4th instar larva of *D. isocrates*. AMA: anterior mandibular articulation, AN: antenna, AS: antennal socket, CA: cardo, CL: clypeus, GA: galea, GE: gena, HL: hypostomal lobe, HR: hypostomal ridge, HY: hypopharynx, LH: left half of cranial capsule, LR: labrum, LP: labial palp, MC: membranous conjuctiva, MN: mandible, MXP: maxillary palp, OA: ocellar area, PA: parietals, PG: postgena, PLR: pleurostomal ridge, PMA: posterior mandibular articulation, POC: post occiput, POM: Postmentum, POR: postoccipital ridge, POS: postoccipital Suture, PRM: premental sclerite, RH: right half of head capsule, SN: spinneret, ST: stipes, VE: vertex.

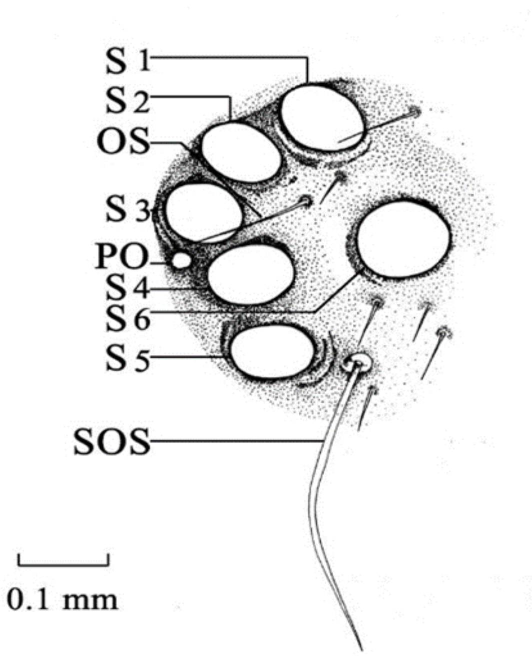

Fig. 6 Lateral ocelli of 4th instar larva of *D. isocrates*. OS: ocellar seta, PO: pore organ, S1 to S6: stemmata 1 to stemmata 6, SOS: subocellar seta.

The lateral ocelli (stemmata) (Fig. 6) are dome shaped having transparent corneal lens. At the base, it is circular and protrudes out slightly from the basal surface. The stemmata 1st to 5th are arranged in a semicircular fashion and are more or less equidistant from one another. The 5th stemma of the anterior semicircle is less shifted anterior than the remaining four stemmata. The 6th stemma is shifted away towards the posterior side from the main group. In relation to the 1ststemma, it is situated posterioventrally. There is a pore organ situated in anterioventral position in relation to the 3rd stemma. Not all stemmata are equal in size. On the basis of the difference in size, the stemmata form two groups. The 1st and 6th stemma form one group while 2nd, 3rd, 4th and 5th stemmata form other group. The average diameter of the stemma of first group is 0.096 mm while it is 0.086 mm of the other group (n = 10). The ocellar area shows the presence of a few setae, which are distributed randomly. One of the setae, which is placed posterioventral to the 5th stemma is the longest. It is referred as subocellar seta in the present studies.

The posterior view (Fig. 7), most on the dorsal and the mesal part of the cranial capsule is occupied by the occipital foramen. The foramen is bound dorsally by posterior extension of postfrons and notched vertex, laterally by postocciput and ventrolaterally by postgena and hypostomal lobes. All these regions show postoccipital suture, postoccipital ridge and posterior extension of hypostomal ridge at their respective places. The posterior view also shows the presence of maxillo-labial hypopharyngeal complex with the spinneret. In general, though the posterior surface of the cranial capsule is reddish brown. It is not highly pigmented.

The ventral surface view (Fig. 8) of the cranial capsule is roughly oval. Anterioposteriorly, the lateral half of the cranial capsule shows the regions of gena and postgena. The ocellar area is situated in the gena. Mesally, the postgena is delimited by hypostomal suture. The mesal portion of the posterior half of the cranial capsule shows presence of maxillolabial-hypopharyngeal complex with various structures like stapes, galea, and maxillary palpi of the maxillae while postmentum, premental arm, palpiger, labial palpi and spinneret of labium. In the complex maxillae lie laterally, the labium takes the central position while hypopharynx is situated anteriorly. The mesal portion of the anterior half of the cranial capsule shows a pair of mandibles, each with the clearly marked anterior and posterior articulation. Internally, at the posterior mandibular articulation the hypostomal ridge continues with the pleurostomal ridge. The mandibles are not interlocked at the resting state. The left mandible is placed anterior to the right one. The antennal socket appears lateral to the mandibular base on either sides. The most anterio-mesal portion shows the posterioanteriorly arranged labrum and the membranous conjunctiva. The clypeus is observed with its ventral sub marginal area only.

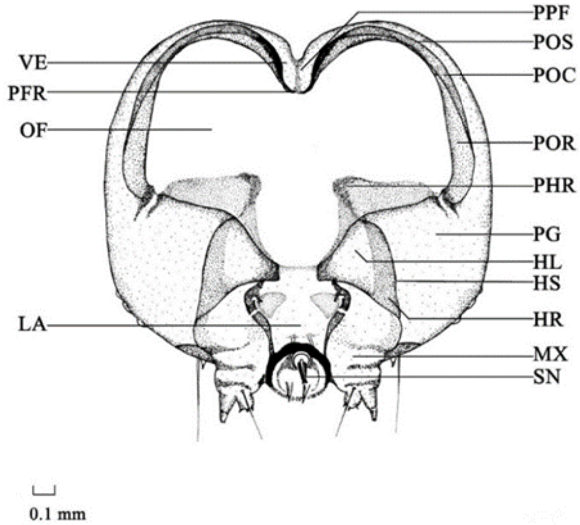

Fig. 7 Posterior view of cranial capsule of 4th instar larva of *D. Isocrates*. LA: labium, MX: maxilla, OF: occipital foramen, PFR: posterior end of frontal ridge, PG: postgena, PHR: posterior end of hypostomal ridge, POC: post occiput, OR: post occipital ridge, POS: Post occipital suture, PPF: posterior extension of postfrons, SN: spinneret, VE: vertex, HL: hypostomal lobe, HR: hypostomal ridge, HS: hypostomal suture

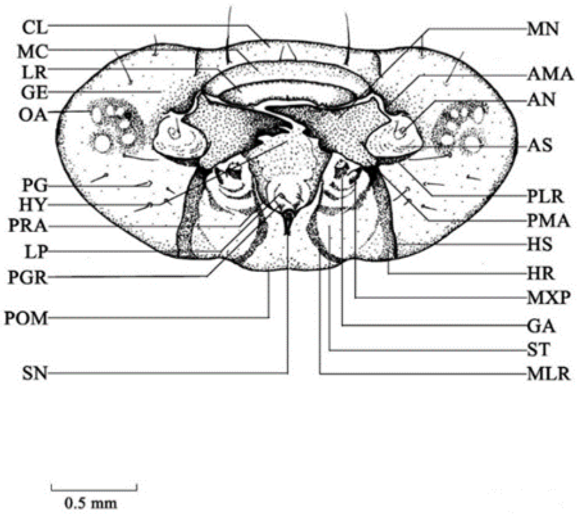

Fig. 8 Ventral surface view of cranial capsule of 4th instar larva of *D. isocrates.* AMA: anterior mandibular articulation, AN: antenna, AS: antennal socket, CL: clypeus, GA: galea, GE: gena, HR: hypostomal ridge, HS: hypostomal suture, HY: hypopharynx, LP: labial palp, LR: labrum, MC: membranous conjuctiva, MLR: maxillolabial ridge, MN: mandible, MXP: maxillary palp, OA: ocellar area, PG: postgena, PGR: palpiger, PLR: pleurostomal ridge, PMA: posterior mandibular articulation, POM: postmentum, PRA: premental arm, SN: spinneret, ST: stipes.

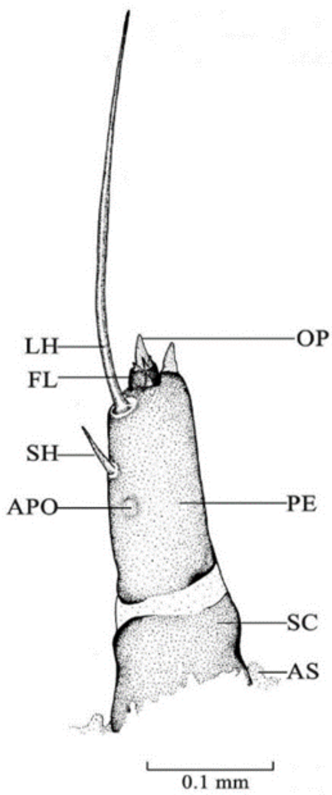

Fig. 9 Antenna of 4th instar larva of *D. isocrates* APO: antennal pore organ, OP: olfactory peg, FL: flagellomere, AS: antennal socket, LH: long hair, PE: pedicel, SC: scape, SH: short hair.

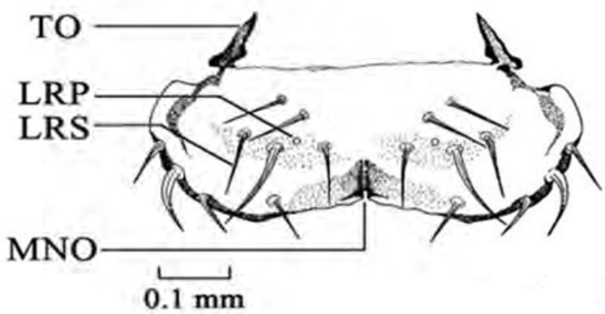

Fig. 10 Frontal surface of labrum of 4th instar larva of *D. isocrates*. LRP: labral puncture, LRS: labral seta, MNO: median notch, SB: sensilla, TO: tormae.

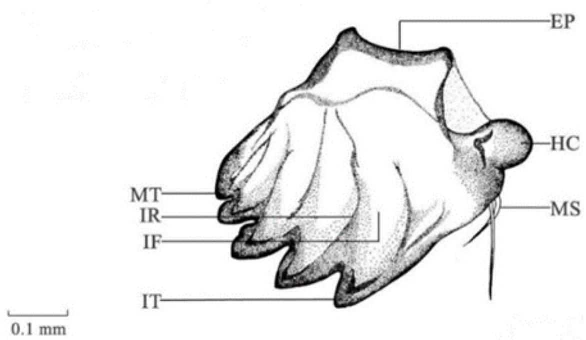

Fig. 11 Inner view of right mandible of 4th instar larva of *D. isocrates*. EP: epichondyle, HC: hypochondyle, IF: inner furrow, IR: inner ridge, IT: incisor teeth, MS: mandibular seta, MT: molar teeth.

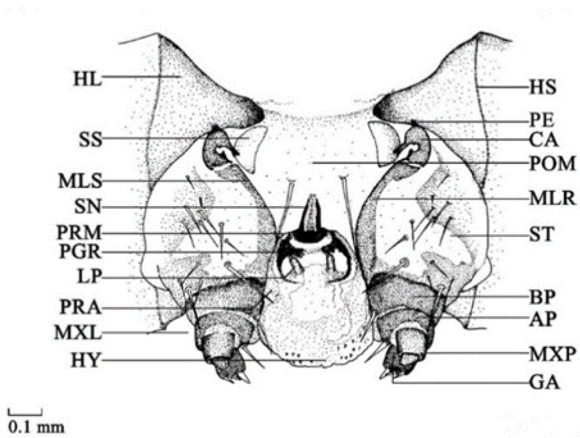

Fig. 12 Maxillolabial-hypopharyngeal complex of 4th instar larva of *D. isocrates*. AP: apical plate, BP: basal plate, CA: cardo, GA: Galea, HL: hypostomal lobe, HS: hypostomal suture, HY: hypopharynx, LP: labial palp, MLR: maxillolabial ridge, MXL: maxillary lobe, MXP: maxillary palp, PGR: palpiger, POM: postmentum, PRA: premental arm, PRM: premental sclerite, SN: spinneret, SS: submental sclerite, ST: stipes.

3.2 Head appendages

In *D. isocrates* a pair of minute antennae (Fig. 9) is situated in the antennal socket. The antennal socket is situated between the pleurostomal ridge and the base of mandible. The antenna is measured 0.299 mm long. The antenna consists of three annuli, scape, pedicel and single flagellomere. The scape is small pale brown basal annulus. The pedicel is the second annulus, which is relatively much longer than the other annuli.

Two socketed hairs lie distally on the lateral part of the pedicel, one of which is very long and protrude anteriorly. The other hair is microscopic, short and heavily sclerotized. On intersegmental membrane between the pedicel and the flagellomere, there are olfactory pegs. These pegs are arranged around the base of flagellomere. There is a pore organ which lies posterior to the socket of the short sclerotized hair. The small flagellomere lies distal to the pedicel. The tip of the flagellomere is also provided with the olfactory pegs. The socketal membrane is unsclerotized, colorless and flexible.

The mouthparts consist of the labrum, the mandibles, the 1st maxillae and labium. Except the labrum other appendages are paired. 1st and 2nd maxillae more or less are fused together so as to form the maxillolabial-hypopharyngeal complex. The morphological observations of the mouthparts result into general lepidopterous biting and cutting type with some special taxonomic features. Labrum is plate like lobe is notched medially at anterior edge. The anterior surface (Fig. 10) bears 3 lateral setae, 3 median setae and a labral puncture.

Mandibles (Fig.11) are masticatory organs articulated to the cranium through anterior (epichondyle) and the posterior (hypochondyle) articular points. The mandibular setae are distributed on the outer surface while the inner surface is produced into distal incisor and proximal molar teeth. The maxillolabial-hypopharyngeal complex is the composite structure (Fig. 12) resulted due to the fusion of maxillae, labium and hypopharynx. In the present study the labial component has been designated as submental sclerite, postmentum and prementum.The prementum has premental sclerite and distal lobe which provides the base for spinneret. A pair of labial palpi is situated one on either side to it and based on palpigers. Each palp is segmented and shows anteriorly projected setae. The hypopharynx present as posterior dorsolateral extension of prementum laterally delimited with premental arms. The surface is covered with transverse rows of spines. The maxilla consists of cardo, stipes and dorsal free lobe. The free lobe bears the inner galea and the outer maxillary palp supported by incomplete sclerite namely basal plate and apical plate.

4 Discussion

The larval stage of the lepidopteran pest is the most harmful to the host plant. In larval *D. isocrates* the hypognathous head bears well developed antennae and gnathal appendages, which are useful for boring the fruit rind and to feed on the seeds of pomegranate fruit.

The cranial features of *P. brassicae* (Eassa, 1963), *P. demoleus*, (Zaka-Ur-Rab, 1964) and *P. xanthorrhoea* (Kumar, 2008) have been studied earlier. The presence of occipital suture in the head of *P. brassicae* is also reported (Eassa, 1963). In present study on *D. isocrates* the cranial peculiarities have been found to be similar to those which are observed *in P. brassicae* (Eassa, 1963). The stemma 1st to 5th are arranged in a semicircle and the stemma 6^{th} is slightly shifted anterior wards of a circumscribed ocellar semicircle in *S. albiguttalis* (Center, 1982) this observation is different from that of *D. isocrates*. Both larvae and adults in Lepidoptera use their olfactory system to detect various compounds, yet their antennae are morphologically distinct (Chang et al., 2017). The antenna has three main parts, a scape, a pedicel and a flagellum which is the main parts of the antenna, and have most of sensory structure (Schneider, 1964). The antenna of Cossidae larva shows antenna at both the sides of the head and lateral to the mandible (Xu et al., 2017). The antennal morphology of *D. isocrates* is found similar to *G. molesta* (Song et al., 2014), and *C. sasakii* (Liu et al., 2011). In *P. xylostella* presence of hypognathous mouthparts consists labrum, mandibles, maxilla, labium and hypopharynx has been reported (Li et al., 2018). These observations are similar to those observed in present study on *D. isocrates*.

The labrum in *A.assamensis* is kidney shaped with two slightly pointed and protruding outer ends (Goldsmith, 2013). Labrum in *B. choui*, is trapezoid and membranous apically and shows two pair of setae. (Tan, 2008) while in *D. isocrates* the labrum is plate like, notched at medially and shows the 3 lateral and 3 median setae. Mandibles are mechanosensory and meant for determining the hardness of the food (Zacharuk, 1980). Each mandible bears distal incisor and proximal molar teeth. These results are in concurrence with the studies on various other lepidopterous species (Richards and Davies, 1977; Center et al., 1982; Desai, 1994; Chapman, 1998). The structural components of maxillolabial- hypopharyngeal complex in *D. isocrates* appear general characteristics of larval lepidopteran mouthparts (Doerkson and Neunzing, 1975; Richards and Davies, 1977; Kumar, 2008; Song et al., 2014). The terminology used to describe morphological feature of these mouthparts is adopted after studies in *P. brassicae* (Essa, 1963)

A more focused research on the sensilla of cranial capsule is needed to understand its phylogeny and taxonomy. The results of the above study have improved our knowledge regarding the morphology of the cranial capsule, antennae and mouthparts of larval *D. isocrates*.

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