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

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Abstract
Deudorix Isocrates (Fab.) is a polyphagous horticultural pest. This is the first study has been done on morphology of adult cranium and its appendages. The cranial surface with gray scales, ring of white scales around compound eyes, alternate ring of black and white scales on antennal flagellum and orange scales on club of the antennae were found as identification marks of adult. The frontal cranial surface reveals central frontoclypeus which extends lower side up to ventral furrow, laterally located gena around the compound eyes. The posterior face of cranium is contributed by rim like post occiput around occipital foramen, postgenal-occipital area, postgenal -occipital protuberance. These areas are separated from each other and the cranial wall is strengthened by postoccipital suture and temporal sulcus as a lycaenid characteristic. Ventrally the cranial surface has hypostomal area with which the siphoning type of gnathal appendages are articulated. The distal part of galea possesses sickle shaped rasping scales and sensilla chaetica, sensilla basiconica, two rows of ridged sensilla styloconica in support to pest activity. The hair sensilla and sunken pegs are distributed on flagellomeres of antennae. Results add to our knowledge of adult cranium and its appendages and provide a standard for further research on the morphological characters of *Deudorix Isocrates* (Fab.) and other members of Lycaenidae.

Keywords: pest, cranial areas, cranial sutures, gnathal appendages, sensilla

Introduction
The morphological characteristic of many insect groups is unexplored. Studies focusing on the morphological aspects of Lepidopteran adults are critical for the filling the gaps in group's systematic and taxonomic knowledge. Results of morphological studies are crucial for analysis of intraspecific variation and useful for classification. There are some major studies on external morphology Papilionidae (Leite *et al*., 2010) [27], Pieridae (Eassa, 1963) [15], Lycaenidae (Sorensen 1980; Duarte *et al*., 2001) [51, 13]. Nymphalidae: Brassolinae (Casagrande, 1979), Morphinae (Bilotta 1992) [4], Ithomiinae (Bizarro *et al*., 2003) [5], Charaxinae (Mielke *et al*., 2004; Dias *et al*., 2010) [33, 11] and Hesperiidae (Miller, 1971) [4].
The cranium of adult butterfly is crucial for feeding and sensory functions. The cranium mainly consist cranial suture, compound eyes, antennae, and mouthparts. The cranium and the sensilla present particularly on the antennae and mouthparts, play key roles in many actions, including host selection, feeding, mate attraction, oviposition, defense, and migration (Schoonhoven *et al*., 1998; Anton *et al*., 2003) [46, 3]. The chemical and mechanical senses are sensed by the receptors on the cranium (Davis & Tammaru, 2019). The lepidopteran mouthparts are best studied in terms of anatomy, morphology and evolutionary biology (Krenn, 2005). The mouthparts of butterflies are diverse in terms of morphology and function. Differences in mouthpart structure can be used as a key feature in identifying and classifying organisms (Brożek, 2014) [6]. The antennal sensilla of insects perform significant roles in insect behaviours (Skiri *et al*., 2005) [49]. The study of cranial morphology is crucial for understanding the behavior and ecology of butterfly. Due to the lack of studies in the majority of the families, the number of descriptive external morphological studies that illustrate cranial morphology is still minimal. Lycaenidae is the second largest butterfly family in the world, with over 6000 species. (Pierce *et al*., 2002) [42]. Significant progress in systematics and taxonomy of Lycaenidae has been hindered due to a lack of data (Duarte, 2007) [12]. The pest *Deudorix Isocrates* is belongs to family Lycaenidae. *D. Isocrates* is a serious pest that attacks pomegranate, guava, peach, and apple crops (Khandare *et al*., 2018) [21]. The majority of *D. Isocrates* research is focused on its life cycle and ecology (Devi & Jha, 2017) [10]. The cranial and mouthpart morphology of *D. Isocrates* larvae are investigated (Patil & Dethe., 2021; Patil & Dethe, 2022). Because the morphology of adult *D. Isocrates* is still obscure, a comprehensive morphological study is required.
The objective of this study is to describe the exterior cranial morphology of adult *D. Isocrates*. In order to improve morphological knowledge and identify features that are more informative that can be employed in systematics. There is currently no data on the cranial morphology of adult *D. Isocrates*. This study provides the morphology of cranium and its appendages in detail, with the goal of better understanding the behavior and morphology of the adult *D. Isocrates*.

Materials and Methods

The infested fruits due to last stage larval instar of *D. isocrates* were collected from pomegranate field and reared in plastic jars up to eclosion of adults. The eclosed adults were observed under dissecting binocular to report general morphological features of cranium. The specimens then preserved in 10% formalin for further use. The light microscopic details were studied using permanent mounting preparations of cranium, compound eyes and cranial appendages like gnathal appendages and antennae. For permanent preparations the cranium was dissected out from the rest of the body, rinsed in distilled water and boiled in10% KOH to make them translucent. As a whole the cranium was processed further to study cranial areas, sulci and articulation of cranial appendages while appendages were dissected out from the cranium to study their morphological details through separate preparation. The cranium and these organs were washed in Distilled water and dehydrated with ethanol at increasing concentrations (30%, 50 %, 70 %, 90% and 100 % for 1 hr. each). The clearing was done with xylene and mounted in DPX. The observations were made under low (10x) and high (45x) magnification of light microscope. Micrometry was used for morphometric report and illustrations were made by camera lucida.

Results

The cranium is covered densely with gray scales. The compound eyes take lateral position and occupy major part of the head. The ring of white scales encircles the compound eyes on anterior face. The antennae show alternate ring of white and black scales and orange scales on the flagellomeres of club. The vertex and posterior face of head capsule both are clothed with brown hairy scales. The labial palpi project anteriorly in front of the anterior face of cranium. Dorsally, each labial palp is clothed with black scales while lateroventrally with white scales. The sclerotized pale brown proboscis is coiled and laterally concealed by labial palpi. The ventral cranial surface is clothed with white scales.

Light microscopic details of cranium and its appendages

The anterior view of cranium (Fig.1) reveals large and bulged compound eyes on lateral portion of the head. The frontoclypeus covers most of the portion of anterior face of cranium. Truly there is no distinguishing mark between frons and clypeus. The gena appears curved strip like around the eye. Ventrally, the clypeal portion is delimited by ventrally arched furrow. The labrum is concealed below the ventral furrow. The anterior tentorial pits are situated immediately, at dorsolateral ends of ventral furrow. Dorsally, the clypeal portion is extended beyond and between the bases of antennae. It reaches up to the median depression of cranial wall. The cranium if viewed anteriodorsally, it reveals the dorsal extension of clypeal portion, frontal portion, median depression at the center of vertex, the transverse groove between antennal bases. The posterior side of cranium (Fig.2) exhibits occipital foramen, postocciput and postgenal-occipital area and the postgenal-occipital protuberance along with temporal sulcus and postoccipital suture. The occipital foramen lies centrally, which is divided in to dorsal and ventral half due to transverse tentorial bridge. the postocciput encircles the occipital foramen and separated from postgenal-occipital area by postoccipital suture. The postgenal-occipital area extends dorsally in the form of postgenal-occipital protuberance which is divided from postgenal-occipital area by temporal suture. The ventral half of occipital foramen is ventrally limited by posterior edge of the labium. The conspicuous compound eyes bear hexagonal facets with circular corneal lens in each facet of an ommatidium. The interommatidial portion is sclerotized and shows interommatidial setae. The ventral surface of head capsule is occupied by ‘hypostoma’ to which the gnathal appendages are articulated. Anteriorly, the hypostoma reaches up to ventral furrow.

Cranial appendages

The cranium of adult *D. Isocrates* possesses siphoning type of gnathal appendages and clavate type of antennae.
The gnathal appendages include an unpaired labrum, paired maxillae give rise to lepidopterist siphoning type of proboscis and labium. The labrum is very narrow, transverse plate. The ventral edge of labrum is bluntly pointed at the median axis. There is straight, pointed, translucent setae like hair are distributed submarginally to the ventral edge. Proximally, the labrum has thick, sclerotized plate. The Maxilla from each side contributing siphoning type of mouthparts. Each maxilla consists of proximal sclerite like cardo, stipes and distal elongated galea with ‘c’ shaped concave structure. The ‘c’ shaped galea from both the sides faces each other and articulated leaving a tubular food channel in between the two. The cardo is oval sclerite. It articulates with the hypostoma. Stipes is large elongated sclerotized plate. It is articulated with cardo by a narrow proximal end. With wide distal end, it is articulated with galea. Ventral surface of stipes bears a central dark brown groove, which runs from proximal to distal end of the stipes and joins itself to the base of galea. Elongated galea bears transversely placed alternate chitinized ring and a membranous band. The formation runs up to distal tip of the galea. Distally the galeae bears two rows of styloconic sensilla distributed on dorsal surface of the tube. Each styloconic sensillum (Fig.3) consists of two parts: i. sensillum and ii. cuticular ridged projection. The sensillum is situated on the tip of cuticular projection, which bears 5-6 lengthwise ridges. Dorsal surface of galea in this region is also provided with sharp edged sickle shaped rasping scales. Sensilla chaetica and sensilla basiconica are also evident on distal part of proboscis. The labium is roughly triangular plate, which occupies central part of the ventral surface of the head capsule. The labium is immediately adjacent to the occipital foramen. The posterior edge of labium is arched posterior wards slightly. A pair of labial palpi is articulated with the labium at proximolateral angle of the labial plate. The labium is much extended anteriorly and reaches up to the base of proboscis. The small portion of forwardly extended region is erected. So that it appears as vertical wall. The edge

of this erected wall is provided with a tuft of hair. The microscopic sensilla are present at the distal end of labium. The labial palp consists of three palpomeres. With the proximal palpomere, the labial palp is articulated to the labium. This segment is roughly curved. The middle palpomere is elongated and tapers gradually towards distal end. The distal palpomere is spatulate. The paired antennae are fixed in antennal socket through the scape. It is comparatively large and somewhat dome shaped. The annulus distal to scape is the pedicel. It is articulated to scape by its proximal end while by its distal end it is articulated to the flagellum. Flagellum is the rest portion of antenna made up of flagellomeres. The number of flagellomeres in male ranges from 36 to 39 (n = 25) whereas in female the range is between 37 to 42 (n = 25). The basal portion of flagellum consists of rectangular flagellomeres but some distally situated flagellomeres become broad and the region is then transformed into a "club". In general, from first basal flagellomere, each flagellomere shows single hair like sensillum, which lies on middle region. The hair like sensillum is transparent and straight. It is directed towards the club. Besides the said sensilla, the flagellomeres of club bear much blunt hair sensilla. The number of these sensilla goes on increasing from basal flagellomere of club to the apical flagellomere. Similarly, the sunken pegs are present on the flagellomeres of the club. The pegs are situated in the circular depressions.

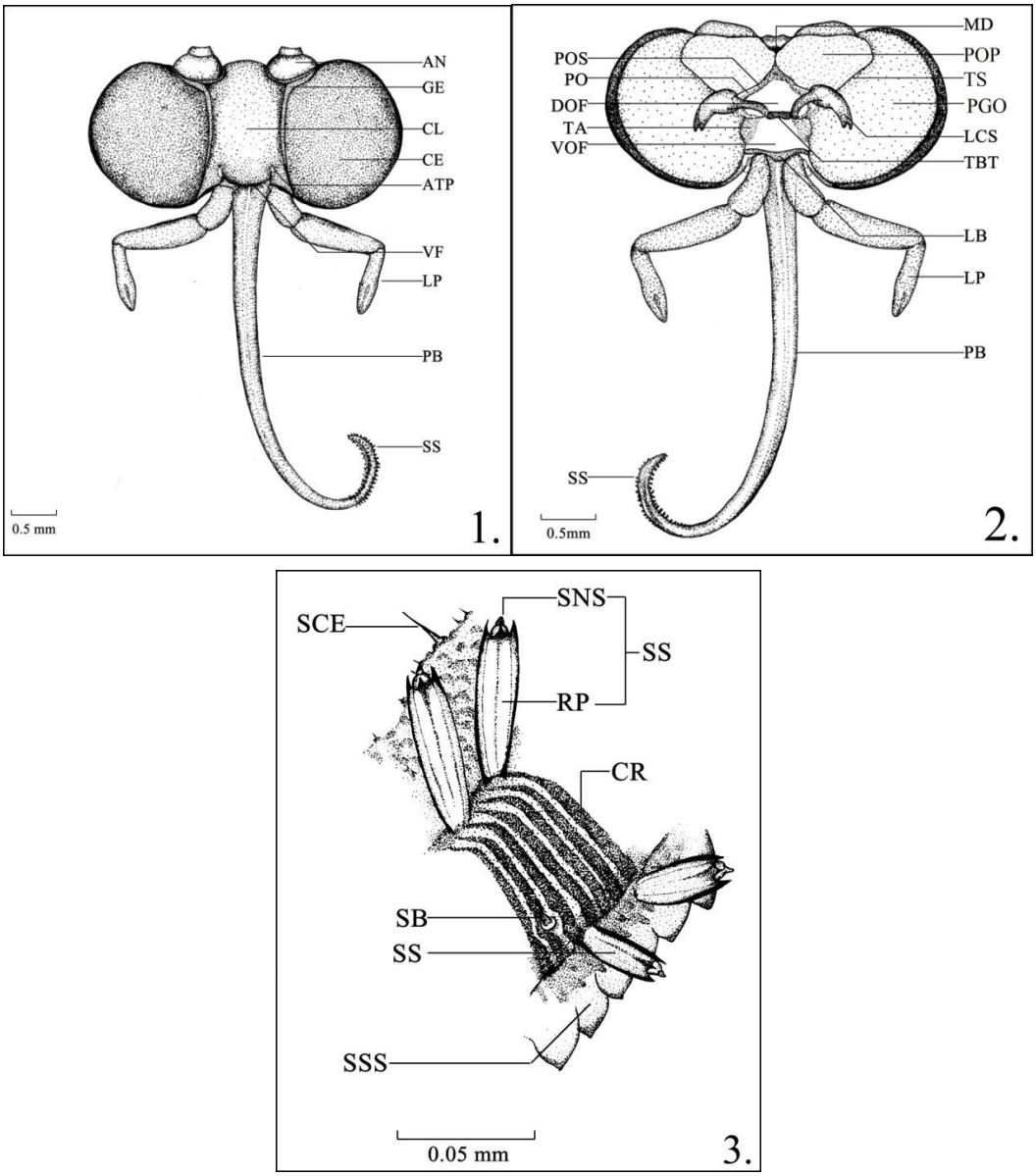

Fig 1-3: 1. Frontal view of cranium of adult *D.isocrates* (Flagellum of antenna removed) 2.Posterior view of cranium of adult *D.isocrates.* (Flagellum of antenna removed) 3. Distal region of galea (in part magnified) of adult *D.isocrates*. AN: Antenna, ATP: Anterior tentorial pit, CE: Compound eye, CL: Clypeal portion of frontoclypeus, CR: Chitinized ring, DOF: Dorsal half of occipital foramen, GE: Gena, LB: Labium, LCS: Lateral cervical sclerite, LP: Labial palp, MD: Median depression, PB: Proboscis, PGO: Postgenal-occipital area, PO: Post occiput, POP: postgenal-occipital Protuberance, POS: Post occipital suture, SB: Sensilla basiconica, SCE: Sensilla chaetica SNS: Sensillum, SS: Sensilla styloconica, RP: Ridged projection, SSS: Sickle shaped scales.TA: Tentorial arm, TBT: Transverse tentorial bridge, TS: Temporal sulcus, VF: Ventral furrow, VOF: Ventral half of foramen

Discussion

The cranial surface is densely clothed with various types of scales and their different patterns like uniform distribution of gray scale on anterior surface, white ring around each compound eye, orange scales on distal annulus of antennae and black scales on dorsolateral part of labial palpi were found as a species-specific characteristic in *D. isocrates*. However, the cranium covered with scales is the general lepidopterist feature of adult cranium. To study microscopic details of cranium necessarily the scales were removed before proceeding for permanent slide preparations. In present study the details of cranial areas and sutures are described by the terms have been adopted from the fundamental classical work on insect morphology (Sharplin, 1963; Matsuda, 1965, 1970, 1976; Kristensen, 2003) [48, 25]. The frontal view of cranium exhibits frontoclypeus as an anterior face of cranium delimited dorsally by antennal suture and ventrally by clypeolabral boundary as it is reported in the Nymphalidae (Kawahara *et al*.,2012) [20] and Papilionidae (Leite *et al*.,2010) [27]. An inverted 'V' shaped elevated frons around median depression is appeared as a special feature of frontal area in *D. isocrates*. There is no trace of frontoclypeal suture (Duporte, 1946) in *D. isocrates*. This observation is supported by previous work on lepidoptera (Casagrande, 1979; Sorensen, 1980) [8, 51]. That is why in the present work the clypeus and frons are appeared without distinction. A pair of anterior tentorial pits located. The anterior tentorial pits are located laterally along the frontoclypeal sclerite, at dorsolateral ends of ventral furrow which acts as clypeolabral boundary to separate clypeus from ventrally located labrum. It is clypeal-labral articulation (Miller, 1971) [4]. In present work, this structure is referred as clypeolabral sulcus (Sorensen, 1980) [51] which is present in the form of ventral furrow in *D. isocrates*.

Dorsolateral large and bulged compound eyes of *D. isocrates* exhibit hexagonal ommatidia with circular corneal facet in each ommatidium and interommatidial setae under the light microscope. The interommatidial setae have also been reported in a member of Lycaenidae, *G. lygdamus* (Sorensen, 1980) [51] and in *A. unifascia* (Kristensen, 2014). The same structure has been termed as 'Setaeform hair' (Adamski & Peters, 1982) [1]. The gena on the anterior face appears band likearea runs parallel to the eyes, which may be reduced because of the development of conspicuous eyes (Michener, 1953) [32]. Some authors described this band in lepidoptera as ocular sclerite (Casagrande, 1979; Bilotta, 1992) [8, 4]. and paraocular area (Sorensen, 1980) [51].

The most of the posterior face of cranium is occupied by postgena (Snodgrass, 1960, Kawahara, 2012; Queiroz-Santos *et al*., 2018) which covers the back of compound eyes and expanded centrally towards occipital foramen. In Lycaenidae, this cranial area has been referred as postgenal-occipital area (Sorensen, 1980) [51]. This area is cleaved dorsolaterally by temporal sulcus which delimits the temporal protuberance. These features are in concurrence with the present study on *D. isocrates.* The postocciput is appeared as the most restricted ring like cranial area around the occipital foramen (Leite *et al*., 2010) [27] and bound with postoccipital sulcus are the usual lepidopterists characteristics found in *D. isocrates*. The occipital foramen is divided in to dorsal and ventral halves due to transverse tentorial bridge. This observation is supported by earlier work on papilionid species, *Heraclides anchisiades capys* (Leite *et al*., 2010) [27]. Ventrally the cranium exhibits hypostomal-postgenal sulcus which separates the laterally located postgena and central hypostoma. Bases of gnathal appendages lie in the centre of the cranium.

Siphoning mouthparts, consisting of proboscis and labial palps, are the exclusive feeding organs and important chemosensory organs in most adult Lepidoptera (Guo *et al*., 2018) [18]. The present study on gnathal appendages revealed the microscopic details of siphoning mouthparts in the form of proboscis which is contributed by maxillae and particularly composed with galea. The cardo and stipes are clearly evident while maxillary palps are absent. Absence of maxillary palp in *D. isocrates* is supported by earlier work on Hesperiidae (Carneiro, 2012). The present study revealed three types of sensilla like Sensilla chaetica, sensilla basiconica and sensilla styloconica with their particular pattern of distribution on the galea. Four types of sensilla have been reported in various lepidopterans, such as Nymphalidae (Molleman *et al*., 2005; Ômura *et al*., 2008, Ômura *et al*., 2009) [35, 38, 37]. Micropterigidae (Krenn, 2010) [23], Noctuidae (Guo, *et al*., 2018) [18] Plusiinae and Noctuinae (Xue *et al*., 2016; Wang, 2012). According to previous studies, sensilla chaetica, sensilla basiconica, and sensilla styloconica are common types of sensilla in Lepidoptera (Krenn, 1998; 2010; Faucheux, 2013) including Lycaenidae (Ma *et al*., 2019). These reports support the present observations in *D. isocrates*. However, ultrastructural studies on these sensilla are needed to find their details. The studies on sensilla reflects taxonomic relationships and has been used to infer phylogenetic relationship (Paulus & Krenn, 1996; Castro Gerardino & Liorente Bousquets, 2019) [41, 9]. Besides the sensilla the tip of galeae bears rasping sickle shaped scales which are also reported in (Goldware & Barnes, 1973) [17]. The present study also revealed absence of mandibles, labium in the form of reduced small plate (Richards & Davies, 1977) [44], segmented labial palpi with three palpomeres (Kawahara *et al*., 2012) [20] as the usual lepidopterist mouthparts. However, more emphasis is needed to find details of sensory organs on labial palp. The clubbed antennae of *D. isocrates* shows two types of hair sensilla and sunken pegs under the light microscope.

Present study on antennae of *D. isocrates*, revealed hair sensilla and sunken pegs on flagellomere of its antenna with the aid of light microscope. However, details of hair Sensilla and sunken pegs could not be observed under the light microscope. The antennae of insects have various types of sensilla that play important roles in insect behaviors, including host location, feeding, mate attraction and oviposition (Skiri *et al*., 2005) [49]. Recently antennal morphology and sensilla have been recorded in different lepidopteran species including the crop pests (Seada, 2015; Yan *et al*., 2017). The hair sensilla observed in the present work are correspond to the trichoid sensilla reported in these earlier reports and Presence, of sunken pegs on flagellomeres in present study concur

with the observations reported in *D. gilippus berenice* (Myers,1968) and in *Plutella xylosella* (Yan *et al*.,2017). But still the ultrastructural studies on the antennal sensilla in *D. isocrates* is needed.

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