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Scanning electron morphological studies of *Tribolium confusum* Jacquelin du Val (Coleopteran: Tenebrionidae)

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Abstract

Background: The confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleopteran: Tenebrionidae) is the most destructive pest of stored products worldwide. It is the most common pest of wheat flour.

Results: This study describes and illustrates the larvae, pupae, and adults of *T. confusum* using scanning electron microscopy. The first larval instars are 5.0–5.1 mm long and 0.5–0.6 mm wide whereas the last larval instars are 5.75–6.9 mm long and 0.75–0.95 mm wide. Adults of *T. confusum* are reddish brown elongate beetles (4.0–4.5 mm in body length and 1.0–1.2 mm in width). Electron micrographs revealed the structure of the mouth parts during the larval, pupal, and adult stages as well as the structure of thoracic and abdominal appendages. Results indicated that the setiferous sex patches which were reported in males can often be used for sexing specimens. A specific feature of the first instar larvae of *T. confusum* is the extreme shortened antenna with a reduced number of antennomeres and the presence of well-developed and moderately long legs.

Conclusion: SEM examination may help us not only discover and understand new morphological details as the pits with spine on the elytra and the spikes on the membrane wings which will facilitate the identification of this species but also clarify the functions of various body parts.

Keywords: Coleoptera, Tenebrionidae, Larva, Pupa, Adult, Scanning electron microscopy, *Tribolium confusum*

Background

Tenebrionidae is one of the most diverse families within Coleoptera (beetles) and is very difficult to classify (Aalbu, Triplehorn, Campbell, Brown, Somerby, et al., 2002).

Studies of immature and adult stages of insects are very important and useful for classification of particular groups which were seeing several times (Beutel & Friedrich, 2005).

Taxonomic studies on immature stages of the family Tenebrionidae are rare because the knowledge about the developmental stages of this family is very limited (Jia, Ren, & Yu, 2013). This is particularly true for tenebrionid pupae and larvae. For the tenebrionid pupae, several workers have made their contributions as (Daggy, 1946; Abdulla, 1964; Spilman, 1966, 1969; Bouchard & Steiner, 2004; Cherney, 2006; Gosik, 2007; Dunford & Steiner, 2007; Simoes et al., 2009; Matthews et al., 2010 and Jia et al., 2013), but few studies including the tribe Optrini,

was reported by Cherney (2005) and Cherney and Fedorenko (2006).

The red flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae), is the most widespread and withering major insect pest of stored cereals in the world. Hana (2013) had reported the percentage of damages of these insects ranging from 5 to 30% of the world's total agriculture production. Freeman (1973) had reported that the success of the spreading of these insects is due to different evolutionary adaptations to the actions of humans who transport them through the world and give a protective habitat for stored food stuffs.

The aim of the present work was to study the morphology of different stages of *T. confusum* to explore new morphological details which will facilitate the identification of different stages of this species.

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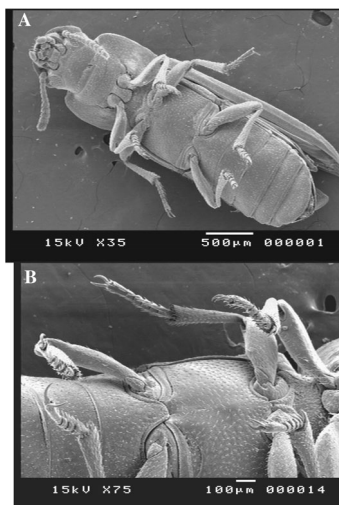


Fig. 1 **a** SEM micrograph of the adult of *T. confusum*. **b** Higher magnification of the adult *T. confusum* showing the legs consists of coxa, trochanter, femur, tibia, and tarsi

Methods

All experiments were done according to the guidance and animal care ethics of Sohag University, Egypt. Larvae, pupae, and adults of *T. confusum* were collected from the flour in the laboratory and fixed in 70% alcohol. Pupae were examined using binocular microscope to differentiate between male and female pupae. Specimens were prepared for examination by subjecting them to dehydration series of 90%, 95%, and absolute alcohol. Specimens were then critically point dried and coated with gold using sputter coating for examination using a scanning electron microscope (SEM), JEOL, JSM 5300.

Results

Adults of *T. confusum* are reddish brown elongate beetles varying from 4.0 to 4.5 mm in body length and from 1.0 to 1.2 mm in width (Fig. 1a). Males and females are morphologically indistinguishable by light microscopy. Using scanning electron microscopy, the setiferous sex patches which were reported in males (Fig. 2b–d) can often be used for sexing specimens. The setiferous patch was found on the fore, middle, and hind femur of males. The patches lie in a nearly circular depression on the ventral surface of the femur of fore and hind femur, but small in size in the middle femur (Fig. 2b–d). The setae present in the patch are nearly of the same size. The depression containing the setae is deeper and has almost vertical walls and a strong ring separates it from the surrounding integument (Fig. 2b). The setae are slope such that their tips project through the opening of the depression. The integument beyond the ring has a minisculpture composed of transverse polygons (Fig. 2b).

The head of adult *T. confusum* is visible from above, it has no beak, and the thorax is slightly parallel on its sides (Fig. 1a). Legs consist of coxa, trochanter, femur, tibia, and five tarsi in the fore and middle legs and four tarsi in the hind legs ending with two claws (Fig. 1b) which are simple, curved apically, and are equipped with sensillae (Fig. 2a). The antenna of adult *T. confusum* is composed of 11 annuli, the scape, the pedicle, and a flagellum of nine annuli (Fig. 3a). The annuli are cylindrical in shape and generally the length decreased near the distal end of the antennae (Fig. 3a).

The adult mandible is short, and the posterior edge have many spines (Fig. 3b). The base of the maxilla consists of two segments, the cardo contains a single sclerite which is semicircular in shape in adults; the main sclerite of the

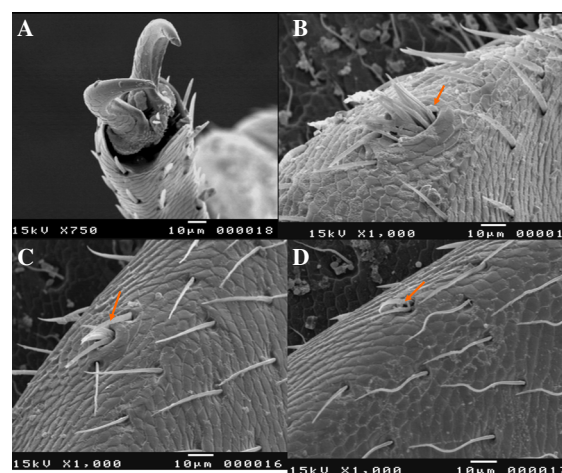


Fig. 2 **a** Higher magnification of the legs of *T. confusum* showing two claws which are simple, curved apically, and are equipped with sensillae unequally distributed. **b–d** Higher magnification of the legs of the male of *T. confusum* showing the setiferous patch found on the ventral surface of the fore, hind, and middle femur, respectively (arrows)

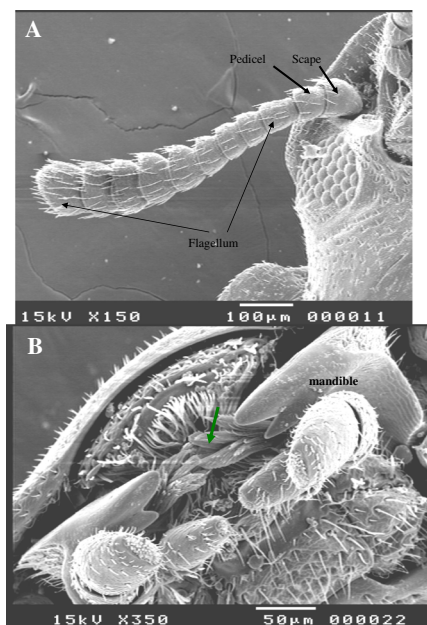


Fig. 3 **a** SEM micrograph of the antenna of the adult of *T. confusum*. **b** Higher magnification of the head of the adult of *T. confusum* showing the mouth parts, the mandible with highly sclerotized appendage. The galea with brush of spines distally (green arrow)

stipes is triangular and articulates with the cardo along its diameter (Fig. 4a). The galea has a brush of spines at its apex (Fig. 3b). The lacinia is short, not extending beyond the galea with bidentate apical (Fig. 4a). The maxillary palp consists of four segments (Fig. 4a). The distal palp segment is elongated and terminates in a field of sensory receptors (Fig. 4a, b).

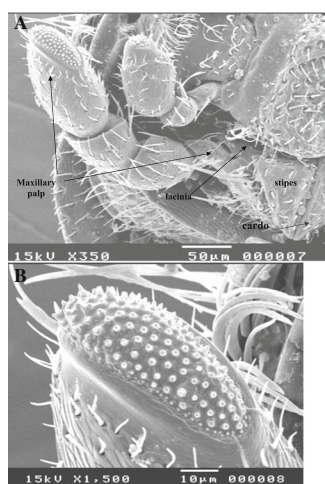


Fig. 4 **a** Higher magnification of the mouth parts showing the maxilla which its base consists of two segments, the cardo, and the stipes. The lacinia is short with bidentate apical. The maxillary palp consists of four segments. **b** Higher magnification of the distal palp segment which is elongated and terminate in a field of sensory receptors

The adult labium is a midventral structure; its base consists of the mentum, which contains a single sclerite (Fig. 5a). Distal to the mentum is the prementum to which the lateral labial palps are tied (Fig. 5a). Between the labial palps is the ligula, a structure fringed with long distal setae (Fig. 5a). The adult labial palps consist of three segments and, like the maxillary palps, terminate in a field of sensillae (Fig. 5b).

The structure of last larval maxilla differs from the adult maxilla in having only three palp segments (Fig. 6a). Two larval endites are present in the two maxillae. In larval labium two segmented labial palps are present (Fig. 6a).

The antenna of the larval stage of *T. confusum* consists of three segments inserted in a membranous structure called antacorium which connects it to the head capsule. The antenna consists of a basal subcylindrical segment (antennomere I), a segment equipped with a lobe (antennomere II) and an apical segment (antennomere III) which is both tiny and elongated internally situated on the apex of antennomere II and bearing a long seta (Fig. 6b). Most last larval instars are varying from 5.75 to 6.9 mm long and 0.75 to 0.95 mm wide. The head, thorax, and abdomen are covered with minute spines (Fig. 7a). The first larval instars are 5.0–5.1 mm long and 0.5–0.6 mm wide. A specific feature of the first instar larvae is the extreme shortened antenna with a reduced number of antennomeres. Another feature is the presence of well-developed and moderately long legs (Fig. 8a, b) and (Fig. 9a).

General body shape of the larvae is as follows: elongate, cylindrical, body mostly white, weakly sclerotized surface with sparse vestiture of whitish setae, segment IX dorsally

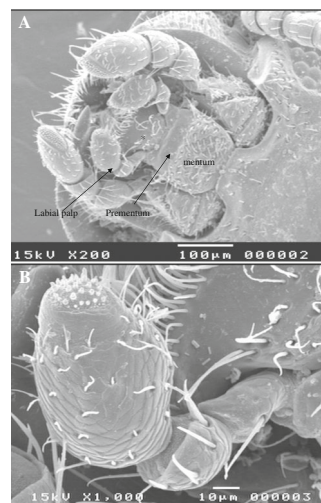


Fig. 5 **a** Ventral view, of SEM of the mouth parts of adult *T. confusum* showing the labium which includes the mentum, prementum, ligula (asterisk), and the labial palps. **b** Higher magnification of the adult labial palp which consists of three segments terminates in a field of sensillae

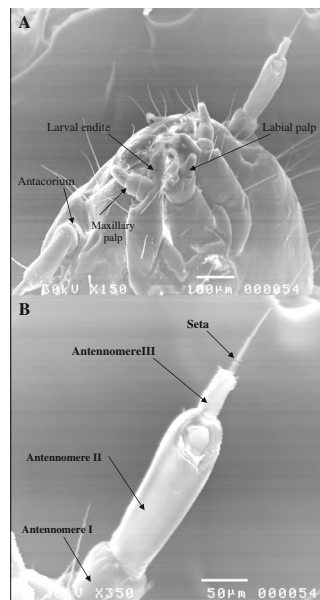


Fig. 6 **a** Higher magnification of ventral view of the last larval instar of *T. confusum* showing the mouth parts showing the maxilla having only three palp segments, the larval endites, the labial palps consists of two segments and the antacorium. **b** Higher magnification of the antenna of the last larval instar which consists of three segments terminates in a long seta

forming divided sclerite (pygidium) (Fig. 7a–d). Urogomphi are appendages of tergum IX of beetle larvae of *T. confusum* which are reported for the first and last larval instars (Fig. 7c, d). Segment X is not visible in dorsal view; it is inserted on the ventral side posterior to sternum IX and may be represented by two lobes (Fig. 7c, d) and (Fig. 9b) which is probably developed as a pygopod. In the larval stage, there were three pairs of legs each of them consists of five segments (Figs. 7a and 9a); these are coxa, trochanter, femur, tibia, and tarsus, and a single claw is present in the first and last instar larvae.

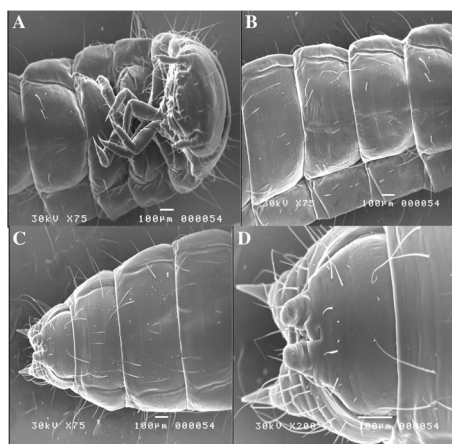


Fig. 7 **a–d** SEM of last larval instar of *T. confusum*

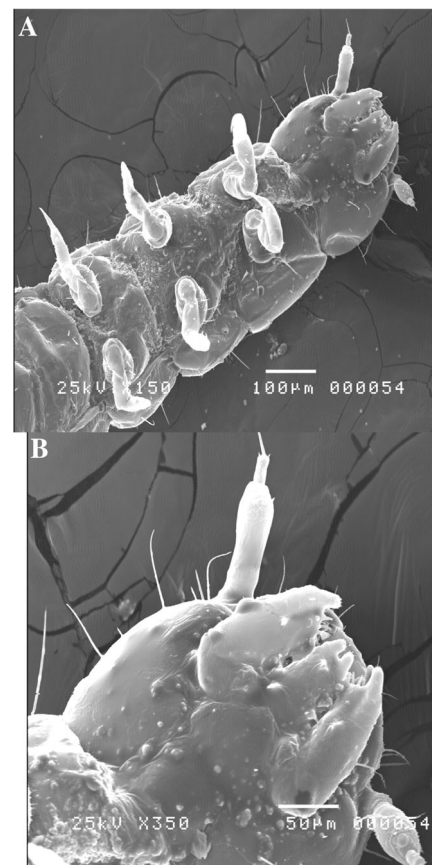


Fig. 8 **a** SEM of ventral view of the first larval instar of *T. confusum* showing the head, thorax, and the legs. **b** Higher magnification of the ventral view of the first larval instar of *T. confusum* showing its mouth parts and antenna

The mature formed pupa is white to light yellow and the head is depressed beneath the pronotum (Fig. 10a). The male pupa measured about 3.25–4.15 mm in length (from the anterior edge of pronotum until before the urogomphi) and 0.95–1.25 mm in width and the female pupa measured about 3.6–4.0 mm in length and 1.0–0.25 mm in width. The pupal abdomen was conical and the last segment has two pointed structures, these are the urogomphi (Fig. 10b). The female genital papillae, which are much larger than those of the male, are two finger-like structures just anterior to the pointed urogomphi (Fig. 11a, c). The male papillae are small enough that they look like just fingertips (Fig. 10b). Pupae of beetle *T. confusum* have jaws called gin traps (Fig. 11b) on the lateral margin of their jointed abdominal segments from segment 1 to 6 (Fig. 11b, c).

A beetle's first line of defense against environmental factors is their mesothoracic elytra, rigid, and protective forewings. The original elytra of *T. confusum* revealed hexagonal structuring and their vestiture, setose decumbent, curved, smooth-sided, tapering to a point, and

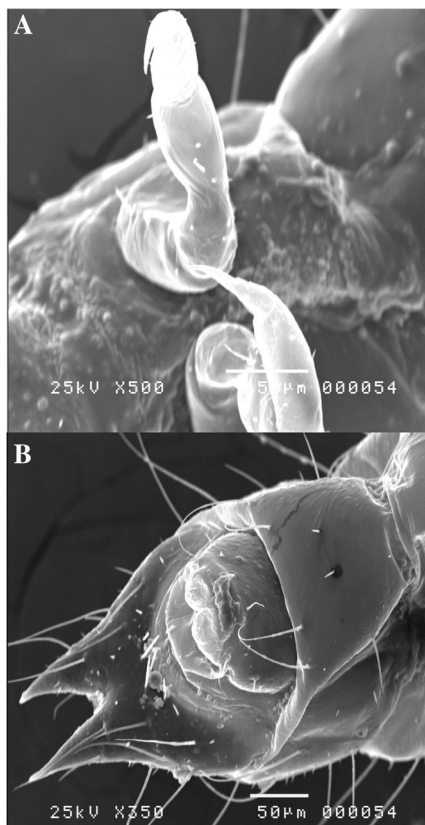


Fig. 9 **a** Higher magnification of the legs of the first larval instar of *T. confusum*. **b** Higher magnification of the last abdominal segments of the first larval instar of *T. confusum*

arising centrally in the pits. The pits have dimple-like depressions with polygonally sculptured sides and floor, with large pores posteriorly close to the base of each seta or the pores are largely close to the margin of each pit (they lie inside the pit). The pores on the *T. confusum* elytra are abundant and simple in structure (Fig. 12a, b and Fig. 13a). SEM image of the upper surface of the hind wing membrane of *T. confusum* contains numerous spikes. Figure 13b shows that these spikes are randomly distributed and vertical on the surface of the wings.

Discussion

Experiments carried out by Huet and Ienoir-Rousseaux (1976) and Daly and Sokoloff (1965) on the tenebrionid beetle *Tenebrio molitor* demonstrated that the entire larval appendage developed to the adult appendage and that specific adult structures within each appendage generally arise by transformation of their corresponding larval structure. Thus on the basis of its relatively close relationship with *Tenebrio* (Daly & Sokoloff, 1965) on *T. castaneum* (David, Frank, Ariel, Moto, & Elizabeth, 2012), the adult mouth parts are assumed to be formed

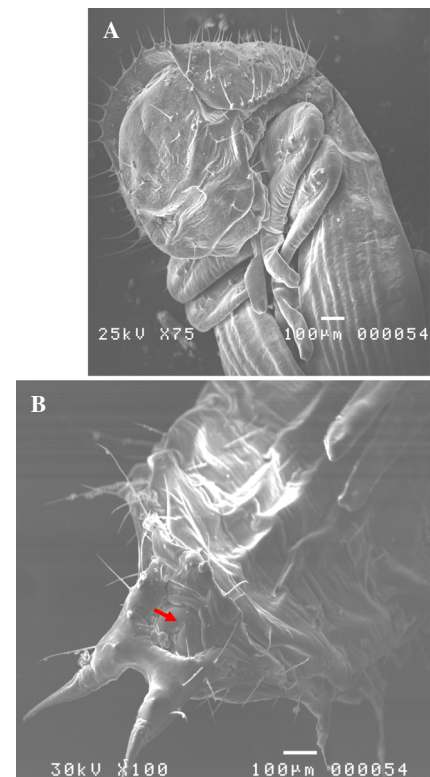


Fig. 10 **a** SEM of ventral view of mature formed pupa showing the head is depressed beneath the pronotum. **b** SEM of ventral view of the last abdominal segments of male pupa showing its papillae look like fingertips (red arrow)

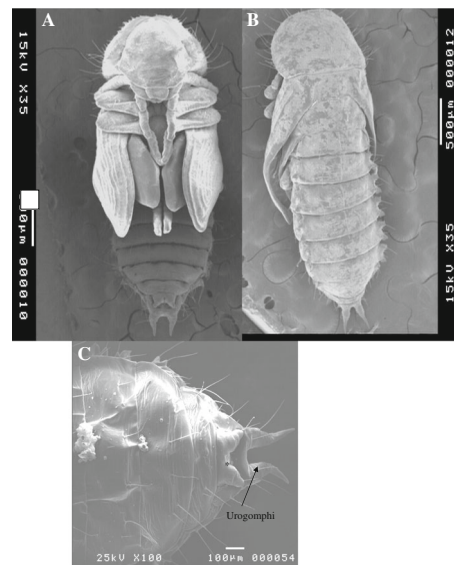


Fig. 11 **a** SEM of ventral view of female pupa of *T. confusum*. **b** SEM of dorsal view of female pupa of *T. confusum*. **c** Higher magnification of the ventral view of the last abdominal segment of female pupa showing the genital papillae (asterisk), are two finger-like just anterior to the pointed urogomphi

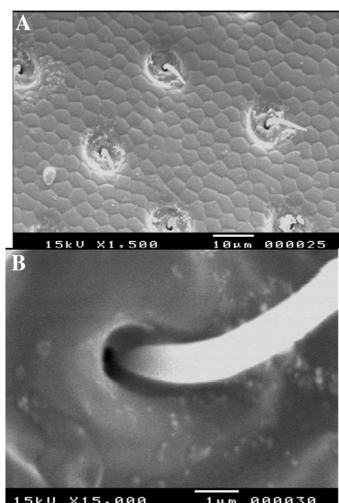


Fig. 12 **a** SEM of the elytra showing the pits. **b** Higher magnification of the elytra of *T. confusum* showing the seta with large pore posteriorly close to the base of the seta

by transformation of their larval precursors. This contrasts with *Drosophila*, where larval appendages exist as small sensory organs and adult appendages develop from imaginal discs that are set aside during embryonic development (David et al., 2012).

In the present study, a specific feature of the first instar larvae of *T. confusum* is the extreme shortened antenna with a reduced number of antennomeres; these results agree with that reported by Ross and Potheary (1970), Beutel and Hornschemeyer (2002), and Margarita et al. (2015). Another feature is the presence of well-

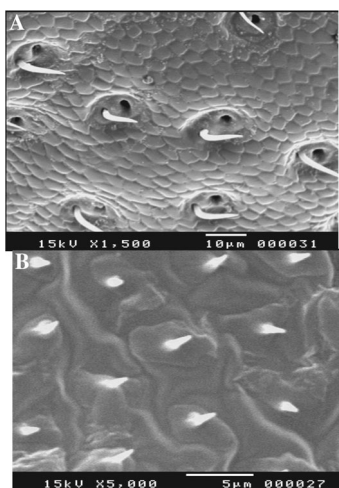


Fig. 13 **a** Higher magnification of the elytra showing the seta with large pore close to the margin of each pit. **b** SEM of upper surface of the hind wing membrane of *T. confusum* contains numerous spikes

developed and moderately long legs; these results agree with that reported by Ross and Potheary (1970) and Margarita et al. (2015).

Urogomphi are appendages of tergum IX of beetle larvae of *T. confusum* which are reported for the first and last larval instars. Urogomphi are prominences of tergum IX of beetle larvae (e.g., Lawrence et al., 2010). In some alticine (e.g., *Chaetocnema* and *Psylliodes*) and galerucine (e.g., *Diabrotica duodecimpunctata*, *D. vittata*) larvae, a pair of simple curved urogomphi is present. A single median terminal process occurs in larvae of *Phyllotreta cruciferae*, *P. atra*, and *P. vittula* (Boving, 1930; Cox, 1988; Cox, 1981). Yi et al. (2014) reported that segment IX is dorsally forming undivided semicircular and subtriangular sclerite (pygidium) for galerucine and alticine larvae.

In the present study, after describing the different stages of *T. confusum*, the urogomphi of the pupae are diverging from each other, these results agree with that reported by Jia et al. (2013) who identified two main types of urogomphi in the Optartini pupae. The urogomphi in *Scleroporum horridum horridum*, *Gonocepholum reticulatum*, *Opatrum (Opatrum) subaratum*, *Penthicus (Myladion) alashanicus*, *Penthicus (Myladion) nojonicus*, and *Melanesthes (Optronesthes) rugipennis* are identical. In these species, the urogomphi are diverging from each other. Also, Jia et al. (2013) reported that the urogomphi in *Eumylada potonini*, *Eumylada punctifera*, *Melanesthes (Melanesthes) maxima maxima*, *Melanesthes (Melanesthes) jintaiensis*, and *Myladina unguiculina* are parallel to each other.

Also, Steiner (2014) described the pupa of *Glyptotus cribratus* which is bearing large divergent urogomphi. The urogomphi of pupa of *Cibdelis blaschkei* are long, wrinkled at base, gradually tapered to divergent sharp apices.

In the present study, *T. confusum* on the first and last larval instar, segment X is not visible in dorsal view, it is inserted on the ventral side posterior to sternum IX and may be represented by two lobes which are probably developed as a pygopod. These results agree with that reported by Margarita et al. (2015) who reported the structure of segment X for the first larval instar of *Tenomergera mucida* that the greatly reduced segment X is inserted on the ventral side posterior to sternum IX, it is represented by two pairs of flattened, sclerotised anal flaps, the larger flap bears two long setae, the smaller ventromesal flap one long seta, and several short ones along its rounded posterior edge; the latter are fused basally but divided by a deep median cleft.

In contrast to Steiner (2014) who reported the absence of pygopods on the mature larva of *Glyptotus cribratus*, the abdominal segment X is small, ventral, transverse, semi-circular, convex, three times wider than long, with

row of six fine setae across width. Also, Steiner (2014) described the mature larva of *Cibdelis blaschkei* in which abdominal segment X is small, ventral, transverse, semi-circular, convex, 2.5× wider than long, with a row of six fine setae across width, and pygopods absent.

Beutel and Hornschemeyer (2002) reported that the eversible ventral lobes of segment IX are composed of a weakly sclerotized proximal part and a largely membranous distal element is present in larvae of *Micromalthus* and Cupedidae (Lawrence, 1991). Beutel and Hornschemeyer (2002) reported that the eversible ventral lobes of segment IX may facilitate locomotion in galleries. Also, Beutel and Hornschemeyer (2002) reported that segment X is not visible externally in all larvae of Archostemata. It is exposed in larvae of most other groups of Coleopteran (e.g., Adephaga, Staphyliniformia) (Frank, 1991; Newton, 1991).

Beutel and Hornschemeyer (2002) reported the absence of urogomphi from tergum IX of all larvae of Archostemata but they are present in most larvae of Adephaga, in Torridincolidae (Beutel, 1999b) and in larvae of many groups of Polyphaga (e.g., Hydrophiloidea, Histeroidea, and Staphylinoidae) (Frank, 1991; Newton, 1991).

In the present study, the presence of setiferous patches on the fore, middle, and hind femur of the legs were reported in male of *T. confusum*. Their basic structure, comprising a depression-containing dense, fluted or grooved setae, and numerous pores, is similar to that found in the setiferous sex patches of other Coleoptera (Faustini & Halstead, 1982).

Conclusions

So, using SEM examination add some knowledge about morphological details of different stages of *T. confusum*, the setiferous sex patches in males, the structure of the mouth parts of adult and larval stage, the pits with spine on the elytra, and the spikes on the membrane wings which will facilitate the identification of *T. confusum* and may help to clarify the functions of various body parts.

Based on the current results, the future work will concern mainly with the morphological, histological, and biochemical studies of this species and others.

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I agree to the publication.

Competing interests

The author declares that he/she has no competing interests.

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