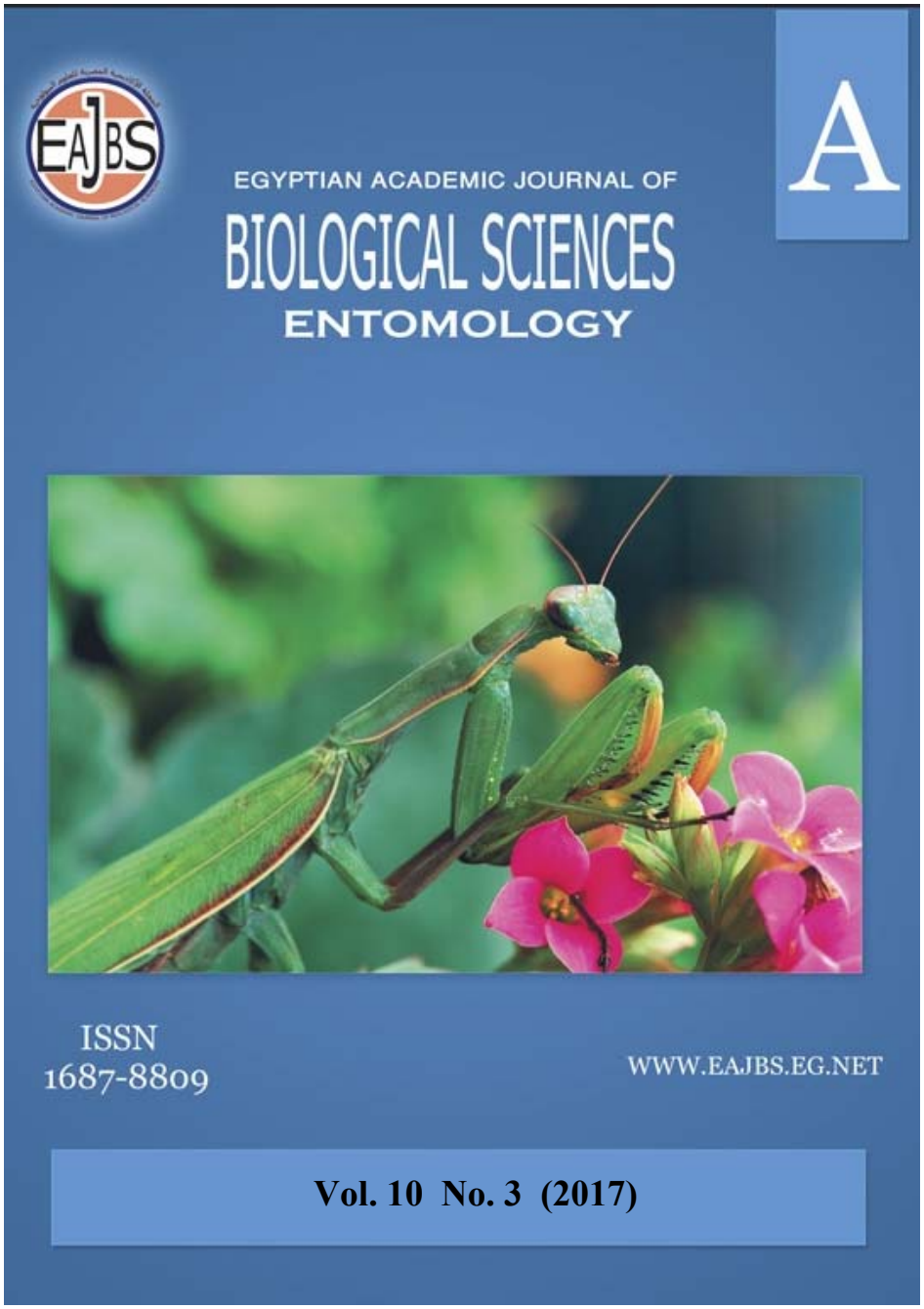


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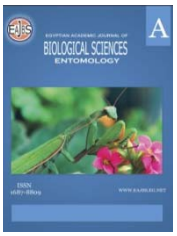

# Scanning Electron Microscope of Genus *Leichenum* Dejean, 1834 (Coleoptera: Tenebrionidae: Tenebrioninae: Pedinini) in Egypt

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## ABSTRACT

In Egypt genus *Leichenum* is represented by two species, *Leichenum mulleri* Grid., 1939 and *Leichenum pulchellum* Kust**.,** 1849. Few studies have reported on both species all over the world. Both species are difficult for species identification so, the present study was examined specimens by SEM and found microstructural characters that facilitate identification of them. A number of new characters were added to the previous e.g. shape of setae and other vestitures, shape and structure of the intercoxal processes and submentum, also some old characters were emended.

## INTRODUCTION

Alfeiri (1976) and El-Torkey *et al.* (2009) classified genus *Leichenum* within subfamily Opatrinae, tribe Leichenmini. Iawn and Löbl (2008) recorded the genus as belongs to subfamily Tenebrioninae, tribe Pedinini. The opatrine native to Ethiopian and Palearctic faunal regions (James and Warren, 2007). Spilman (1959) stated that species of *Leichenum* have no economic damage. In recent studies James and Warren (2007) recorded *L. canaliculatum* on grass, cotton, soil among turnips and associated with damaged peach trees. Hagstrum (2009) recorded *L. canaliculatum* on soybeans feed on grass roots. *Leichenum pulchellum pulchellum* was found in a narrow strip of the seashore beside a sand dune (Fattorini 2002). Mouna *et al*. (2009) recorded this species in the sandy beaches.

Using of Scanning Electron Microscope (SEM) is extremely useful for studying the detailed morphology of very small structures on insect cuticle (Watson, 2015). The SEM enables quick, accurate representation with high resolution, three-dimensional clarity, and great depth of field.

Legner and Kogan (1969) confirmed that, the rigid exoskeleton of insects is especially suitable for electron microscopy because the specimen usually retains its shape under vacuum.

The SEM provides an excellent and detailed morphological overview (Zhang *et al*., 2012).

Ubero-Pascal (2010) reported the reasons of limitation of using SEM technique and stated that it is expensive and requires specialized preparation of the material to be studied.

Taxonomic studies on the species of genus *Leichenum* very scarce, especially *L. mulleri* which is probably only briefly studied by Penrith (1984), and other few studies carried out *L. pulchellum.* Hence the present study aimed to present an attempt to review the taxonomic status of the Egyptian species of genus *Leichenum* and contribute towards confirming or cancelling the old taxonomic characters, previously suggested by ancient taxonomists, also the study used SEM technique which offer high magnification and high-quality micrographs enabling the researchers to describe precisely and take the right decisions.

## MATERIALS AND METHODS

***Insects***

The present study is carried out on preserved insects of two species which belong to genus *Leichnum,* obtained from the side collection of the Plant Protection Research Institute, Dokki, Giza governorate, Egypt.

***Scanning Electron Micrograph***

The specimens were coated by gold sputter coater (PSI-Module, USA). A fully computer-controlled scanning electron microscope (Model: JSM-5500 LV; JEOL, Ltd.- Japan). Using high vacuum, it was scanned with the electron beam at 30 kV from different angles. Images of dorsal surface, mouthparts, eyes, mentum, pronotum, fore tibia, elytra, ventral surface, intercoxal process and the abdominal segments were taken. The SEM photograph was conducted at the Regional Center of Mycology and Biotechnology, Al-Azhar university, Cairo, Egypt.

## RESULTS

### Genus *Leichenum* Dejean, 1834

*Leichenum* Dejean, 1834: 176-256.
*Endothina* Carter, 1924: 521-544.
*Lichenum* Agassiz, 1846: 209

**Distribution**

Australia, Botswana, Cameroon, Chad, Cuba, Egypt, France, Gambia, India, Italy, Japan, Kenya, Liberia, Mozambique, Namibia, Niger, Nigeria, Senegal, Somalia, South Africa, Spain, Sri Lanka, Sudan, Tanzania, Thailand, and Vietnam (Marcuzzi 1984, Penrith 1984, Ferrer 2002, Peck 2005, Steiner 2005).

**Diagnosis of genus *Leichenum.***

Body scaly, size 3-5 mm. Clypeus rounded, obtuse or oblong, greatly emarginated; mentum small, plan, slightly transverse, more or less emarginated; last segment of palpi obconic and obtuse at the tip; labrum emarginated and committed in the clypeal emargination; eye sub-rounded with scales; antennae with ratio of flagellomeres length 2.7:2:1.7:1.7:1.5:1.3:1.3:1:1:0.7:1, apical six flagellomeres gradually enlarged forming the club. Pronotum strongly transverse, rounded at sides, bi-sinuate basally, with projecting and sharp posterior angles. Elytra subparallel, rounded at apex. Fore legs compressed, tibia denticulate at outside margin; the middle and hind legs cylindrical, 1$^{st}$ tarsal segment shorter than the apical.

**Key to the species of genus *Leichenum* Dejean**

**1-** Pronotum with sparse setae dorsally, with incised anterior margin (Fig.1 a); lateral margins of pronotum with long setae slightly broadened at apex (Fig.1 b); inner apical angle of anterior tibia provided with a small outgrowth, perpendicular with axis of the tibia (in male) (Fig.1 c) and small and straight (in female)…..*mülleri* Grid.

-Pronotum with very dense setae dorsally, with not incised anterior margin (Fig.1 d); lateral margins of pronotum with obviously broadened at apex (Fig.1 e); inner apical angle of anterior tibia in male and female without such outgrowth (Fig.1 f) … ……..……..*pulchellum* Kust.

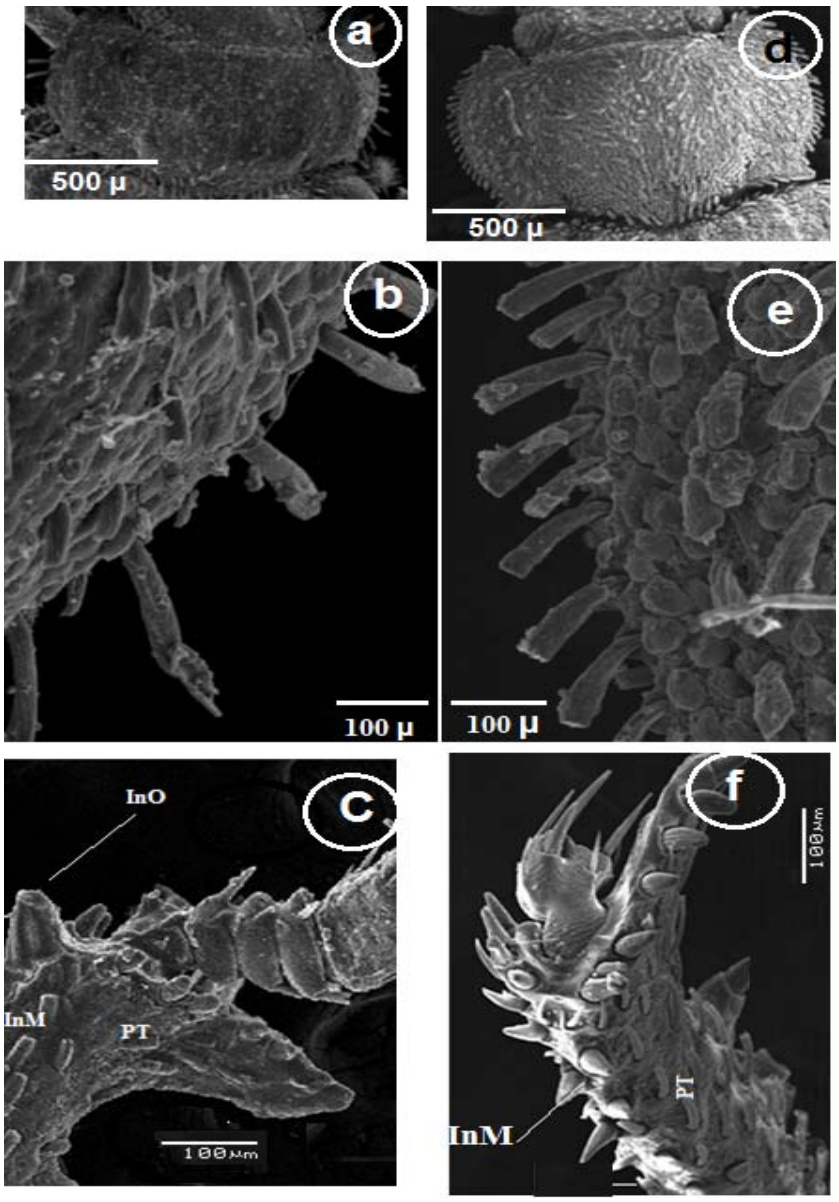


Fig. 1: Scanning Electron Micrographs. *Leichenum mulleri*, a- Dorsal view of pronotum, b- Lateral margin of pronotum showing the lateral setae, c- Protibia, d- Dorsal view of pronotum, e- Lateral margin of pronotum showing the distinct setae, f- Protibia. *Leichenum pulchellum*,. InM, inner margin; InO, inner outgrowth; PLS, Pronotal lateral setae; PT, Protibia.

### ***Leichenum mulleri* Gridelli, 1939**
### **(Figure 2)**

*Leichenum mulleri* Grid., 1939: 222.
Type locality: Eritrea.

**Description:**

Vertex bare, wrinkled medially, with widely sparse setae laterally above the dorsal margin of eyes, gena and above the antennal insertion with dense setae longer than that at vertex; eye with dark brown, erect scales surrounding each eye facet (Fig.

3 a); pronotum with widely sparse, greyish seta, brown at midline, anterior margin bordered with dense seta directed forwardly, sub-lateral strip with sparse setae, slightly broadened to apex, laterally with sparse setae, longer than that at sub-lateral strip, cylindrical, slightly gradually thickened to apex (Fig.1 b); posterior margin with row of dense setae, longer than that of anterior margin, surface of pronotum wrinkled (Fig. 3 b).

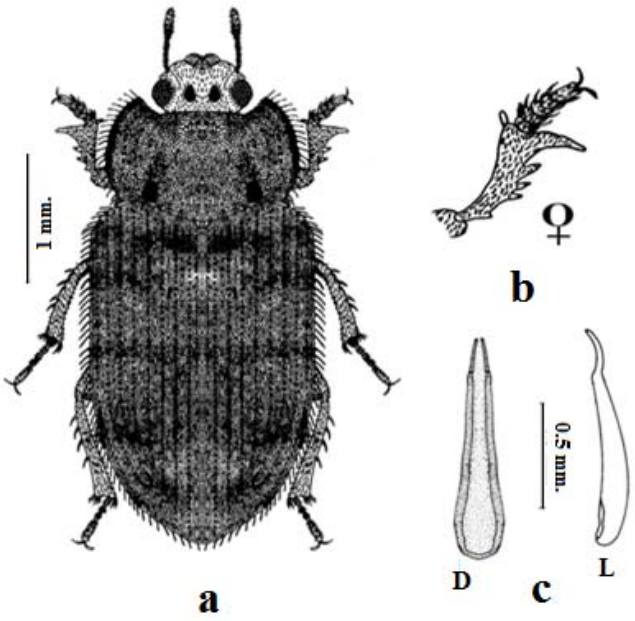


**Fig. 2:** ***Leichenum mulleri*****, a- Dorsal view of adult, b- protibia and protarsus, c- Aedeagus, D. dorsal view, L. lateral view.**

Elytra with whitish-grey and yellowish-brown setae, arranged in rows, equidistant from each other, intervals between rows broad (Fig. 3 c); laterally with setae similar to that at the lateral margin of pronotum but longer (Fig. 3 d), elytral surface ornamented with scale –like shapes. Ventrally, submentum with sparse setae, mentum, maxillae, labial and maxillary palpi with dense setae; labium with widely sparse setae (Fig. 3 e); prosternum with dense setae, apical margin with row of setae, prosternal process bare, wrinkled, with sparse punctures; mesosternum and mesosternal process densely punctate, metasternum slightly dense punctate with few number of setae; abdominal segments bare, slightly dense punctures. Head relatively small (about 0.7 time length of pronotum). Vertex convex, without depressions (Fig. 3 a). Eyes rounded, slightly prominent, its diameter about half length of head. Gena brown and thick at apex. Pronotum transverse (width 1.8 times its length), maximum width is at the middle, slightly arched laterally, incised anteriorly with sharp, prominent anterior angles; anterior margin narrowly serrate; posterior margin shallowly bisinuate, with obtuse posterior angles, not project laterally; pronotum with lateral depressions at its basal fourth, scutellum subquadritic, visible, flat (Fig. 1 a & Fig. 3 b). Elytra with raised striae, intervals between striae wide and concave, punctate laterally and basally; anterior angles rounded and prominent. Legs with setae and truncated at apex; anterior tibia with large outer tooth in both sexes; in male with two stout pointed teeth externally at middle and a perpendicular tooth at inner apical angle; in female, the fore tibia with stout teeth externally at middle and straight tooth at inner apical angle. Ventrally, submentum convex medially, sloping

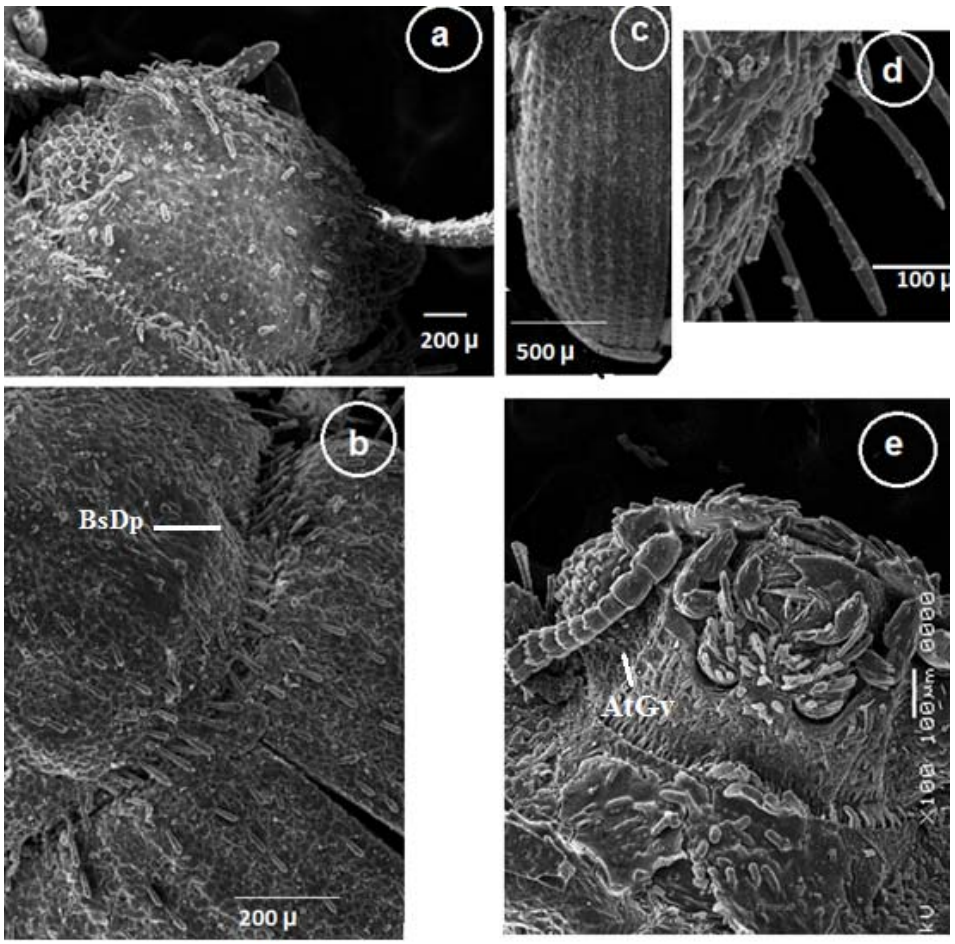


Fig. 3: *Leichenum mulleri* a- Head and eyes; b- Pronotum and elytra; c- Elytral striae and intervals; d- Elytra, lateral margin and setae; e- Head, ventral view showing submentum and mouth

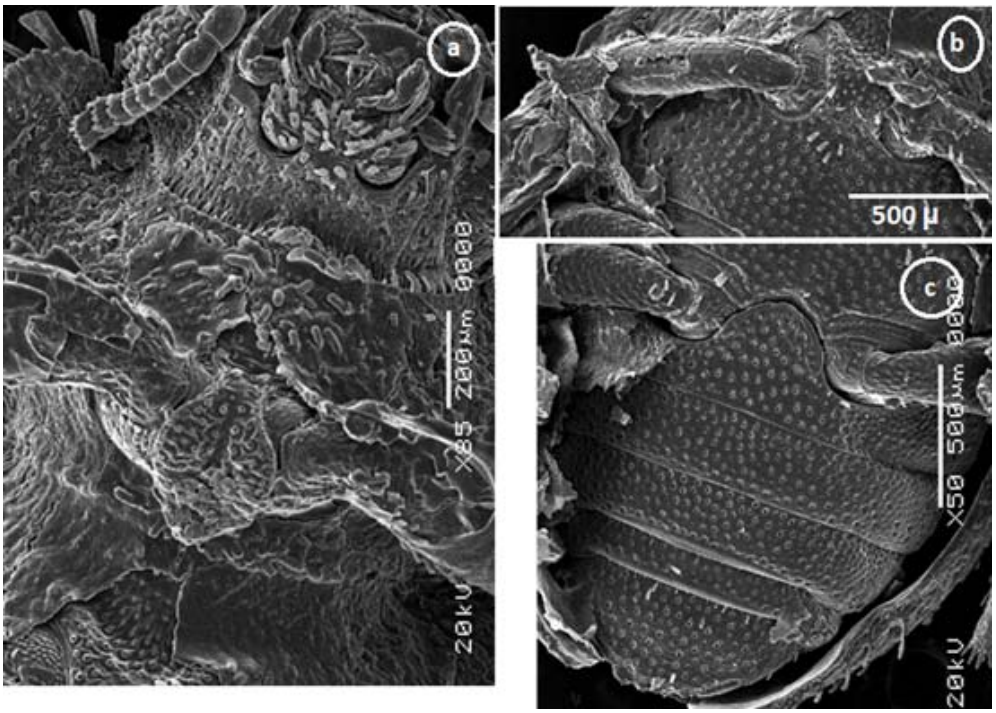


Fig. 4: *Leichenum mulleri* a- Prosternal intercoxal process; b- Mesosternal intercoxal process; c- Abdominal sternites and mesosternal basal strip.

laterally forming broad antennal groove beneath eyes (Fig.3 e). Prosternal intercoxal process cordate, passes above procoxae, raised above level of prosternum, arched above procoxae, not bent down apically, reaches to mesosternun, with shallow median sulcus at its apical half, pointed apically (Fig. 4 a). mesosternal intercoxal process trapezoidal, slightly depressed, raised above the level of mesosternum, contiguous with metasternum (Fig 4. b); mesosternum with narrow basal strip, bordered anteriorly by fine and raised line, separated by broad median arch, smooth and devoid of punctures (Fig. 4 c); first abdominal sternite narrow laterally, medially extended forwardly between hind coxae reach to metasternum beyond hind coxae. Abdomen oval, length ratio of abdominal segments (1.5:2.5:2.1:1:2.7) (Fig. 4c).

**World distribution**: Eritrea, Egypt.

**Material examined:** 75 specimens

Karm Alam (Sinai) April 1940 ……..……. **{15, CUC}**

Borgash (Giza) July 1932; G. Elba (Red Sea) Jan. 1933; Borgash (Giza) July 1932; …… ……... **{55, MAC}**

Gabal Elba (Red Sea) Jan. 2003……..……...…**{5, MAC.}**

### *Leichenum pulchellum pulchellum* Küster, 1849
### (Figure 5)

*Leichenum pulchellum* Küster, 1849, p. 65.

Type locality: Egypt.

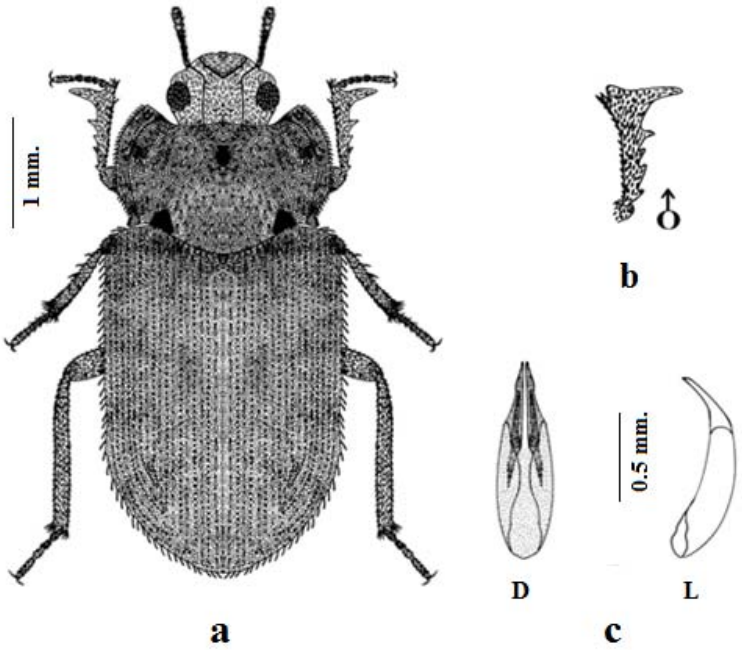


Fig. 5: *Leichenum pulchellum;* a- Adult; b- Protibia; c- Aedeagus, D, dorsal view, L, ventral view.

**This species differs from *L. mulleri* in the following characters:**

Vertex covered with very dense setae-like scales. Eye with dark brown, with recumbent scales cover most of eyes (Fig. 6 a). Pronotum furnished with very dense, oval, recumbent scales; with sparse, medium, suberect, greyish seta, anterior margin bordered with very dense seta, not directed forwardly (lie horizontally along the

margin); laterally pronotum with slightly sparse setae, depressed dorsoventrally broadened to apex; posterior margin with widely sparse setae, as long as that of anterior margin, surface of pronotum scaly (Figs. 1 b). Elytra, intervals between rows of setae narrow (Fig. 6 b); elytral surface invisible, laterally setae similar to that on pronotum but longer (Fig. 6 c). Ventrally, apical margin of prosternum with row of setae longer than that on surface of prosternum (Fig. 6 d); prosternal process with slightly dense, very dense posteriorly, broadened at apex setae; without punctures (Fig. 6 d). Mesosternum and mesosternal process with slightly dense setae, without punctures; metasternum with slightly dense setae, without punctures (Fig.7 a); abdominal segments with slightly dense setae, without punctures (Fig. 7 b). Head relatively small (about 0.5-time length of pronotum). Vertex with broad, slightly deep lateral depressions. Eyes, prominent, surrounded by deep groove (Fig. 6 a). Gena brown and thick at apex. Pronotum transverse (width 1.5 its length), strongly arched laterally, constricted basally, anterior angles extended anteriorly; anterior margin not serrate; posterior margin slightly deeply bisinuate, with acute posterior angles, forming spine-like projection extend laterally; pronotum with very small depression at posterior margin (Fig. 1 d); scutellum shield-shape, hardly visible. Elytra, with flat striae; intervals narrow and flat, punctat (Fig. 6b). Legs, fore tibia broad and toothed outside, its inner apical angle with small spines and teeth at middle of external margin; meso- and metatibiae externally with relatively strong and pointed spins.

Ventrally, submentum convex medially, sloping laterally forming antennal broad groove beneath eyes (Fig. 7 c) abdomen, prosternal intercoxal process oblong, passes between procoxae, bent down apically, convex, without median sulcus, rounded apically; mesosternal intercoxal process convex (Fig. 7 a).

**World distribution**: Egypt.

**Material examined:** 256 specimens

Luxor June 1908 & June 1909; Warrak (Giza) July 1909; Barrage(Qalyubiya) Jan. 1909 & May 1913; Rod El Farag (Cairo) June 1905; June 1907; Helwan (Cairo) July 1895; Marg (Cairo) Sep. 1914; Abu Rawash (Giza) May 1914; Beni Mazar (Menia) March 1916; El Roda (Cairo) Sep. & Aug.1913; Gabal Elba (Red Sea) May 1949; W. Garawi (near Helwan) April 1928; Menia July; Alexandria 1931; Giza May 1914; Barrage (Qalyubiya) May; El-Alag (Qalyubiya) March 1914; Oratyein Jan.; W. Morra (Sinai) April 1930; Egypt July 1909; Egypt; Kitchner Island (Aswan) March 1931; Cairo 1912…….…....…. **{213, EESC}**

Aswan April 1921; Barrage (Qalyubiya) Aug. 1933; Abu Rawash (Giza) Aug. 1935 ……...……..……..…… **{9, MAC}**

Tisfa (Giza) Sep. 1945; Madinat El-Tahrir (Behera) Feb. 1956; Abu Rawash (Giza) March 1958 ……...……..…**{3, CUC}**

Sidi Bishr (Alex.) May 1922; Maadi (Cairo) March 1912; El Mahala (Gharbiya) July 1911; El-Roda (Cairo) Aug. 1913; G. Kathrin (Sinai) April 1940; Osis Feiran (Sinai) May 1935; Bir Isla (Sinai) April 1940; W. El-Kontela (Sinai) April 1940 ……..……...…..…… **{21, ALFC}**

W. El-Gedeirat (Sinai) June 2005……..…….....…….. **{10, MAC}**

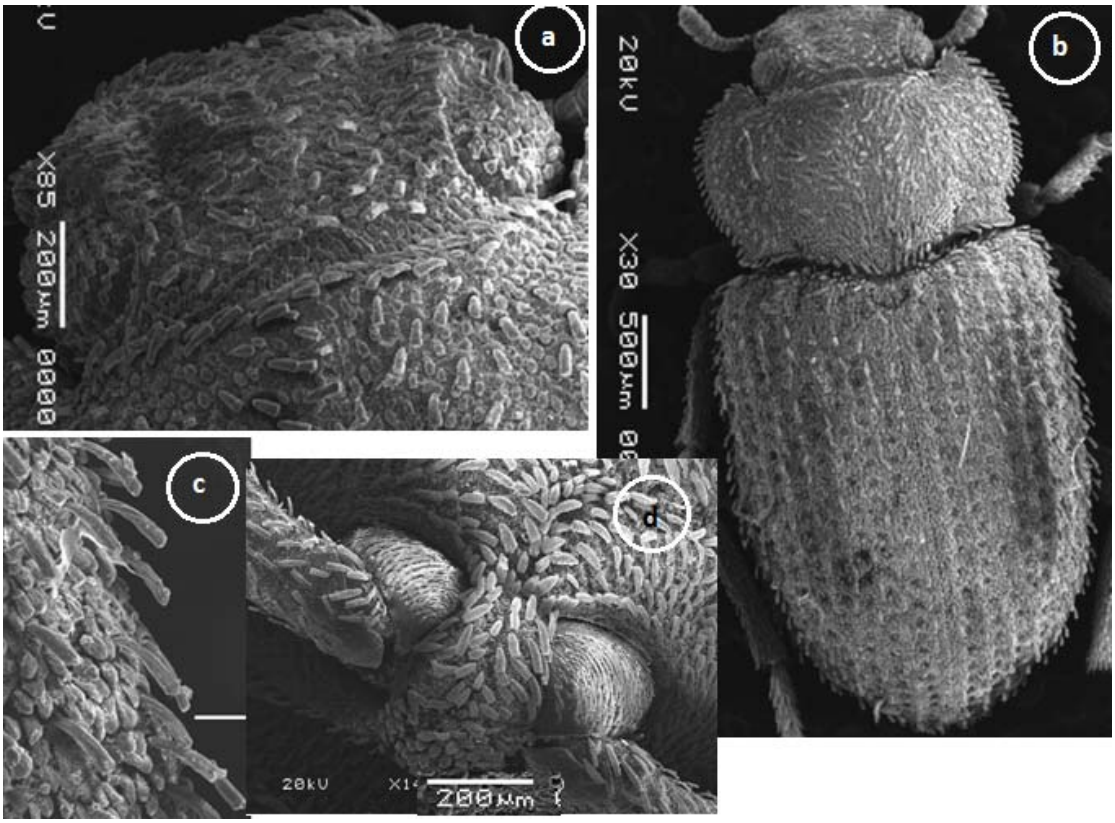


Fig. 6: Scanning Electron Micrograph. *L. pulchellum* a- Dorsal view of head; b-Dorsal view showing elytral striae; c- lateral margin of elytra and lateral setae, d- Prosternum; & Prosternal intercoxal process.

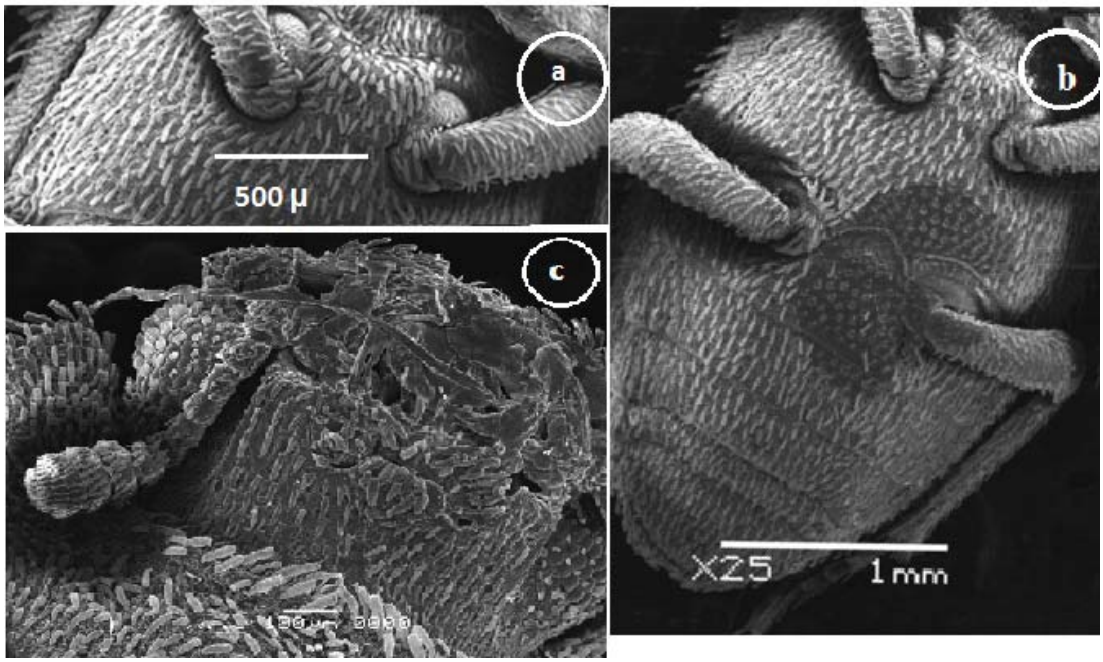


Fig. 7: Scanning Electron Micrograph. *L. pulchellum*,; a- Mesosternal intercoxal process, b- Abdominal sternites;. c - submentum and mouthparts.

## DISCUSSION

The members of the genus *Leichenum* are cosmopolitan, small and scarcely studied insects. These beetles occur at sandy areas, along beaches (Mouna *et al.*, 2009), lake and river shorelines (James and Warren, 2007).

Alfeiri (1976) and El-Torkey *et al.* (2009) classified genus *Leichenum* within subfamily Opatrinae, tribe leichenmini, while Aalbu and Triplehorn (1985) removed *Leichenum* from Opatrini and the genus is currently the only representative of the tribe Leichenini (Aalbu *et al.* 2002).

The Leichenini are distinguished from the Opatrini by the possession of ocular scales, short antennae with a four-segmented club, and a unipartite tegmen of the aedeagus (Penrith, 1984). Medvedev (1973) concluded that the setae located between the facets of the *Leichenum c. variegatum* are to protect the eyes from damage when they burrow into the sand. This can be applied on the two Egyptian species of *Leichenum* as their eyes provided with scales. The ocular scales are found in other Tenebrionidae, as Drosochrini and Cryptochilini, and also occur in other families, e.g., Dasytidae, Colydiidae (Penrith, Australian Faunal Directory (2007) stated that *Leichenum pulchellum* Küster, 1849 is the synonym to the Madagascar beetle, *Leichenum canaliculatum variegatum* (Klug) 1833, and described the latter as having eyes slightly emarginated, with erect setae between facets, the present study found that eyes not emarginated and eyes facets surrounded by scales instead setae.

Applying SEM micrographs in the present study adding a number of distinguishing characters differentiating the two Egyptian *Leichenum* spp. as, detail description of the body vestitures i.e. setae and scales shapes, body topography, i.e. depressions, grooves, convexity etc.

Present knowledge of *Leichenum* is not sufficiently extensive for a serious consideration of relationships within the genus, but the evidence so far available is presented.

## CONCLUSIONS

The scanning electron microscope is a very useful tool for observing minute, three dimensional structures of small insects, and some of these details can only be seen under the SEM. Like setae and hairs. Although the preparation of specimens for viewing under the SEM is time-consuming and expensive, the effort is worthwhile because the detailed information obtained is useful to solve some difficult aspects of tenebrionids beetle's taxonomy.

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## ARABIC SUMMERY

# استخدام الميكروسكوب الالكتروني الماسح في توصيف جنس ليتشنوم ديجاني ١٨٣٤ ( رتبة غمدية الاجنحة: فصيلة الظلاميات : بدييننى) في مصر

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١- معهد وقاية النباتات بالدقي جيزة - القاهرة

٢- جامعة الازهر كلية العلوم - القاهرة

يتمثل جنس *ليتشنوم* في مصر بنوعين هما *ليتشينوم مولري* جريد.، ١٩٣٩ و *ليتشينوم بولشيلوم* كوست.، ١٨٤٩. وينتمي هذا الجنس لخنافس الظلاميات وهي من الانواع الهامة للتوازن البيئي.

ولم يتم تناول النوعين سابقا بالوصف او الدراسة الا من خلال عدد قليل جدا من الابحاث في جميع أنحاء العالم ولم يتم ذكر النوع *مولري* الا من خلال بحث واحد فقط ولم يتم وصفه وصفا كاملا حيث جاء كمقارنة مفتاحية فقط . وكلا النوعين يصعب تفرقتهم علي مستوي الانواع نظرا للتشابه الكبير بينهما.

ولذا هدفت الدراسة الحالية لدراسة النوعين بإستخدام الميكروسكوب الالكتروني الماسح للتوصل للصفات الدقيقة التي تسهل التعرف عليهما. وقد تم إضافة عدد من الصفات الجديدة والتي لم يتم تناولها من قبل في وصف الانواع الشبيهة في الفصيلة فقد تم تناول شكل الاشواك والتراكيب الدقيقة الاخري ، شكل وتركيب النتوءات بين حراقف الارجل ، منطقة ما تحت الذقن كما قام الباحثين بعمل تحديث للوصف الكلاسيكي ووصف نوع مولري بالتفصيل مع مقارنة النوعين لسهولة التعرف عليهما.