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Description of Surface Morphology in Male and Female Genitals of *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae: Lixinae) a Scanning Electron Microscope Study

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Abstract: The internal genitalia of both sexes of *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae) were examined by optical and scanning electron microscope (SEM). A detailed description is provided. In lateral view, the aedeagal tube is bented and its lateral surface is strongly sclerotised. In dorsal view, the ostium which fromendophallus (internal sac) goes out, seems clearly. The suroundings of the ostium has little sclerotised membrane. The spermatheca appears like a hook shape. It consists of receptaculum seminis, spermathecal duct and accesory gland.

Key words: aedeagus, spermatheca, ovipositor, tegmen, SEM.

Introduction

In insects, male and female genital structures are often used in distinguishing at the species level. The genitalia of both sexes have been recognised for diagnoses by several authors (Hoffmann 1950, 1954, Morimoto 1962, Zimmermann 1968, Tuxen 1970, Pajni *et al.* 1977, Ter-Minasyan 1978, Barker 1989, Clark 1990, Pesarini 1979, Caldara 1985, 1990, Kojima, Morimoto 1996, Sert, Çağatay 1999, Sert 1997, 2006, Rubio *et al.* 2008). However, accurate knowledge of the morphology and functions of the genital structures in Curculionidae is still lacking in many taxonomic groups, including Lixinae. Generally, the male genitalia occurs annulate type in Curculionidae, and consists of the aedeagus and the spiculum gastrale (called '9th sternite' by Wanat 2007) (Tuxen 1970).

The aedeagus consists of two main parts: aedegal tube and tegmen which are connected by the first connecting membrane.The ejeculatory duct enters the aedeagal tube through the basal orifice. It goes out from the ostium (apical orifice) which is, exceptions, situated on the dorsum of the median lobe near the apex (Tuxen 1970). The tegmen consists of a flattened and usually bilobed tegminal plate, V-shaped basal piece and an apodeme (Manubrium) (Wanat 2007). The tegmen located the basal part of median lobe (called 'aedeagal tube' by Wanat 2007) form a ring. In some groups, dorsal part of tegmen is reduced, and it is not enveloping the aedeagal tube (Tuxen 1970). The function of the weevil tegmen is primarily mechanical in two respects. The tegmen limits the range of

eversion of the aedeagus and serves as a point of attachment for the post-tegminal membrane or it assists with eversion and retraction of the genitalia through the action of the muscles attached to the apodeme (Wanat 2007). The 9th sternite (spiculum gastrale) in different shapes is originated from 9th abdominal sternite (Tuxen 1970) and used as a special taxonomic character for species by many researchers (Clark 1990).

The female genitalia of Curculionoidae is constituted by the recracted 8th and 9th abdominal segments, as in other Coleoptera (Tanner 1927). The 8th segment is usually reduced and its sternite is usually assosiated with a chitinous apodeme of variable size and shapes. It is accordingly designated as 'genital spicule. The 9th segment forms a short or long tube and its sternum generally bears a pair of short or long sternites each with or without a stylus (Tanner 1927). Stylus has normally sensorial function (armed with bristles) (Tuxen 1970). The spermatheca is strongly chitinized since it is of ectodermal origin; this arrangement is common in all coleopterans (Crowson 1981). Sperm stored within the spermatheca passes through the spermathecal duct into the bursa copulatrix where the eggs are fertilized (Khan, Musgrave 1969). Accesory gland is regularly attached to the spermatheca (Tuxen 1970). The accessory gland secretion of insects is involved in spermatozoa activation, spermatozoa transfer to the spermatheca, and maintenance of spermatozoa (Davey, Webster 1967, Bhatnager, Musgrave 1971, Grodner, Steffens 1978).

Herein, we presented details of the genital structures of male and female *Lixus cardui* Olivier, 1807 (Curculionidae) by optical microscopy and SEM.

Material and Methods

Ten specimens were selected from dried museum materials obtained from the field

(Boğazpınar village, Çamlıyayla, Mersin, Turkey, 7. VI. 2006, 495 m). The male and female genitals were prepared by first softening the abdomen in 10% KOH for 24 h at 300° C.

Thereafter, tissues were carefully removed and the male and female genitals were placed in glycerin. Observation was made using a stereomicroscope (Olympus SZX12 Photo-microscope at 40X). For scanning electron microscope (SEM), rinsed and dried genitals were mounted with double-sided carbon tape on SEM stubs, coated with gold in a Polaron SC 502 Sputter Coater, and examined with a JOEL JSM 6060 SEM operated at 15 kV. Nomenclature follows Tuxen (1970), Pajni *et al.* (1977), Wanat (2007) and classification follows Alonso-Zarazaga, Lyal (1999). A list of abbreviations is given in Table 1.

Table 1. Abbreviations used in figures for male and female genital structures:

Aa: Adeagal apodemes
Ad: Aedeagus
Ae: Aedeagal tube
A: Accesory gland
As: Abdominal sternum
Tc: Aedeagal tectum
Gs: Genital spicule
Ma: Manubrium
Os: Ostium
Ovip: Ovipositor
Pe: Adeagal pedon
Sd: Spermathecal duct
S9: 9th sternite
Sp: Spermatheca
Sty: Stylus
Tg: Tegmen

Results

Male genitalia

The male genitalia (aedeagus and 9th sternite) of *Lixus cardui* are drawned in Figs. 1A, B. The aedeagus consists of two main parts: aedegal tube and tegmen. In lateral view, the aedeagal tube is bented and its lateral surface is strongly sclerotised (Figs. 1B, C). The adeagal pedon is widens and cornered medially, very narrow basally, and narrows distally. There are some short spines on surface of pedon (Fig. 1E). The great basal part of aedeagal tube seems membranous (Fig. 1F). The manubrium is clearly bented in apically, and its surface has design wrinkles (Figs. 2C, D). The aedeagal apodemes are very short and strongly sclerotised. They are approximately 1/5 lenght of aedeagal tube (Figs. 1B, 2C). In dorsal view, the aedeagal pedons is strongly sclerotised. The basal and apical parts of aedeagal tube appear as

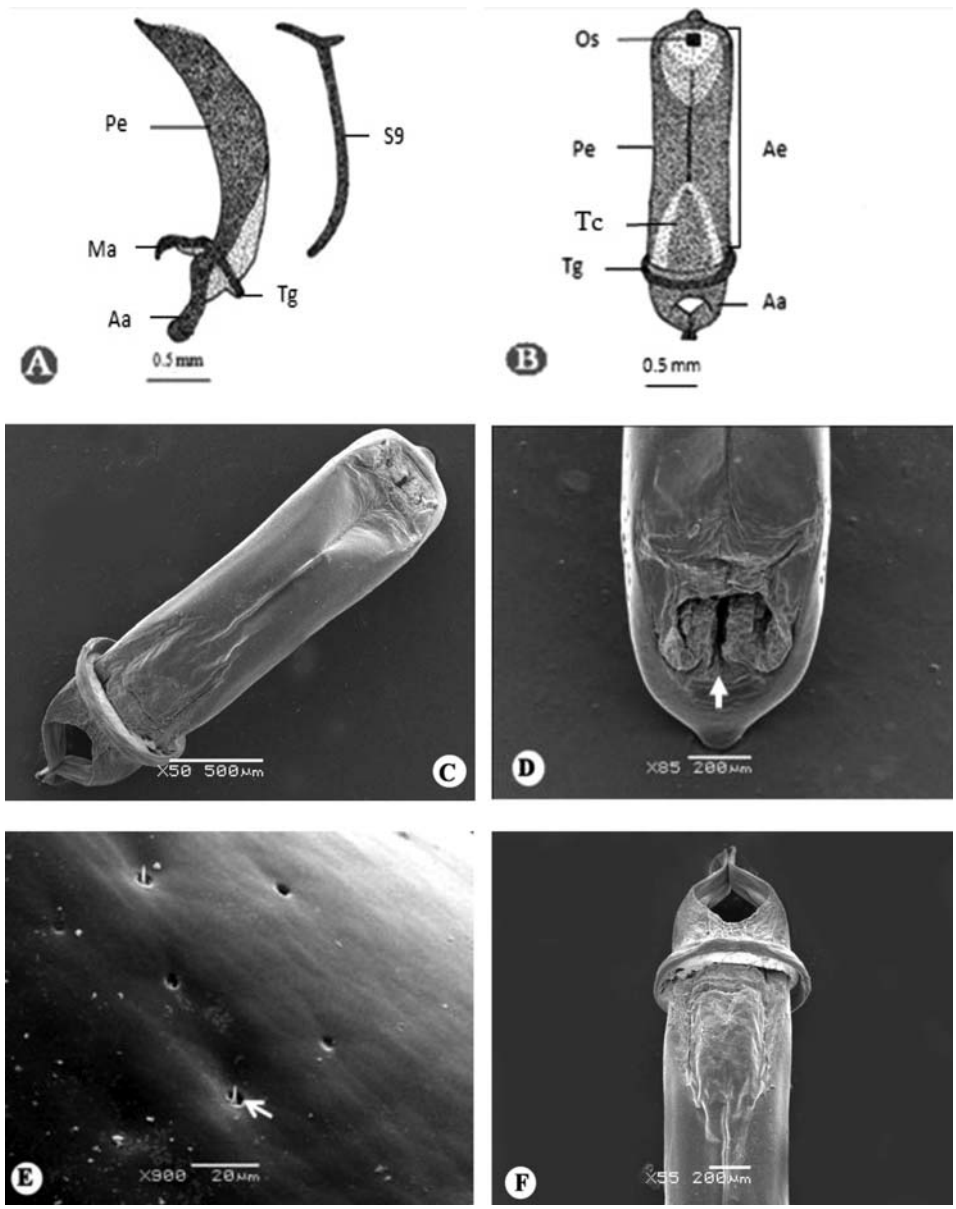

Fig. 1. Drawing and SEM photos of male genitalia of *Lixus cardui*: A – drawing of the aedeagus (lateral view) and 9th sternite (spiculum gastrale); B – drawing of the aedeagus (dorsal view); C – SEM photo of aedeagal tube; D – the ostium on apical of aedeagus (B); E – spines on surface of aedeagal tube (B); F – basal part of aedeagal tube.

membrane plate (Fig. 1B). The ostium which from endophallus (internal sac) goes out, seems clearly. The suroundings of the ostium has a membrane that little sclerotised (Fig. 1D). In the basal of aedeagal tube, the tegmen is located like a ring (Fig. 2A). It is connected to the aedeagal tube by a wide membrane which is the first connecting membrane (Fig. 2A). The chinisation of tegmen seems to be porous structure (Fig. 2B).

The 9th sternite is T-shaped and curved especially at the basal part. It has two assimetrically arms (Figs. 1B, 2E). The body of 9th sternite is strongly sclerotised, but the structure of the arms has densely porous (Fig. 2F).

Female genitalia

The spermatheca appears like a hook shape. It consists of receptaculum seminis, spermathecal duct and accessory gland (Fig. 3A). It is strongly sclerotised (Fig. 3B). The surface of spermatheca has densely porous (Fig. 3C). The spermatheca is widening like a sac in basally.

The ventral side of spermatheca and the surface of sac have many curls (Figs. 3D, 4A). The

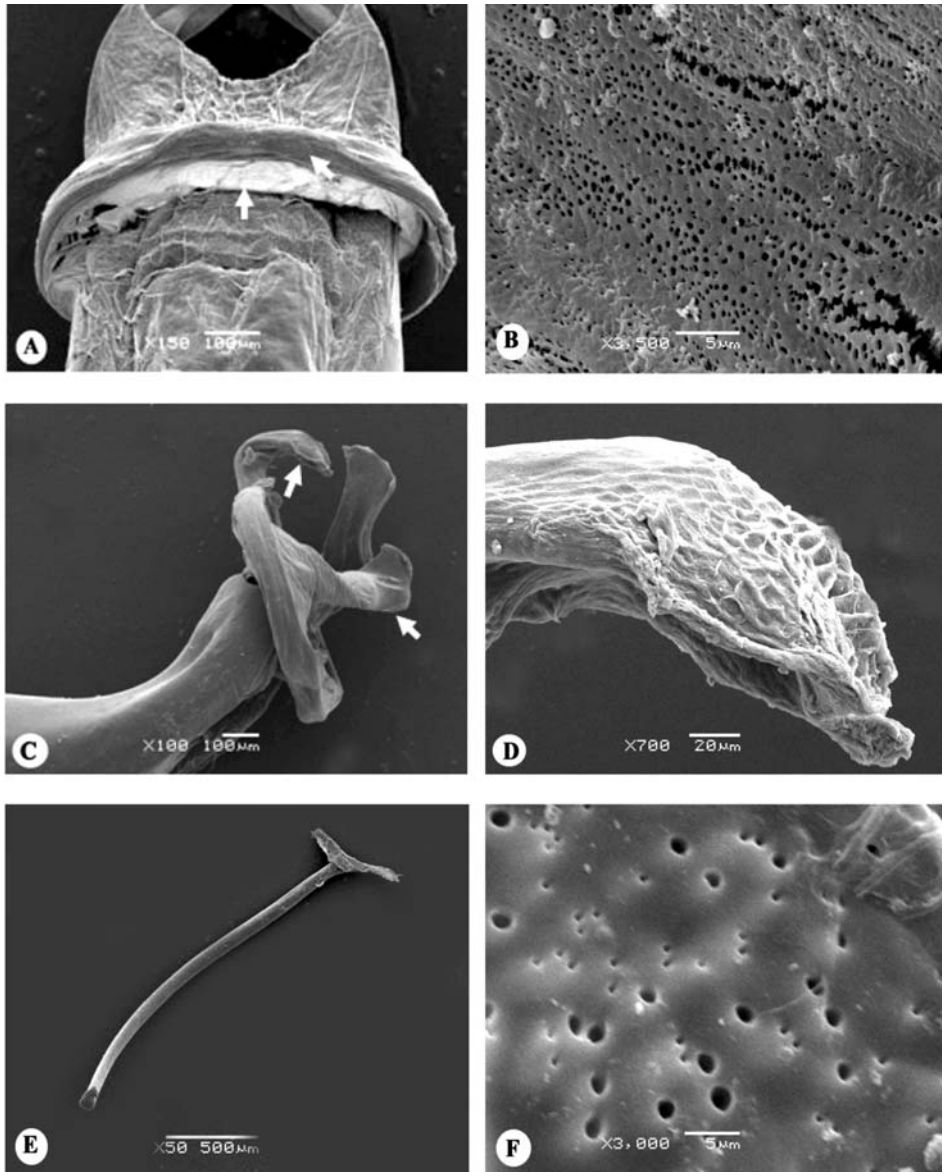

Fig. 2. SEM photos of male genitalia: A – tegmen with a wide membrane (B). B – surface of tegmen; C – basal part of aedeagus with tegmen, manubrium and aedeagal apodemes (B); D – dorsal surface of manubrium; E – view of 9th sternite; F – pores on surface of 9th sternite.

spermathecal duct seems very thin and covered with a thick layer of chitin (Fig. 4B). There are many muscles fibers, which are enveloping the spermathecal duct in the basal part of spermatheca (Fig. 4C). The accesory gland is opened to the dorsal of spermatheca with a short duct which of surface has irregular structure (Fig. 4E). The accesory gland appears transparently in light microscope but in SEM examination its surface is little sclerotised (Fig.4F).

Female genitalia of *Lixus cardui* are with genital spicule and 8th abdominal sternum which are joined and seem Y-shaped. The genital spicule is narrowed in middle, basally and apically, it is little widen (Fig. 5A). Its surface is strongly sclerotised. The 8th abdominal sternum has two plates which are joined with a wide membrane (Figs. 5A, B). It is widen in basal part where there are many long hairs (Figs. 5B, C), but apically it is narrowed gradually. The 8th abdominal sternum has many structures like a funnel shape on back surface (Figs. 5D, E). There are many long fibrils among the funnel structures (Figs. 5E, F). The ovipositor which has two hemisternites is in Fig. 6A. Each sternite bears the stylus which is armed with bristles (Fig. 6B). The stylus is like a short tube that has many long bristles at the apex (Fig. 6C). The sternites appear

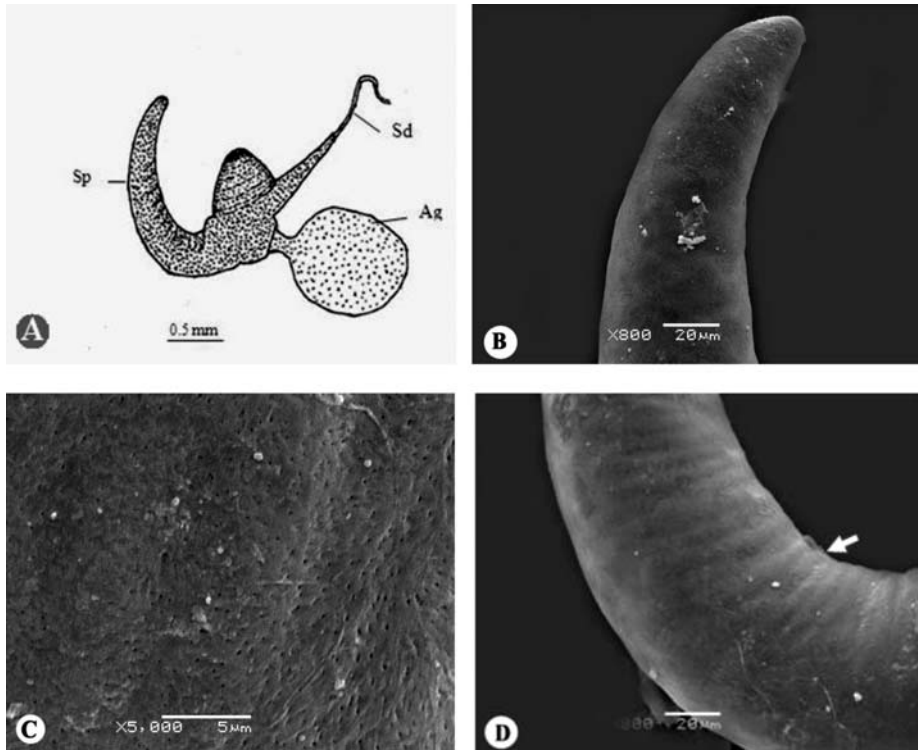

Fig. 3. Drawing and SEM photos of spermatheca of *Lixus cardui*: A – drawing of spermatheca and accesory gland; B – SEM photo of apical part of spermatheca; C – small pores on surface of spermatheca (B); D – the curls on ventral side of spermatheca.

as swollen tube shape. The apex of sternites has many long bristles (Figs. 6B, D). Each sternite has membrane in middle, but it is strongly sclerotised apically, where are many spines and porous (Fig. 6E). The ventral side of sternites appears smooth surface which has not spines and porous (Fig. 6F).

Discussion

The male and female genitalia have been recognised as source of taxonomically important characters in Coleoptera for several years. Many authors (Pajni *et al.* 1977, Sert 1997, Sert, Çağatay 1999) compared the shape and size of the genital structures for species diagnoses but they are not investigated by SEM.

The structure of genitalia seems limited under the light microscope but in scanning electron microscope, all the details appeare clearly. In *L. cardui*, the chitinisation of the structures which has spines and porous are showed appearently. For example, the surface of aedeagal pedon has spines and porous. The surface of tegmen and spermatheca has only porous. In additional, we determined the particular field where surroundings with many muscle fibers at the distal part of spermathecal duct. These muscle fibers play a role as an ejection apparatus which controls the passage of the spermatozoa (Theodor 1976, Rodriquez 1994, Pabalan *et al.* 1996, Yuval *et al.* 1996, Bloch Qazi *et al.* 1998, Gschwentner, Tadler 2000, Fritz, Turner 2002). The 8th abdominal sternum has many structures like funnel-shape and very long fibrils on the back surface and their functions are unknown.

Finally, the morphological characters in both sexes of *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae), as the presence of aedeagus, 9th sternite, spermatheca, genital spicule, 8th abdominal sternum and ovipositor are important in classification higher at the generic level of the Curculionidae taxa and its taxonomic importance were estabilished. The sculpturing of different shapes and structures may provide distinctive characteristics for some of the species. The survey revealed a wide variety of morphological characters of male and female in Curculionidae. More work involving SEM is needed to estabilished clear trends within the taxonomic groups.

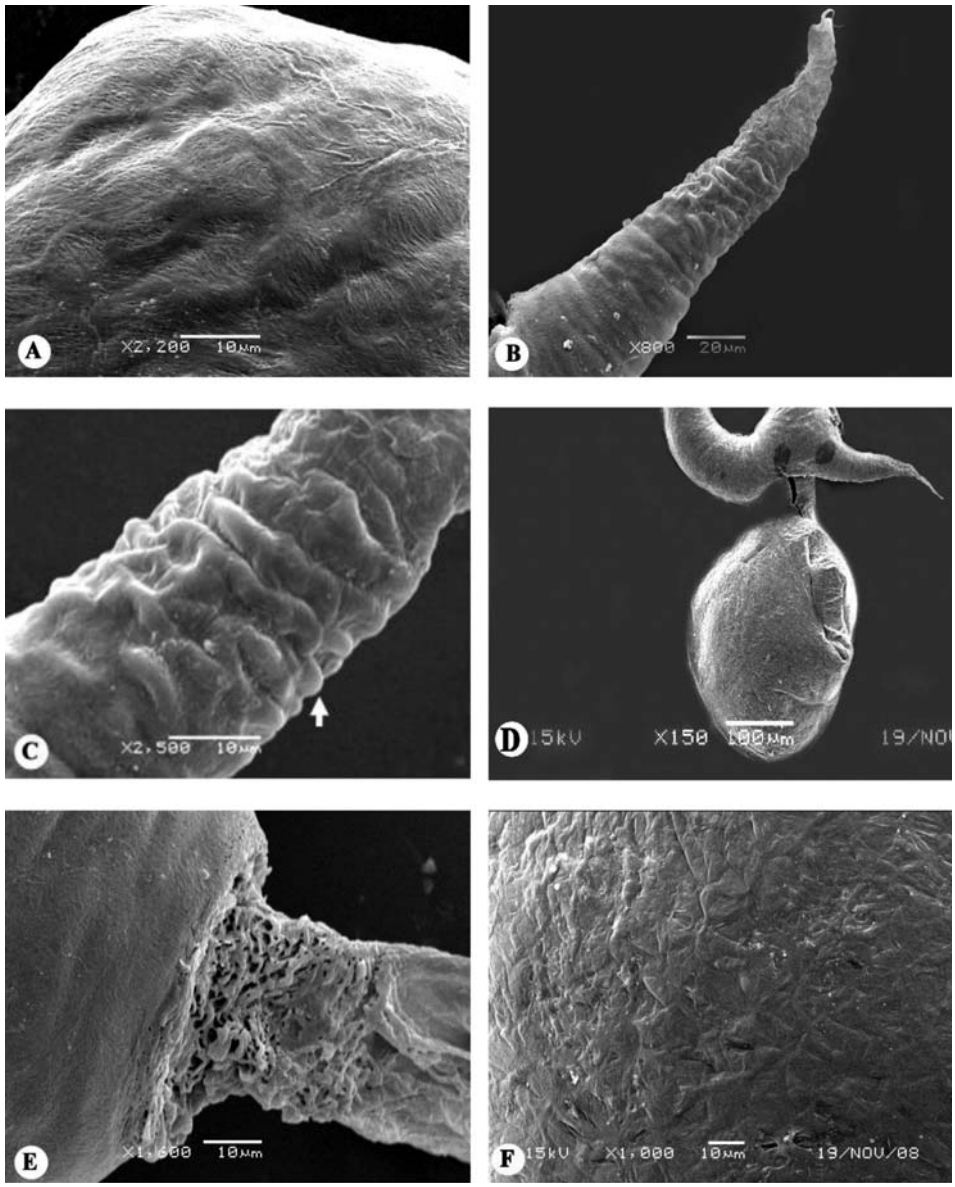

Fig. 4. SEM photos of spermatheca: A – surface of sack; B – view of spermathecal duct with covered chitin; C – surface of spermathecal duct and seem wrinkles like muscle fibers (B); D – accesory gland; E – duct of accesory gland and connected to the area of spermatheca; F – surface of accesory gland.

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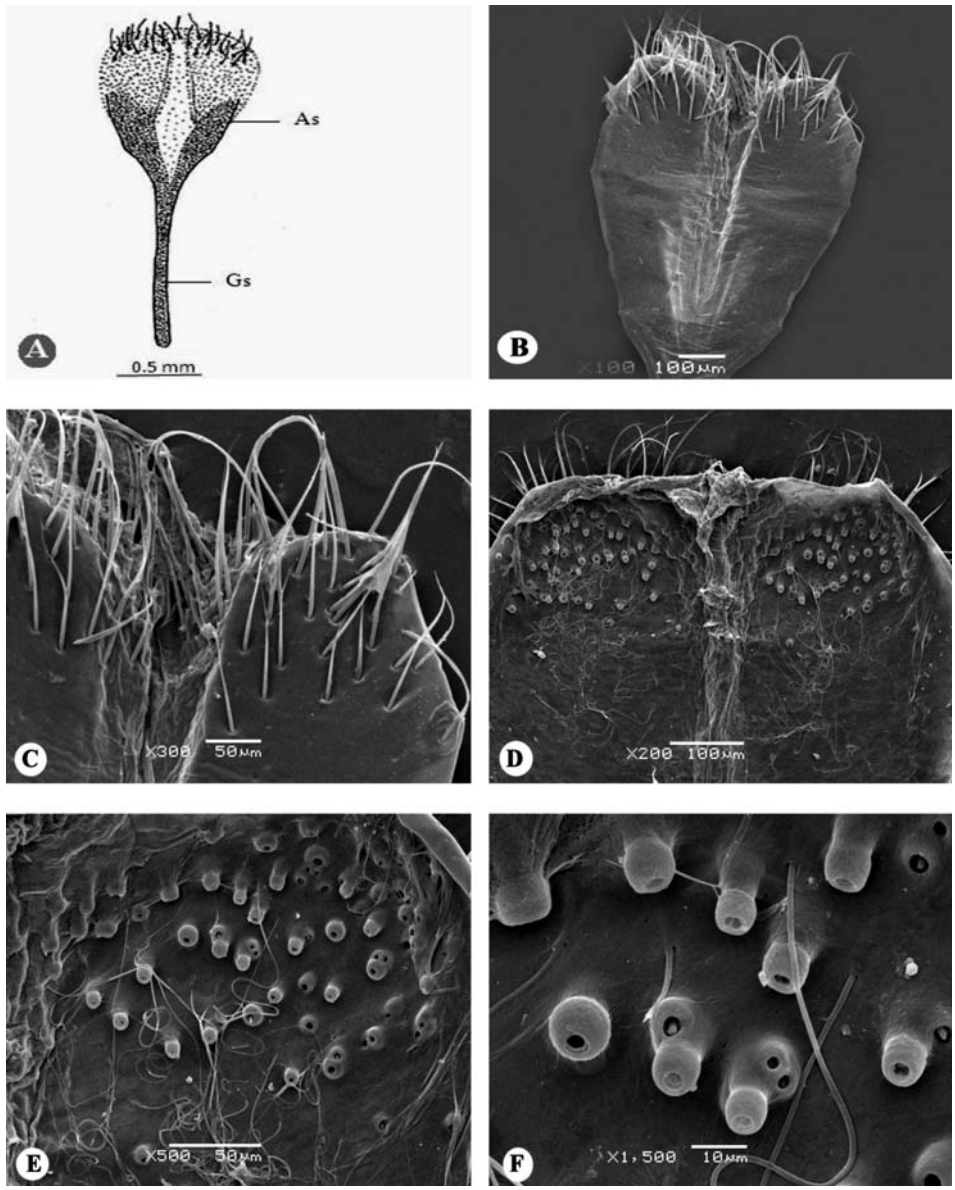

Fig. 5. Drawing and SEM photos of genital spicule and abdominal sternum: A – drawing of genital spicule and abdominal sternum; B – view of abdominal sternum; C – long hairs on posterior of abdominal sternum (front face); D – view of posterior of abdominal sternum (back face); E – funnels and very long fibrils on back surface; F – view of funnels and fibrils.

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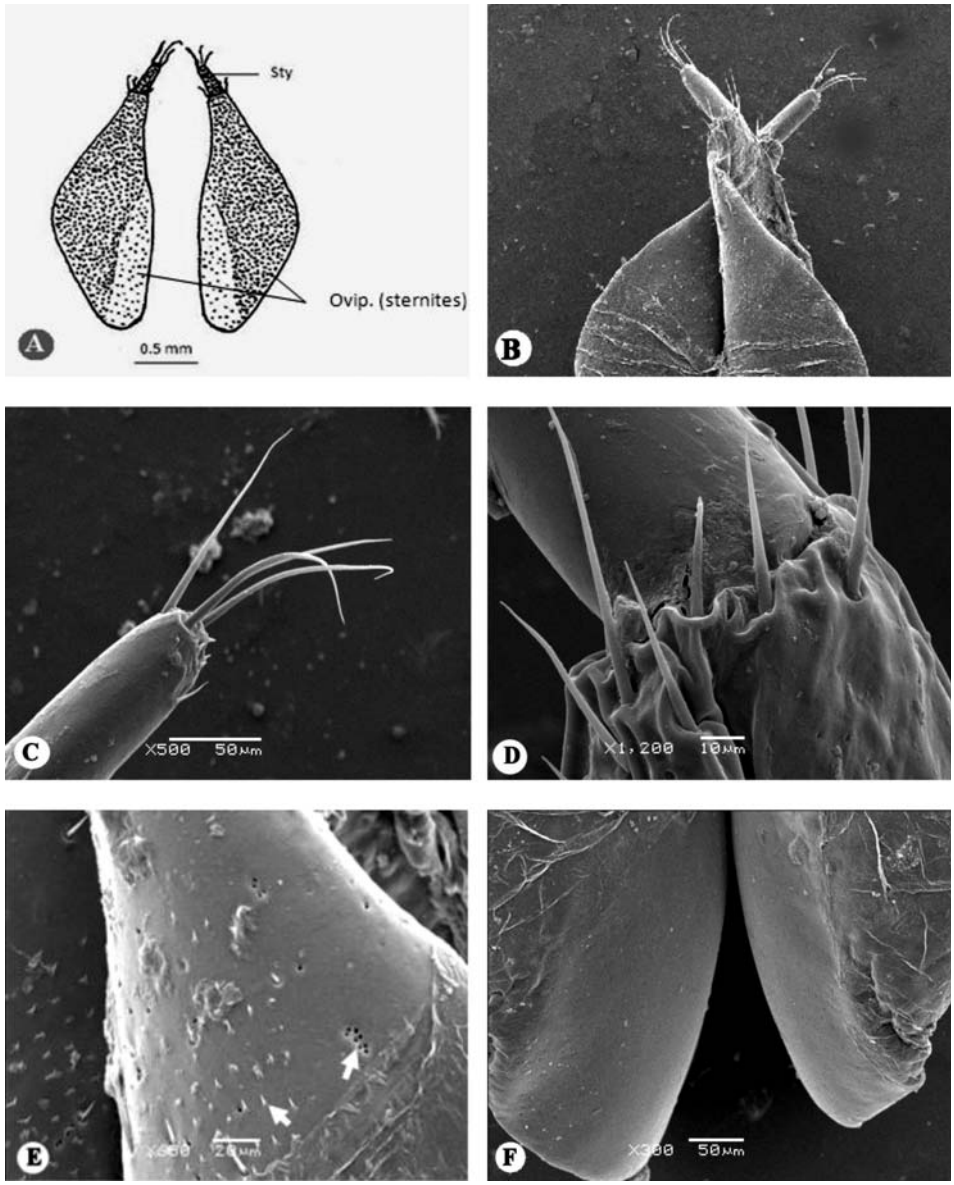

Fig. 6. Drawing and SEM photos of ovipositor : A – drawing of sternite of ovipositor and stylus; B – the sternites of ovipositor and stylus; C – stylus and hairs on apical part; D – basal part of stylus; E – short spines and pores on apical surface of sternite (B); F – ventral side of sternites.

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Морфология на повърхноста на женски и мъжки гениталии на *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae: Lixinae): изследване със сканиращ електронен микроскоп

М. Ербей, С. Джандан

(Резюме)

Със светлинен и сканиращ електронен микроскоп (SEM) са изследвани гениталиите на двата пола на *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae) и е представено е подробно описание. В латерален изглед едеагалната тръбичка е дъговидно извита. Около оскулума се наблюдава малка склеротизирана мембрана. Сперматеката е под формата на кукичка и се състои от семеприемник, спермален канал и допълнителна жлеза.