Ultrastructural analysis of the internal sac in several species of the weevil tribe Phyllobiini (Coleoptera: Curculionidae: Entiminae)

Article *in* Zoology in the Middle East · October 2014

DOI: 10.1080/13546805.2014.970369

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Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (2): 54-56

Received: 01-02-2015
Accepted: 23-02-2015

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Ultrastructural analysis of endophallus in several species of the genus *Larinus* Dejean, 1821 (Coleoptera: Curculionidae: Lixinae)

Mahmut Erbey, Selami Candan

Abstract
The ultrastructures of the endophallus in eight species of the genus *Larinus* Dejean, 1821 (*Larinus scolymi, L. grisescens, L. iaceae, L. latus, L. minutus, L. onopordi, L. sturnus,* and *L. turbinatus*) (Coleoptera: Curculionidae: Lixinae) are investigated. The endophallus was obtained from the aedeagi by dissection. The structures were drawn under a light microscope. The ultrastructures of endophallus (or internal sac) consisting of spines, teeth and papillae were investigated with a scanning electron microscope. Similarities and differences between all species investigated are discussed. The ultrastructures found, e.g. spines, teeth, papillae and hairs are recognised as important for taxonomy, and can be used for separation of the morphologically similar species.

Keywords: Coleoptera, Curculionidae, Lixinae, *Larinus*, endophallus (internal sac), SEM

1. Introduction
In Coleoptera, the male genitalia and associated membranes have been used since long important characters in taxonomy, but the functioning of internal membranes are not yet well understood. Their relative position in the connecting membrane and the genital membrane folding patterns have never been thoroughly investigated [1]. Genital structures provide, in many cases, taxonomically useful characters for distinguishing organisms at species and subspecies level [2]. In Curculionidae, the male genitalia have important structures that have been used to define taxonomic groups [1, 3-7]. The shape of the aedeagus is tubular, often arcuate, sometimes flattened, and normally contains an endophallus. The endophallus becomes everted in to the female vagina (or the bursa copulatrix) during coitus and is thus the functional intromittent organ. The membranous walls of the endophallus often possess an armature of spines, denticles, hairs, scales, papillae (= a set of ultrastructures) which, protruding into the lumen of the endophallus when in repose, serve to fix it in the female genitalia after evagination. The shape of armature of the endophallus is very constant within a species and therefore very useful for taxonomic purposes. Wanat, [1] investigated the structure of the genital chamber in weevils (Curculionoidea) and other Coleoptera and used the term endophallus for the genital membrane in the aedeagus as endophallus, which is synonymous to the internal sac. The endophallus membrane often has various sclerotised outgrowths forming variably shaped sclerites, larger teeth, spinules or miniplates, and usually becomes outwardly prominent when the endophallus is extruded [1].
Here, we determined the ultrastructures of the endophallus in eight species of *Larinus* by light and scanning electron microscope and showed for the first time.

2. Material and methods
The eight species of *Larinus* (Curculionidae: Lixinae) were investigated: *Larinus scolymi* (Olivier, 1808), *L. grisescens, L. iaceae, L. latus, L. minutus, L. onopordi, L. sturnus,* and *L. turbinatus*. Specimens were collected from the different localities in central Anatolia between 2011 and 2013. The specimens were dissected under a light microscope. The aedeagus were obtained after softening the abdomen in 10% KOH for 24 h at 30ºC. The endophallus was then removed from the aedeagus and extended by fine dissection. Observations and drawings were made on the display screen of the microscope (Olympus SZX12) at a magnification of x40. For examination with scanning electron microscope, the specimens were cleaned from the organic churns and mounted with double-sided carbon tape on SEM stubs, coated with gold in a Polaron SC 502 Sputter Coater, The scanning electron microscope used was a JOEL JSM

6060 SEM operated at 10 kV (1000x and 3000x magnification). The terminology used (see Fig 1) follows Wanat [1] and Medina *et al*. [2].

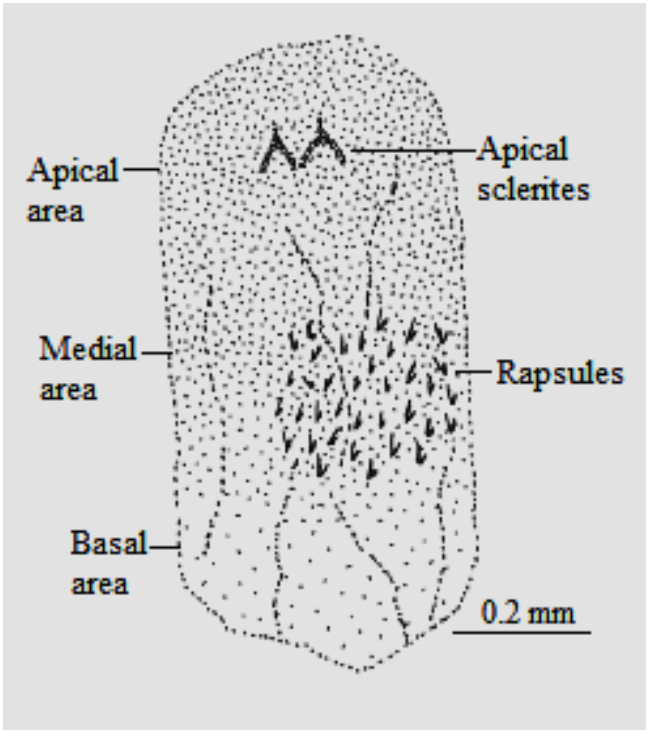
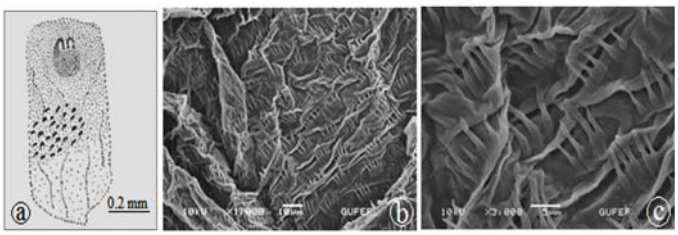


Fig 1: Drawing of endophallus with terminologies in *Larinus grisescens* Gyllenhal, 1836

3. Results

Under the light microscope; The endophallus membraneous and transparent. Surface densely covered with spots. Apical part strongly sclerotised and with two symmetrical sclerites (Figs. 2a, d, g, k, 3a, d, g, k), the armature of teeth, stated rapsules by Medina *et al*. [2], located in medial area, but slightly visible, basal area weakly sclerotised. Apical sklerites oval or little longitudinal (*L. scolymi*) (Fig. 2a), oval (*L. iaceae*, *L. onopordi* and *L. turbinatus*) (Figs. 2g, 3d, k), oval with apical projections (*L. latus* and *L. sturnus*) (Figs. 2k, 3g), triangular with apical projections (*L. grisescens*)(Fig. 2d) or with a hook (*L. minutus*) (Fig. 3a).

Under the scanning electron microscope; the armatures of the endophallus different in each species. Endophallus covered with long teeth which are clustered (*Larinus scolymi*) (Figs. 2b-c); Surface of endophallus with teeth which constituting dual or triple, and few structures that like aeropil appear among teeth (*L. grisescens*) (Figs. 2e-f); surface with sparsely standing long teeth (*L. iaceae*) (Figs. 2h-i), teeth short, widened basally, gradually narrowed from base to apex (*L. latus*) (Figs. 2l-m), surface of endophallus with long and simple teeth (*L. minutus*) (Figs. 3b-c); surface of endophallus strongly covered with teeth that are wrecked to ground (*L. onopordi*) (Figs. 3e-f); short teeth form groups like fringes, and strongly covered to surface (*L. sturnus*) (Figs. 3h-i); teeth simple, short and thick (*L. turbinatus*) (Figs. 3l-m).



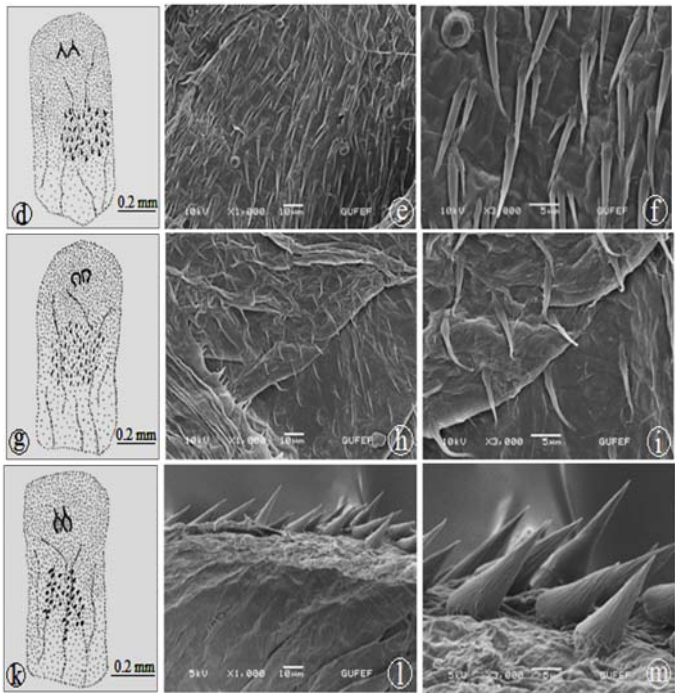


Fig 2: Drawings of endophallus; **a:** *Larinus scolymi*, **d:** *L. grisescens*, **g:** *L. iaceae*, **k:** *L. latus*; SEM micrographs of endophallus (1000x and 3000x magnifications); **b-c:** *L. scolymi*, **e-f:** *L. grisescens*, **h-i:** *L. iaceae* **l-m:** *L. latus*.

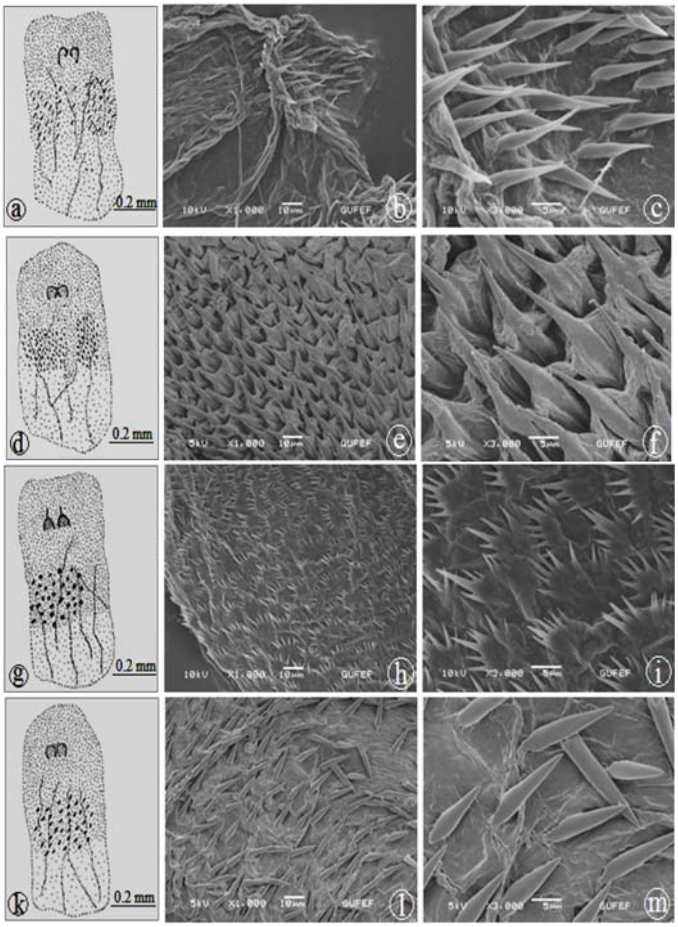


Fig 3. Drawings of endophallus; **a:** *Larinus minutus*, **d:** *L. onopordi*, **g:** *L. sturnus*, **k:** *L. turbinatus*; SEM micrographs of endophallus (1000x and 3000x magnifications); **b-c:** *L.minutus*, **e-f:** *L. onopordi*, **h-i:** *L. sturnus*, **l-m:** *L. turbinatus*.

4. Discussion

The aedeagi is used as an important character in taxonomy. In Curculionidae, several authors used the aedeagi in their

investigations, but did not treat specifically the armature of the endophallus [8-18]. The Endophallus is generally not yet well studied. Tuxen [3], described the internal sac in detail. Recently, some authors studied to the endophallus and drew the simple shapes. Velazquez *et al*. [19], revised the systematics of Sitonini based on extensive investigations of the endophallus. Sert and Çağatay [5] described the endophallus of some species in *Sitona, Bangasternus* and *Larinus* (*Larinus aeruginosus* Hochhut, 1851, *L. atomarius*, *L. latus* and *L. sturnus*). Sert and Çağatay [6], investigated the endophallus in *Cleonus, Coniocleonus, Conorrhynchus, Larinus* (*Larinus jacea*, *L. minutus* and *L. planus*) and *Lixus.* Sert [7], described the male genitalia of *Sitona fairmarei* Allard, 1869. Medina *et al*. [2] examined the external and internal male genitalia Scarabaeinae (Coleoptera: Scarabaeidae) with drawings of endophallus of 37 species. In this study, we show details of the endophallus in the eight species of *Larinus.*

The results of this study may help in finding more characters in the differentiation of similar species. It may be interesting to include the examination of the documented ultrastructures of the endophallus in future studies of *Larinus*, but also in other Curculionidae (Coleoptera) some outlook concerning the use of the results should be given.

5. Acknowledgements

We thank Ahi Evran University (Project Direction Office) (Turkey) for supporting this project (Project no: PYO-FEN.4003/2.13.001).

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