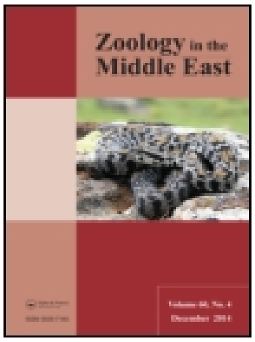

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Ultrastructural analysis of the internal sac in several species of the weevil tribe Phyllobiini (Coleoptera: Curculionidae: Entiminae)

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The ultrastructure of the internal sac was comparatively studied in five species of the genus *Phyllobius* Germar, 1824, two species of the genus *Oedecnemidius* Daniel, 1903 and one species of the genus *Parascythropus* Desbrochers, 1875, all belonging to the weevil tribe Phyllobiini (Coleoptera: Curculionidae: Entiminae). Similarities and differences between the species investigated are discussed. The total shape of internal sac is similar, but the teeth that are located in the ventral part are different in all the species. The details of the internal sac under the scanning electron microscope are important for taxonomy and could be used for separating similar species.

Keywords: Phyllobiini, *Phyllobius*, male genitalia, internal sac, SEM.

Introduction

In Coleoptera, the male genitalia and associated membranes have for long been used as important characters in taxonomy, but the functioning of the internal membranes are still not well understood. Their position in relation to the connecting membrane and the genital membrane folding patterns has never been thoroughly investigated (Wanat, 2007). In Curculionidae, the male genitalia have important structures that have been used to define taxonomic groups (Tuxen, 1970; Thompson, 1988; Sert & Çağatay, 1994, 1999; Sert, 2006; Wanat, 2007). The shape of the aedeagus is tubular, often arcuate, sometimes flattened, and normally contains an internal sac. The internal sac becomes everted into the female vagina (or the bursa copulatrix) during coitus and is thus the functional intromittent organ. The membraneous walls of the internal sac often possess an armature of spines, denticles, hairs, scales, papillae (i.e. 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 the armature of the internal sac is constant within a species and therefore very useful for taxonomic purposes. Wanat (2007) studied the structure of the genital chamber in weevils (Curculionoidea) and other Coleoptera and used the term endophallus (internal sac) for the genital membrane in the aedeagus, which is synonymous with the internal sac. The internal sac membrane often has various sclerotised outgrowths forming variably shaped sclerites, larger teeth, spinules or miniplates, and usually becomes outwardly prominent when the internal sac is extruded (Wanat, 2007).

We examined the surface morphology of the internal sac in eight species of Phyllobiini utilizing both light and scanning electron microscopes to identify differences in the ultrastructure between these species.

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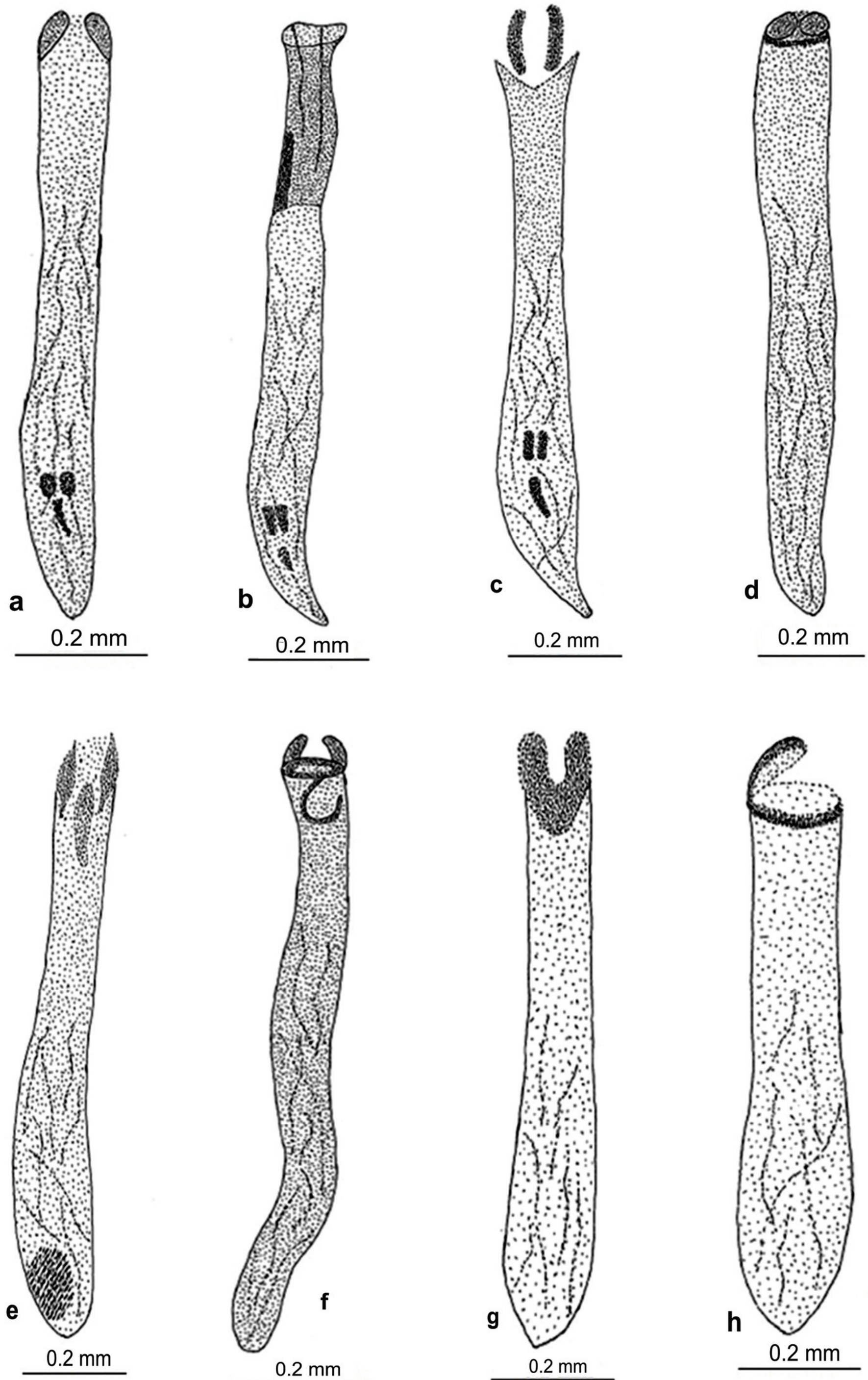

Figure 1. Drawings of the internal sac (endophallus) in species of Phyllobiini; a- *Phyllobius argentatus*, b- *P. fulvago*, c- *P. glaucus*, d- *P. karamanensis*, e- *P. maculicornis*, f- *Parascythropus mirandus*, g- *Oedecnemidius pictus*, h- *O. saltuarius*.

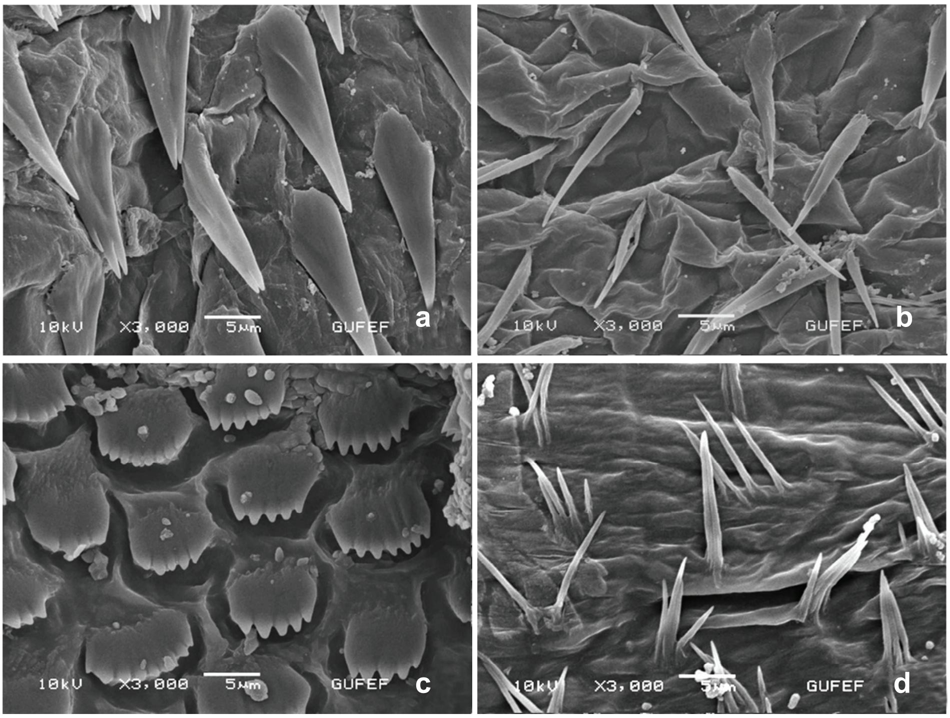

Figure 2. Scanning electron microscope micrograph of the surface morphology of the endophallus; *Phyllobius argentatus* (a), *P. fulvago* (b), *P. glaucus* (c), *P. karamanensis* (d).

Material and methods

Five species of *Phyllobius* Germar, 1824, two species of *Oedecnemidius* Daniel, 1903 and one species of *Parascythropus* Desbrochers, 1875, all in the Phyllobiini (Entiminae), were investigated. Specimens (*Phyllobius argentatus* (4♂), *P. glaucus* (6♂), *P. fulvago* (2♂), *P. karamanensis* (5♂) and *P. maculicornis* (3♂), *Oedecnemidius pictus* (4♂) and *O. saltuarius* (2♂), *Parascythropus mirandus* (4♂♂)) were collected from central Anatolia. The specimens were dissected under a light microscope The aedeagi were removed after softening the abdomen in 10% KOH for 24 h at 30°C. The internal sac was then separated from the aedeagus and extended using fine needles. 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 of their organic debris, mounted with double-sided carbon tape on SEM stubs, and 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 Figure 1) follows Tuxen (1970), Wanat (2007) and Medina, Molano, & Scholtz (2013).

Results

Under the light microscope, the internal sac appears membraneous, transparent and resembles a tube in all species. The armature of teeth, referred to as rapsules by Medina et al. (2013), are located in the medial area on the ventral surface, but are barely visible under the light microscope (Figures 1a-h). In all species, the internal sac has multiple folds apically while the basal portion exhibits a different organisation containing various sclerites (Figures 1a-h). Additionally *P. argentatus, P. fulvago* and *P. glaucus* have

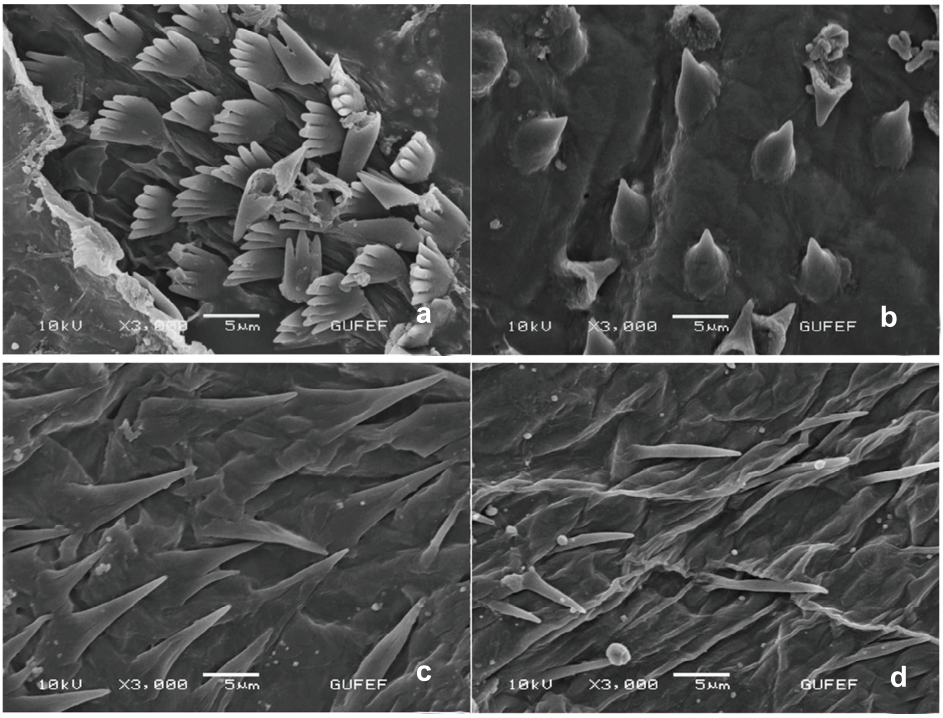

Figure 3. Scanning electron microscope micrograph of the surface morphology of the endophallus; *Phyllobius maculicornis* (a), *Parascythropus mirandus* (b), *Oedecnemidius pictus* (c), *O. saltuarius* (d).

three apical sclerites (Figures 1a-c) while other species lack these sclerites (Figures 1d-h), although in *P. maculicornis* the internal sac has a large apical spot that is strongly sclerotised (Figure 1e). In *P. argentatus* the basal sclerites are elliptical (Figure 1a), *P. fulvago* lacks the basal sclerites (Figure 1b), and in *P. glaucus* the basal sclerites are longitudinal and the ventral margin slightly convex (Figure 1c). In *P. karamanensis*, the basal sclerites are semi-elliptical (Figure 1d), and in *P. maculicornis* the basal sclerites are leaf-like (Figure 1e). *Parasyctropus mirandus* has two short basal sclerites plus a strong hook-like structure (Figure 1f). In *Oedecnemidius* species, the internal sac is smooth and shorter than seen in the other genera (Figure 1g-h), in *O. pictus* the basal sclerites are V-shaped (Figure 1g), while in *O. saltuarius* the basal sclerites resemble the rim of a cover (Figure 1h).

Under the scanning electron microscope, the teeth of the internal sac in the medial area are different for each species. The internal sac is tube-like and the teeth are located on the ventral surface, and thus are not visible. When the ventral surface is opened by forceps, the teeth appear (Figure 3). The internal sac is covered with short teeth some of which are triangle-like apically, whilst others have teeth that are fork-like (*P. argentatus*) (Figure 2); the surface of the internal sac has long teeth which are simple (*P. fulvago*); short teeth form plate-like groups (*P. glaucus*); the surface of the internal sac has teeth which are dual or triple (*P. karamanensis*) (Figure 2); short teeth form groups like fringes, and strongly cover the surface (*P. maculicornis*) (Figure 3); the surface of the internal sac has short and simple teeth, gradually narrowed from base to apex (*Parascythropus mirandus*); teeth short, widened basally, gradually narrowed from base to apex (*O. pictus*); teeth simple, long and thin (*O. saltuarius*) (Figure 3).

Discussion

In the Curculionidae, several authors used the aedeagi in their taxonomic work, but did not treat specifically the armature of the internal sac (e.g. Angelov, 1976; Caldara, 1984, 1990; Dieckmann, 1980; Erbey & Candan, 2010; Pesarini, 1980; Ter-Minasyan, 1978; Yunakov & Korotyaev, 2007). The internal sac is generally not yet well studied. Tuxen (1970, figure 73) provided a rough line drawing of the internal sac; he noted that “the membraneous walls of the internal sac often possess an armature of spines, denticles, hairs, scales, papillae, etc.” Velazquez de Castro, Alonso-Zarazaga, and Outerelo (2007) revised the Sitonini based on extensive investigations of the internal sac. Sert and Çağatay (1994) described the internal sac of some species in *Sitona, Bangasternus* and *Larinus* (Curculionidae), and Sert and Çağatay (1999) investigated the internal sac in *Cleonus, Coniocleonus, Conorrhynchus, Larinus* and *Lixus* (Curculionidae: Lixinae). Sert (2006) described the male genitalia of *Sitona fairmarei* Allard, 1869. Medina et al. (2013) examined the external and internal male genitalia of Scarabaeinae (Coleoptera: Scarabaeidae) with drawings of the internal sac of 37 species. In our study, we found that the apical and especially the basal organisation of the internal sac are different in all species (Figure 1). In *Phyllobius argentatus*, *P. fulvago* and *P. glaucus*, the internal sac has similar apical sclerites (Figures 1a-c). An important difference among the species is the teeth which are located in the medial area of the internal sac. They are of different structure among the different species (Figures 2-3).

The results of this study indicate that the ultrastructure of the internal sac, e.g. spines, teeth, papillae and hairs, could be used as a taxonomic character and may also be valid for separating morphologically similar species.

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