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Original araştırma (Original article)

Light and scanning electron microscope investigations on the structure of the proventriculus in *Melanogryllus desertus* (Pallas) (Orthoptera: Gryllidae)[1]

Melanogryllus desertus (Pallas) (Orthoptera: Gryllidae)'ta proventrikulusun yapısı üzerine ışık ve taramalı elektron mikroskobu araştırmaları

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Summary

This study was carried out between the years 2005 and 2008 in the Invertebrate Culture and Research Laboratory at Ege University, Turkey. In this work, we aimed to investigate the proventriculus morphology of *Melanogryllus desertus* using light and scanning electron microscopy. The general morphology of the proventriculus resembles other Gryllidae species in that sclerotized appendices are arranged in six internal rows. Structurally, sclerotized appendices are composed of a middle tooth and two lateral teeth, both adorned with denticles rounded in shape. Although four middle denticles are found in general, their numbers vary to as many as five or six. In addition, there are sclerotized lobes and sclerotized partitions among sclerotized appendices, and they are densely setose.

Key words: *Melanogryllus desertus*, Gryllidae, proventriculus, morphology, denticle

Özet

Bu çalışma 2005-2008 yılları arasında Ege Üniversitesi Kampüsü bünyesindeki Omurgasız Kültür ve Araştırma Laboratuvarında gerçekleştirildi. Bu çalışmada, *Melanogryllus desertus*'ta proventrikulus morfolojisini ışık ve taramalı elektron mikroskobuyla incelemeyi amaçladık. Genel proventrikulus morfolojisi sklerotik apendislerin altı internal sırada düzenlenmesiyle diğer Gryllidae türlerine benzerdir. Yapısal olarak, sklerotik apendisler yuvarlak dişçiklerle donanan tek orta diş ve iki tane lateral dişten oluşmuştur. Genelde dört orta dişçik bulunmasına rağmen sayısı beş ya da altı olabilir. Ayrıca sklerotik apendisler arasında sklerotik loblar ve sklerotik uzantılar mevcuttur ve yoğun kitin kıllıdır.

Anahtar sözcükler: *Melanogryllus desertus*, Gryllidae, proventrikulus, morfoloji, dişçik

[1] This study is a part of the Ph. D. thesis of the first author and a part of the study was presented at the 18th National Biology Congress from 26-30 June, 2006 Kuşadası-Aydın, Turkey

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Introduction

Generally, the digestive system is mainly composed of the foregut, midgut and hindgut in insects. The proventriculus, which is located at the posterior end of the foregut, may be very muscular and have a complex structure containing sclerotized teeth and spines for tearing food to pieces or it may be a simple valve at the entrance of the midgut (Nation, 2002). In Orthoptera, its functions are to break food into small pieces and filter it, to maintain the flow of enzymes into the crop and to form a valve by closing the entrance to the midgut (Snoddgrass, 1935). Morphological differences in the proventriculus are useful for taxonomic studies (Bland & Rentz, 1991; Serrao, 2001; 2005; 2007). The proventriculus is of great importance in terms of taxonomic studies due to its relationship with the feeding habits of insects, which can be quite variable in different species. The relevance of the connection between the morphology of the proventriculus and feeding habits has been correlated in termites (Lebrun, 1985; Lebrun & Lequet, 1985), in bees (Bailey, 1952; Peng & Marston, 1986), in ants (Roche & Wheeler, 1997; Caetano, 1988) and in some Orthopteran species (Judd, 1948; Bland & Rentz, 1991, Fontanetti & Zefa, 2000; Fontanetti et al., 2002).

Melanogryllus desertus, also known as the black cricket, is distributed in South Europe, North Africa, south parts of Siberia and middle Asia, and middle and west Anatolian regions in Turkey (Lodos, 1975). However, there are few studies on the proventriculus of Gryllidae (Judd, 1948; Fontanetti & Zefa, 2000; Fontanetti et al., 2002; Mesa et al., 2004; Li et al., 2011). Although the proventricular morphology was similar in these studies, a slight variety in the shape of the apex of the denticles of lateral teeth and the numbers of denticles of the middle teeth was apparent. As seen clearly in these studies, proventriculus is an auxiliary character for taxonomic studies and its variety of structures helps to determine the different species. The aim of this study was to investigate the morphology of the proventriculus in *Melanogryllus desertus* at the light and scanning electron microscopy level for the first time.

Material and Methods

Insect maintenance and feedings

Crickets were cultured between the years 2005 and 2008 in the Invertebrate Culture and Research Laboratory at Ege University Campus, Bornova-İzmir, Turkey (temperature: 26±2°C; relative humidity: 45±5 %; photoperiod: natural) at Ege University Campus, Bornova-İzmir, Turkey. In their jars, they were fed with lettuce and chicken grain in jars twice a week. Cotton plugged glass tubes filled with water were put into the jars to supply the water needs of the insects. Also, small Petri dishes with their surface covered with moist cotton for mated females to lay eggs on were placed in the jars. The first nymphs emerged within 10-12 days. They became adult crickets nine nymphal stages later. Adults were dissected in insect physiological saline while using a stereomicroscope.

Light microscope analyses

After insect dissection, proventriculi were fixed in Bouin's solution (Strobel et al., 1981) for 24 hours. Dehydration was achieved with graded ethanol (70 %, 96 %,100 %) for 15 minutes in each. They were put in xylol (15 minutes) for transparency and embedded in paraffin (3 x 1 hour). Paraffin blocks were serially sectioned at 5 µm thickness using a microtome. Tissues were stained with Mayer's Haematoxylin & Eosin (H-E) and Mallory's Trichrome (MT) (Humason, 1979) and photographed using a Olympus CX 31 photomicroscope.

Scanning electron microscope analyses

Scanning electron microscopy (SEM) procedures were applied according to Nation (1983). Isolated proventriculi were transferred immediately for 5 minutes to a 1 % glutaraldehyde in 0.1 M

cacodylate buffer of pH=7.0. They were washed in distilled water (5 minutes) and dehydrated through ethanol solutions of 70 %, 85 %, 95 % and 100 %, with 5 minutes in each. They were immersed in HMDS (hexamethyldisilazane) for 5 minutes, air dried at room temperature and mounted on steel stubs with sticky tabs. Specimens were sputter-coated with gold and examined under a JEOL JSM-5200 scanning electron microscope.

Results

The proventriculus is rounded and surrounded posteriorly by two large gastric caeca of the midgut (Fig 1a). Internally, there are six rows of sclerotized appendices very evident in SEM (Fig 1b) and light microscopic examinations (Fig 2a). These appendices are the biggest partitions when compared to other parts of proventriculus (Fig 2b), and are attached to each other by sclerotized partitions (Fig 3a). Each sclerotized appendix is characterized by a central protrusion, called middle tooth and two lateral expansions, called lateral teeth. The middle tooth has apical projections called middle denticles. Similarly, the lateral teeth have apical projections called lateral denticles (Figs 3b, 4a, b).

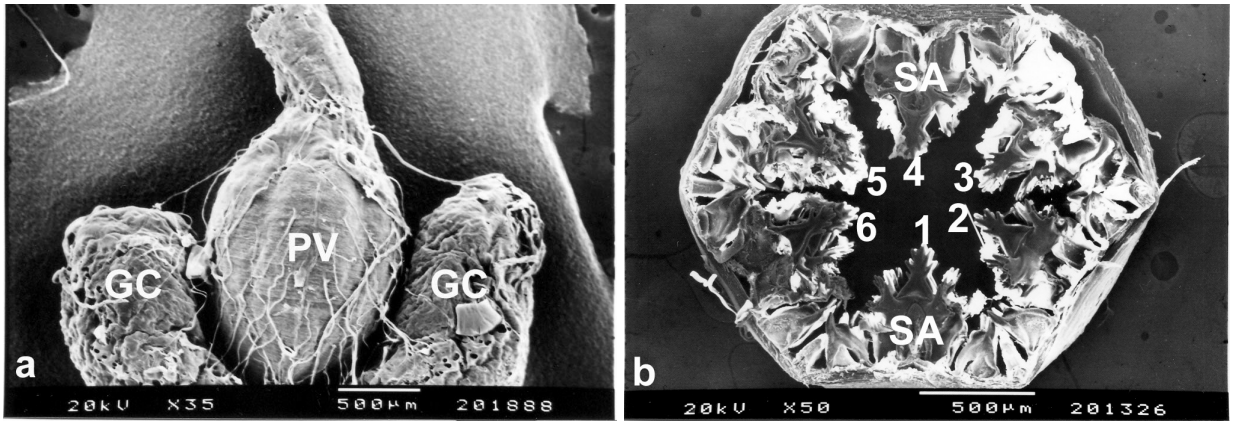

Figure 1. Scanning electron micrographs from *M. desertus* posterior end of foregut and proventriculus. a Posterior end of foregut showing proventriculus (PV) and gastric caecum (GC). b Cross section of proventriculus showing sclerotized appendix (SA); note the six rows of sclerotized appendices.

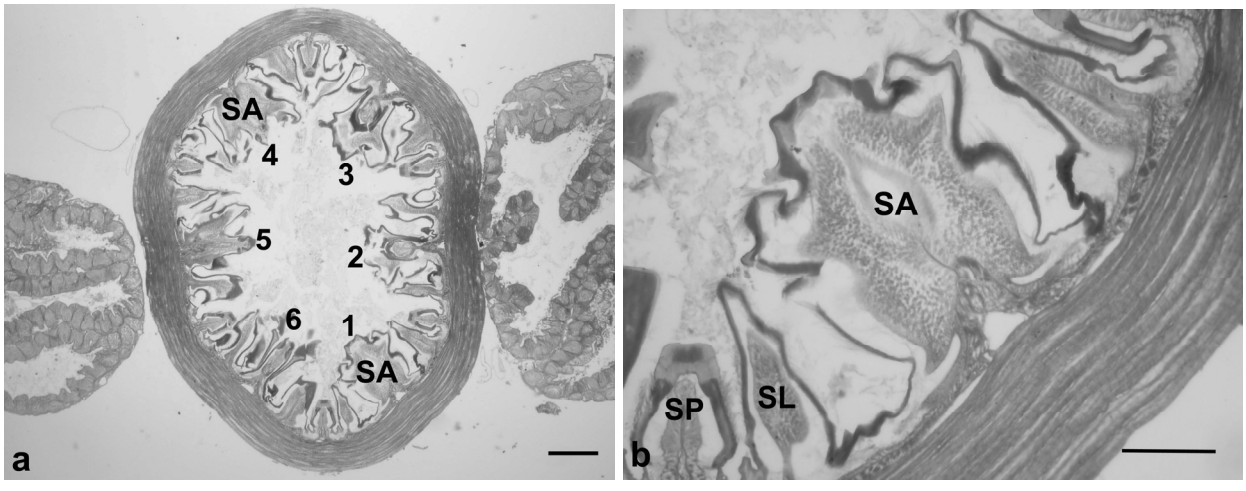

Figure 2. Light micrographs from *M. desertus* proventriculus. a Cross section view of six rows of sclerotized appendices (SA). Bar: 200 µm, Stain: MT; b Detailed view of (a) showing sclerotized lobe (SL) and sclerotized partition (SP). Bar: 100 µm, Stain: MT.

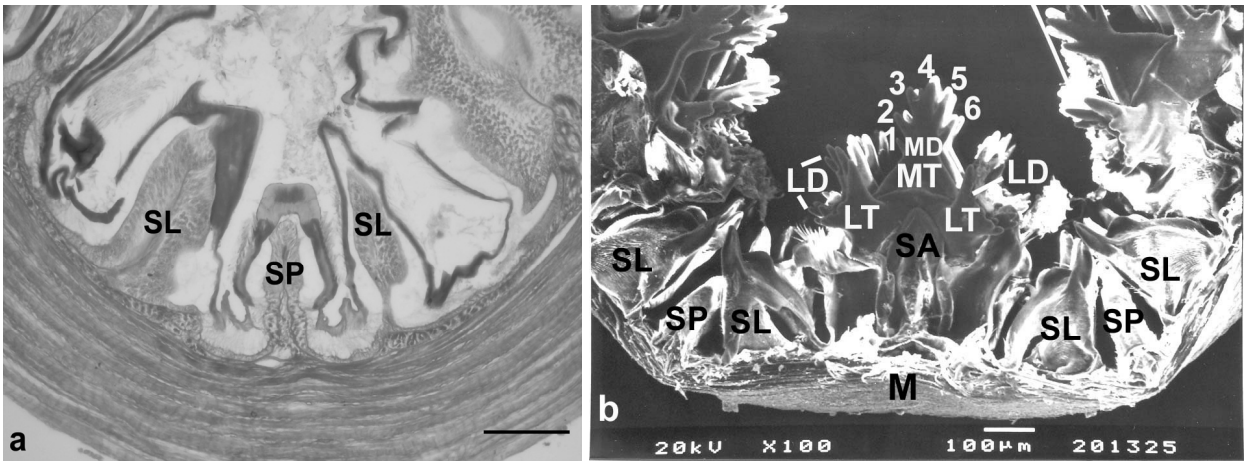

Figure 3. Light and scanning electron micrographs from *M. desertus* proventriculus. a. Sclerotized partition (SP) located between sclerotized lobes (SL). Bar: 100 µm, Stain: MT b. Detailed appearance of proventriculus structure; SA: sclerotized appendix; MT: middle tooth; MD: middle denticles (six); LT: lateral tooth; LD: lateral denticles; SL: sclerotized lobes; SP: sclerotized partitions; M: muscles.

Rounded middle and lateral denticles and triangular middle tooth are quite apparent (Fig 4a). In addition, sclerotized lobes covering sclerotized partitions are easily discerned (Fig 4b). While there are generally four middle denticles in a row of sclerotized appendices (Fig 4b), five or six middle denticles can be found in the same row of sclerotized appendices (Fig 5a). These parts are not clearly seen in histological sections, but some middle denticles can be noticed in some sections, where five and six middle denticles were observed in the sclerotized appendices, respectively (Fig 5b).

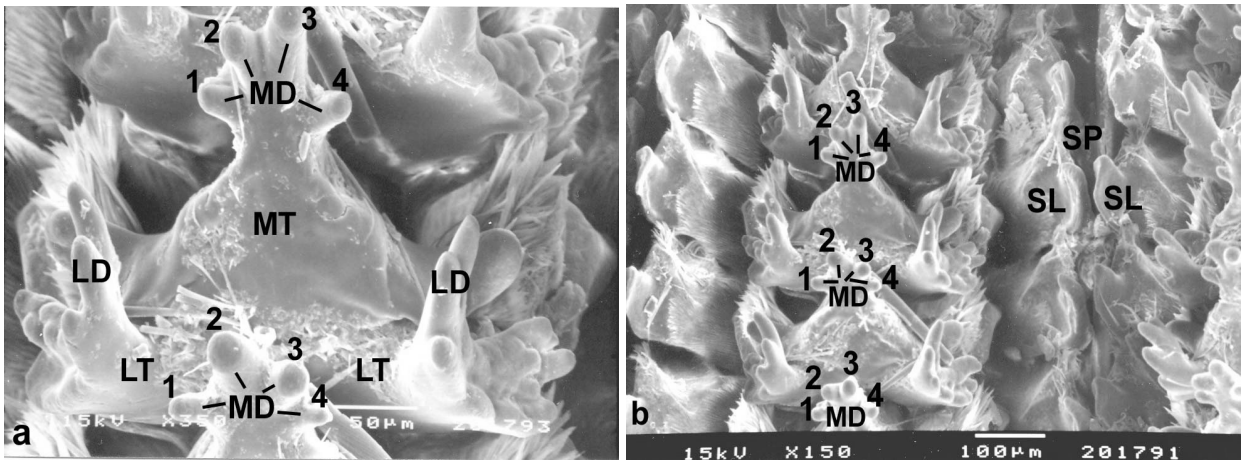

Figure 4. Scanning electron micrographs from *M. desertus* proventriculus. a. Showing rounded shaped middle denticles in two middle teeth; MT: middle tooth; MD: middle denticles; LT: lateral tooth; LD: lateral denticles; b. Four middle denticles in same row of sclerotized appendices. Note that two sclerotized lobes (SL) cover a sclerotized partition (SP). MD: middle denticles; LD, lateral denticles.

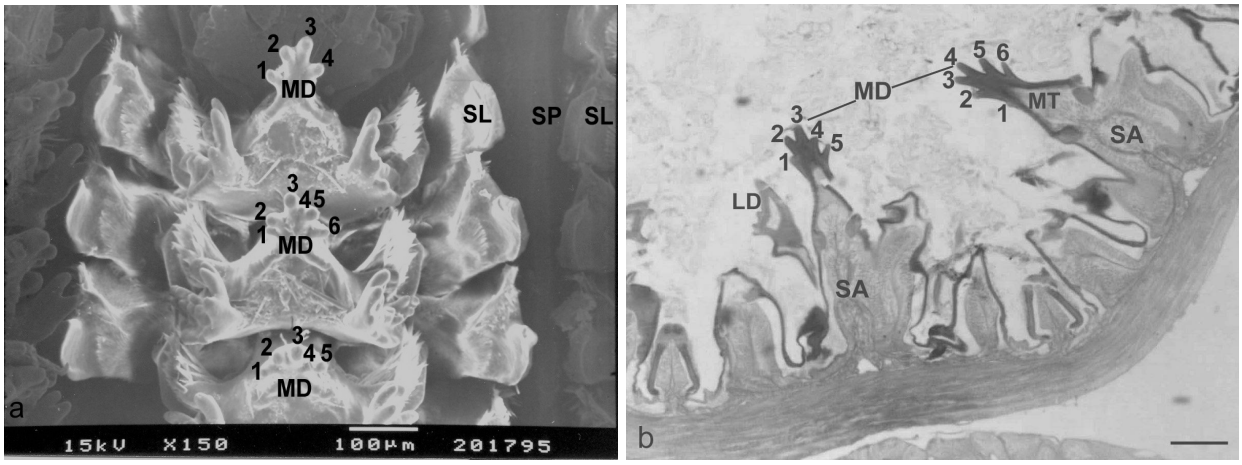

Figure 5. Proventriculus of *M. desertus*: a. Scanning electron micrographs from same individual showing four, five and six middle denticles (MD) in same row of sclerotized appendices. b. Five and six middle denticles (MD) of triangular middle teeth (MT) in different rows of sclerotized appendices (SA); Lateral denticles (LD). Bar: 100 µm, Stain: MT.

The lateral teeth are formed by external and internal partitions (Fig 6a). Similarly, the internal partition has apical projections called lateral denticles and their extremities were also rounded in shape. Sclerotized lobes are densely setose (Figs 6a,b,c).

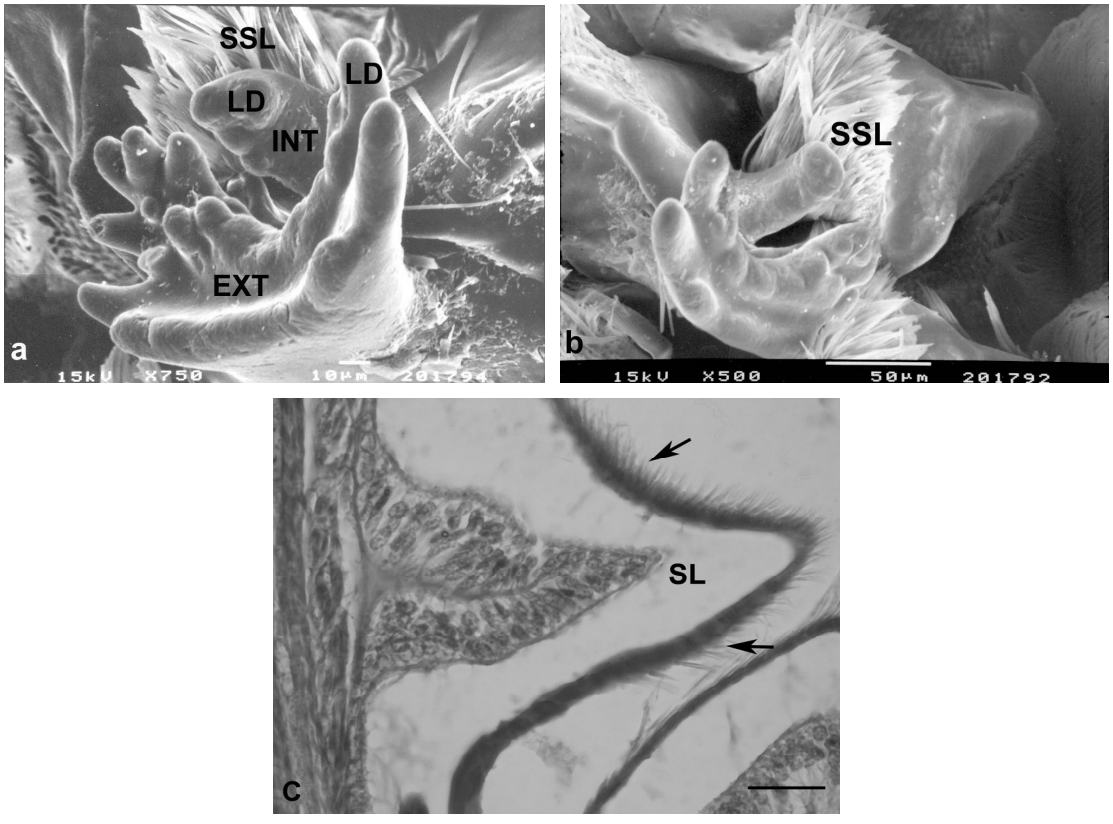

Figure 6. Proventriculus of *M. desertus*. a. Scanning electron micrographs showing external portion of lateral tooth (EXT) and its denticles (LD); internal portion of lateral tooth (INT) and its denticles (LD); sclerotized setose lobes (SSL). b. Scanning electron micrographs showing sclerotized setose lobes (SSL); c. Light micrographs showing cross section details of sclerotized lobes (SL). Note dense setae (arrows). Bar: 20 µm, Stain: H-E.

External layers of the proventriculus are made up of cubic epithelial cells arranged as a single layer. This cell layer is supported by longitudinal and circular muscle layers (Fig 7a). There is a stomodeal valve towards the midgut (Fig 7b).

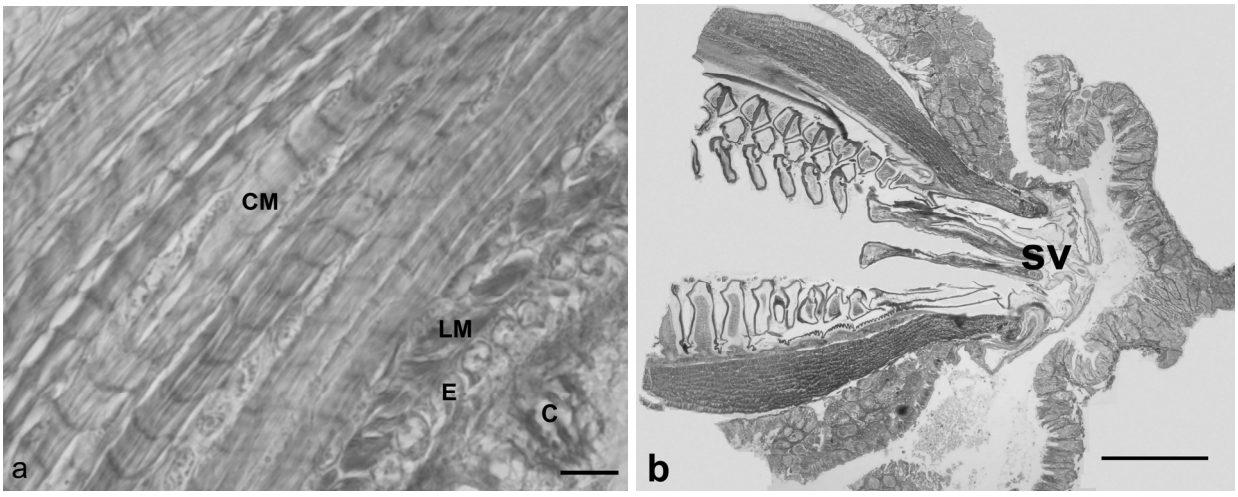

Figure 7. Light micrographs of proventriculus external layers and stomodeal valve of *M. desertus* a. Detail of the external layer of proventriculus. Note cuticle (C), epithelial cells (E), longitudinal muscles (LM) and circular muscles (CM). Bar: 10 µm. Stain: MT b. Longitudinal section view showing the stomodeal valve (SV). Bar: 500 µm. Stain: MT.

Discussion

Many Gryllidae species; *Gryllus assimilis* (Fontanetti & Zefa 2000), *Endecous betariensis*, *E. cavernoculis*, *E. itatibensis* (Fontanetti et al., 2002) and *Miogryllus piracicabensis* (Mesa et al., 2004) have a large and complex proventriculus like *Melanogryllus desertus*. The number of middle denticles varies among Gryllidae species. There were, on average, seven medium denticles in *Gryllus assimilis* (Fontanetti & Zefa, 2000), and six or seven in *Gryllus bimaculatus* (Woodring & Lorenz, 2007). The number of middle denticles in the *E. cavernicolus* varies; some teeth with seven denticles and others with six were observed in the same insect. Similarly, *E. itatibensis* had variations in the number of middle denticles. There were five, six or seven middle denticles in *E. itatibensis*. In contrast to *E. itatibensis* and *E. cavernicolus*, the number of middle denticles in *E. betariensis* was six and did not vary (Fontanetti et al., 2002). However, the number of middle denticles in *M. desertus* showed differences. While the minimum number of middle denticles was four, the maximum number was six in *M. desertus*. Mesa et al (2004) reported that the proventricular median teeth of *Miogryllus piracicabensis* mostly have five denticles (Mesa et al., 2004).

Szinwelski et al (2009) investigated the proventriculus structure in Nemobiinae crickets (*Phoremia* sp., *Zucchiella* sp. and *Amanayara* sp.) and stated that the proventriculus of the three species had the same basic morphology, but there were variations among species in the number and shape of denticles, in the median tooth morphology, and in the shape and location of hair-like projections and lateral projections. In *Amanayara* sp, the maximum middle denticle number was four, whereas it was five in *Phoremia* sp, and six in *Zucchiella* sp as in *M. desertus*.

Li et al. (2011) studied proventricular micromorphological characterization of Grylloidea species from China. In Nemobiinae; *Dianemobius fascipes*, *Polionemobius taprobanensis* and *Pteronemobius*

gifuensis had, on average, 1-2 middle denticles on each medium tooth. On the other hand, in Gryllinae; *Nigrogryllus sibiricus*, *Gryllodes sigillatus*, *Gryllodes supplicans*, *Teleogryllus occipitalis*, *T. emma* and *Velarifictorus micado*, had 6-10 middle denticles on each medium tooth. The number of middle denticles of *G. supplicans* was lowest and of *T. emma* was largest. In addition, *Oecanthus longicauda*, belonging to Oecanthinae, had 5-6 middle denticles on each medium tooth.

The lateral teeth composed of internal and external partitions in *M. desertus* were reported in *E. betariensis*, *E. cavernicolus* and *E. itatibensis* (Fontanetti et al., 2002), *Dianemobius fascipes*, *Polionemobius taprobanensis* and *Pteronemobius gifuensis*, *Nigrogryllus sibiricus*, *Gryllodes sigillatus*, *G. supplicans*, *Teleogryllus occipitalis*, *T. emma* and *Velarifictorus micado* (Li et al., 2011). Middle and lateral denticles were rounded in *Gryllus assimilis* (Fontanetti & Zefa, 2000), and in *E. betariensis* and *E. cavernicolus* (Fontanetti et al., 2002). In addition, in *Nigrogryllus sibiricus* and *Teleogryllus emma*, the medium teeth had large middle denticles and the lateral denticles were more rounded (Li et al., 2011). In the present study, both middle and lateral denticles were rounded in *M. desertus*. However, the middle and lateral denticles in *E. itatibensis* were sharper in general (Fontanetti et al., 2002).

Bland & Rentz (1991), examined the structure of proventriculus in Australian Grylliacrididae species and suggested that herbivorous Grylliacrididae species had lateral denticles longer than those of predatory species. This same characteristic was observed in *Gryllus assimilis* Fabricius, 1775 (Fontanetti & Zefa, 2000) and *M. desertus*. Therefore the authors proposed that these differences in proventriculus structure can be used as taxonomic characters. In addition, the sclerotized lobes are longer in *M. desertus* and cover the sclerotized partition differently from *G. assimilis* (Fontanetti & Zefa, 2000) and *E. itatibensis*, *E. cavernicolus*, *E. betariensis* (Fontanetti et al., 2002). This is another important difference when compared with other Gryllidae species.

Briefly, in adult insects that feed on solid food the proventriculus generally becomes differentiated as a definite part of the digestive system and its inner walls develop a complex structure with teeth (Snoddgrass, 1935). Similarly, many authors mentioned above suggested that the species in question have complex sclerotized appendices containing large middle and lateral teeth with many denticles. Our results are compatible with these findings, and we agree that the insect undertakes multiple food breaking functions by means of this texture of proventriculus. In addition, the proventriculus structure in many Gryllidae species was basicly the same type but there were differences in the shape and number of denticles. As a result, we suggest that our findings are relevant in terms of taxonomic studies in the determination of Gryllidae species.

Acknowledgements

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