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THE COMPARATIVE HISTOLOGY OF THE DIGESTIVE TRACT OF ACRIDA ANATOLICA AND PARAPHOLIDOPTERA SPINULOSA (ORTHOPTERA)

Banu EREN[a*], Fatih BAŞAR[a], Dilek SAĞIR[b], Burcu Demirel YILMAZ[c], Sevcan MERCAN[a], Zafer EREN[a]

[a]*Ondokuz Mayis University, Art and Science Faculty, Biology Department*
[b]*Sinop University, School of Health, Nursing Department*
[c]*Ordu University, Akkuş Vocational School, Hunting and Wildlife Programme*

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*Correspondence: Banu EREN
Address: Ondokuz Mayis University, Art and Science Faculty, Biology Department, Samsun, Turkey
E-mail: banueren@gmail.com

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ABSTRACT

Acrida anatolica and Parapholidoptera spinulosa are two different species of migratory caterpiller in the same family and both are threats to agriculture. They are harmful, they migrate and spread wide areas. Herbivorous *Acrida anatolica* has strong chewy mouth part, its mandibula is considered to be the basic insect mouth type. *Parapholidoptera spinulosa* a carnivorous species, has a strong chewing gut. The purpose of this study is to compare the structures of the digestive tracts of these species. The digestive tract is divided into three parts, the foregut, the midgut, and the hindgut. The prismatic epithelium, the external circular and longitudinal muscles, the connective tissue, the granular structures and the peritrophic membrane are the parts of the foregut. The caecum is also found in this portion. The grasshoppers were collected from the Ondokuz Mayis University Campus and kept in special containers and fed with wheat grass, fresh grass and with different insects. During the dissection process, the digestive tracts were removed and tissues were fixed with 10% buffered neutral formalin solution for 24 hours. After routine histological procedures, the sections were stained with hematoxylin-eosin (H-E). The foregut, the midgut and the hindgut were comparatively examined in terms of peritrophic membrane, epithelial tissue, cell size, nucleus size, circular and longitudinal muscles, connective tissue, regenerative cells and cellular diversity. The knowledge of grasshopper histology and embryology will contribute to the development of more efficient fighting with grasshoppers and the development of agricultural plant protection products.

1. Introduction

Insects that have existed on earth for millions of years have a wide spread. In terms of species, they are the most common species in the kingdom Animalia. They have more than 750,000 species diagnosed today (1).

Orthoptera has more than 22.000 species in 30 families and it has been reported to be an ordo that has spread all over the world. Orthoptera have two sub-order; Caelifera (short antennae grasshoppers) and Ensifera (long antennae grasshoppers). The grasshoppers show hemimetabolous development, their bodies are long and cylindrical, medium-sized, but have members with lengths reaching 12 cm (2).

The grasshoppers have extended back thighs for jumping, downward facing head, chewing mouth, well developed compound eyes, multi-segmented antennas, large prothorax (like a shield), a small mesothorax, a large metathorax, narrow front wings, wide rear wings with a large number of long and diagonally extending veins. The legs are long and cylindrical, have enlarged tarsus with one or four-segment, while the abdomen has eight or nine segments, two or three of the terminal segments are reduced and well developed ovipositors are reported (3).

Courting behavior in locusts is reported that it can be in the form of flashy, communication-receiving sound or with transmitted sound. After the

copulation; Caelifera leaves the eggs into the soil cavities using their ovipositors. It is known that Encifera eggs are placed into the plants one by one and also into the soil (4).

Many different and common features found in *Acrida anatolica* and *Parapholidoptera spinulosa* grasshopper species and the digestive system are compared by making specific measurements. *Parapholidoptera spinulosa* is a long antennae grasshopper from Ensifera sub-order, belongs to Tettigoniidae Familia. *Acrida anatolica* is a short antennae grasshopper from Caelifera sub-order, belongs to Familia Acrida. In both species there are compound eyes at the side and the upper part of the head. *Acrida anatolica* has strong, chewing mouth pieces and are considered as the basic insect mouth type, also the mandible is well developed due to feeding with the plants. *Acrida anatolica* is more capable of flying than *Parapholidoptera spinulosa*. For this reason, *Acrida anatolica* is known to be more capable for migration (5). Nowadays, it is also known that grasshoppers are used as food sources and are imported abroad (3). Forensic entomology has taken its place in the modern sciences and has developed rapidly with the use of insects in the criminal cases (6). Insects can be harmful in various moments; when they come out of their territorial area, when they are fleeing from their enemies or when they are cultivated by humans. An insect is harmless until it becomes a pathogen vector of a plant or animal (including human). Insects can be harmful due to the increase of plant and food sources in their habitats (3).

The majority of insects which feed with plants are pests of agriculture. Knowing the histological and anatomical structures of the insects, will enable the drugs against pests to be developed both more precisely and more economically (7; 8; 9). In the light of this information, nowadays insects continue to be the subject of research.

2. Materials and methods

Acrida anatolica and *Parapholidoptera spinulosa* were collected from the areas located on the campus boundaries of Ondokuz Mayıs University. (Fig. 1A; 1B). For nutrition and care; Hunter and Jones (10)'s cages were used and fed with the split grass, wheat grass and various insects.

Figure 1. ***Acrida anatolica*** **(A) and** ***Parapholidoptera spinulosa*** **(B) species.**

Post dissection, the digestive canal was removed and placed in fixation solution (10% buffered neutral formalin) for 24 hours. After routine histological procedure, sections are taken from paraffin blocks in Rotary microtome, stained with hematoxylin and eosin (H-E) and the histological structure of the digestive tract was examined.

Grass-fed *Acrida anatolica* green in color, *Parapholidoptera spinulosa*, mainly fed with meat, is brownish-blackish in color. The body of *Acrida anatolica* is elongated, like a pipe, flexible and soft compared to other grasshoppers, *Parapholidoptera spinulosa* has been observed to be more rigid, it is thought to be due to the excess amount of chitin.

Histological evaluations were performed on H-E stained slides according to the staining status and number of cells between two types. The cells were counted by using Leica DM1000 light microscope and camera, and a 33.28µm square at 40X magnification as the counting field (Fig. 2A). The section extending from the baseline to the apical

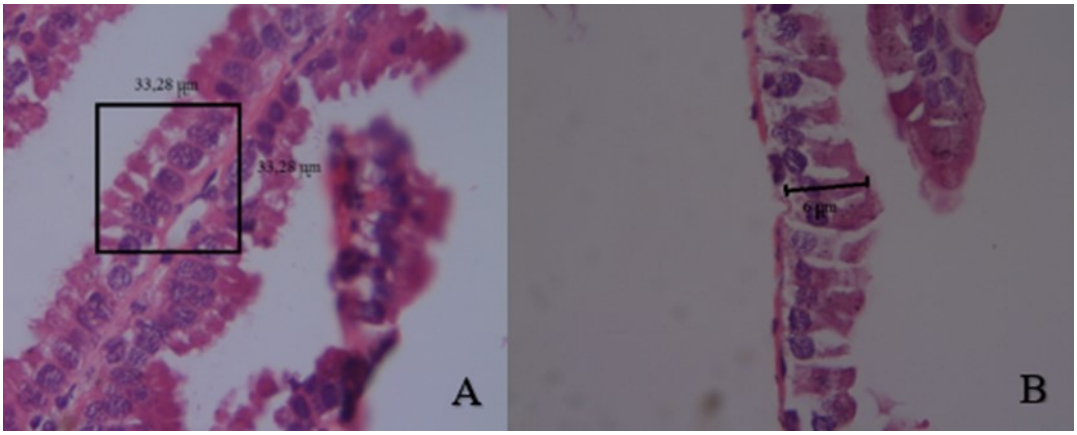

Figure 2. ***Acrida anatolica*** **species digestive tract counting square for epithelial cell and Goblet cell counting (A) (H-E, 400x).** ***Acrida anatolica*** **species digestive tract epithelial height (B) (H-E, 400x).**

direction of the epithelial tissue was measured while measuring the tissue height (Fig. 2B).

Microscopic evaluations:

In the light microscopic examination the curved structure of the digestive tract were detected. The nuclei of the cylindrical epithelial cells observed in stiffened form. The epithelium was composed of single-row prismatic cells, microvilli were observed (Fig. 3, Ab). Nuclei are dark and oval (Fig. 3, Aa).

In the microscopic view, the outer muscle cells are visible (Fig. 3Ae; 3Ad). In the basal part, granular cells are seen between the prismatic cells. These cells are regenerative cells (Fig. 3Ac; 3Bc).

The peritrophic membrane is observed as a thin line, surrounds the food and helps digestion but it is also broken down and later reformed again (Fig. 3A; 3Ah), (Fig. 3Bh).

In the epithelial region, stem cell nests observed and the young cells located in the base of the epithelium are found either individually or several together. They are defined as the regeneration region (Fig. 3A; 3B; 3C; 3D).

Acrida anatolica's digestive tract cells and the muscle structure are thinner than *Parapholidoptera spinulosa*.

In *Parapholidoptera spinulosa*, goblet secretion was more active (Fig. 3Cf; 3Df). Goblet cells and excretion granules were observed (Fig. 3Af; 3Cf). Some secretory granules were also seen in the cells (Fig. 3Ag; 3Cg). Microvilli were observed too (Fig. 3Ab). In the basal section of epithelial cells, the nuclei of regenerative cells were distinguished (Fig. 3Dc). Goblet cells were located between prismatic epithelial cells. The nuclei were oval and markedly stained. Longitudinal muscle cells were seen (Fig. 3Ad).

The average value of the number of epithelial cells in the species *Acrida anatolica* was more than 2 times than the mean value of the epithelial cell number of *Parapholidoptera spinulosa* (P> 0.05). The mean measured value of the goblet number of the *Acrida anatolica* species was more than 3 times the mean measured value of the goblet number of the *Parapholidoptera spinulosa* species (P> 0.05). The epithelial mean value of the *Parapholidoptera spinulosa* species was slightly more than 2 times the mean measured value of the *Acrida anatolica* species (P> 0.05).

Morphometric evaluations:

For the statistical studies, the data obtained from the groups were evaluated and their arithmetic means were taken and the distribution was normal by Shapiro Wilk test. The comparison of the groups was performed using IBM SPSS 20 and One-Way Variance Analysis (ANOVA) and Post-Hoc TUKEY test.

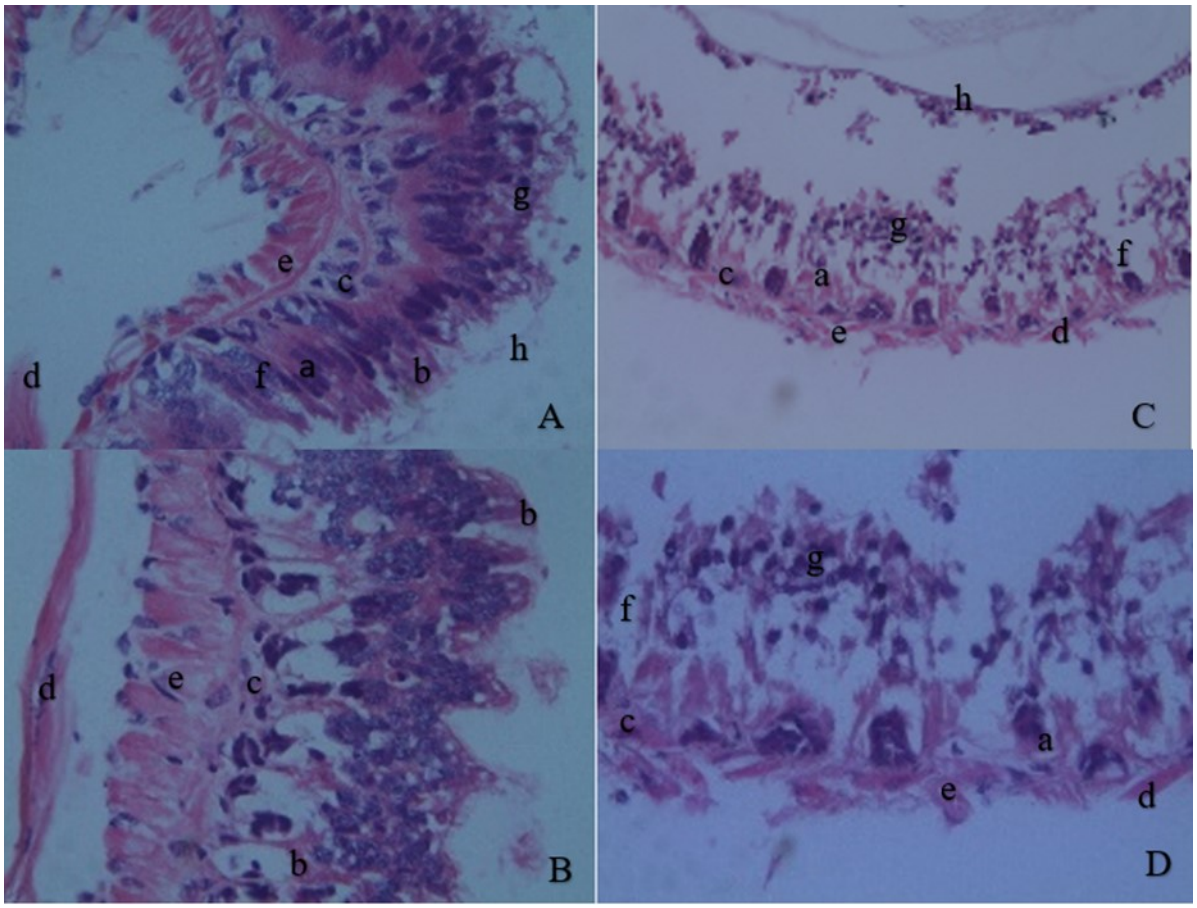

Figure 3. ***Acrida anatolica*** **(A, B) and** ***Parapholidoptera spinulosa*** **(C, D) species digestive tract, (H-E) 400x.**

In our study, herbivorous *Acrida anatolic*a and carnivorous *Parapholidoptera spinulosa* were studied, the number of epithelial cells and the number of goblet cells in the *Acrida anatolica* were higher than the *Parapholidoptera spinulosa* and the height of the epithelial lengths were higher in the *Parapholidoptera spinulosa* species (P> 0.05) (Fig. 4; Fig. 5; Fig, 6).

The df value was 5 and the probability was greater than p >0,05. This assumes that our study was statistically significant (Table 1).

3. Discussion

In this study, in order to contribute to the studies on grasshoppers, the histological structure changes due to feeding among the grasshopper species were investigated.

Acrida anatolica and *Parapholidoptera spinulosa* have a digestive tract with single-row prismatic epithelial tissue, very thin connective tissue, striated muscles with circular and longitudinal structure, microvilli, one-way opening digestive canal valves and peritrophic membrane. These are the main histological structures studied in both grasshoppers. As *Acrida anatolica* is an herbivore, it is observed in greenish color, *Parapholidoptera spinulosa* is carnivorous and brownish-blackish color. It is thought that these colors are a requirement of adaptation (11).

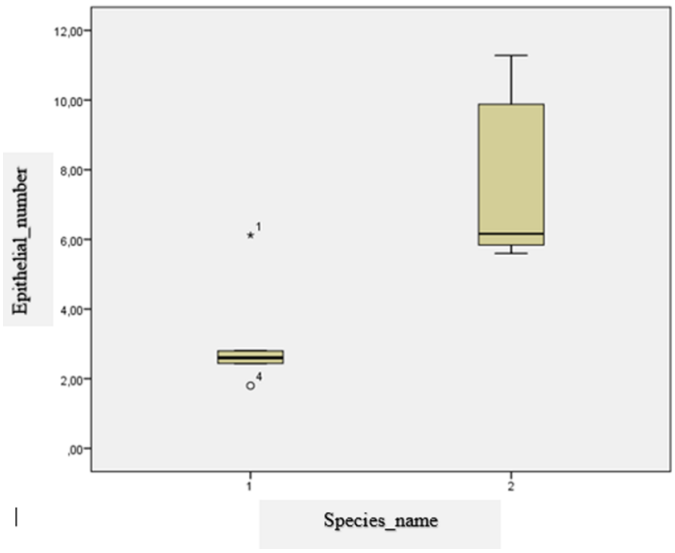

Figure 4. ***Acrida anatolica*** **(1) and** ***Parapholidoptera spinulosa*** **(2) species, number of epithelial cells.**

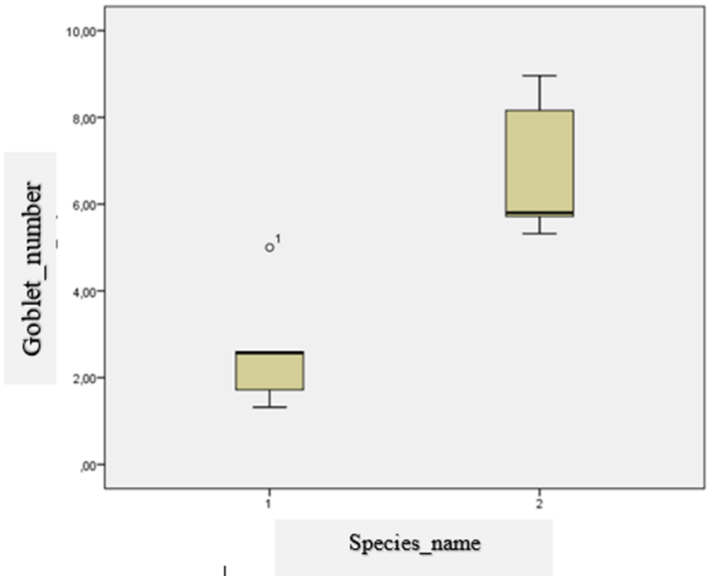

Figure 5. ***Acrida anatolica*** **(1) and** ***Parapholidoptera spinulosa*** **(2) species Goblet cell-number measurement values.**

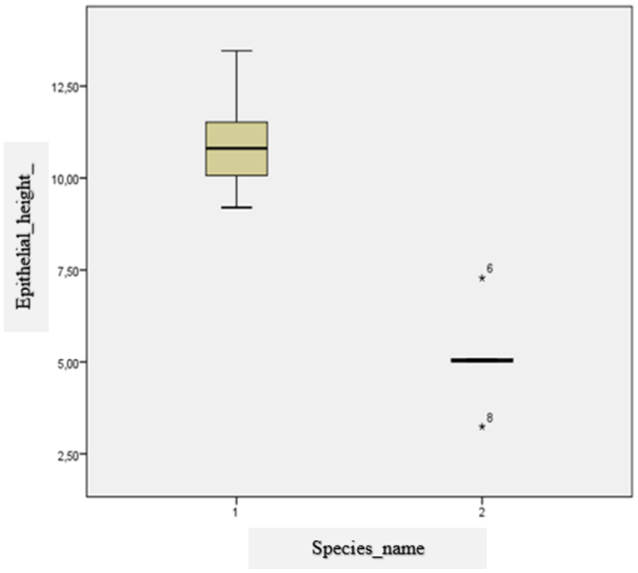

Figure 6. ***Acrida anatolica*** **(1) and** ***Parapholidoptera spinulosa*** **(2) species epithelial height measurement values.**

Bursalı (12), in the study investigating the histological and histochemical structure of *Pezodrymedusa lata* (Orthoptera: Tettigoniidae)'s digestive tract observed a very thin connective tissue under the epithelial tissue. Demirsoy (13), also mentioned about a very thin connective tissue under the epithelial tissue and some parts of the epithelial tissue is reported as a regeneration disk. In our study the presence of a very thin connective tissue under epithelial tissue and regeneration area in the basal regions of prismatic epithelial tissue in both grasshoppers is similar to these studies.

According to Ecevit et al. (11), the length of the digestive tract in insects is related to the diet of the insects and if insects are mostly fed with protein-containing diets, the digestive tract is shorter than those fed with carbohydrates. In other words, carnivorous insects are fed with protein-intensive and solid nutrients and the digestive tract is short. Herbivorous insects feed with fewer nutrients in terms of protein but higher in carbohydrate content and the digestive tract extends almost all over the body. Therefore, the digestive channel of the carnivorous grasshoppers are shorter than the digestive tract of herbivorous grasshoppers.

Table 1. Goblet number, epithelial cell number and epithelial height statistical probability table (1.species ***Parapholidoptera spinulosa*****, 2.species** ***Acrida anatolica*****)**

		Kolmogorov-Smirnov			Shapiro-Wilk		
Goblet_Number	Species_name	Statistic	df	Sig.	Statistic	df	Sig.
	1	.311	5	.128	.865	5	.248
	2	.326	5	.088	.832	5	.145
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Cell_Number	Species_name	Statistic	df	Sig.	Statistic	df	Sig.
	1	.382	5	.016	.756	5	.034
	2	.327	5	.086	.812	5	.101
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Epithelial_Length	Species_name	Statistic	df	Sig.	Statistic	df	Sig.
	1	.177	5	.200*	.967	5	.858
	2	.313	5	.122	.888	5	.347

The epithelial cell number of the herbivorous *Acrida anatolica* and the number of goblet cells were higher than those of the carnivorous *Parapholidoptera spinulosa*, while the height of the epithelial cells were higher in the *Parapholidoptera spinulosa* species. The length of the digestive tract of the species *Acrida anatolica*, was determined to be longer than the digestive tract of *Parapholidoptera spinulosa*. Thus our findings are similar to the work of Ecevit (11).

Secretory granules are abundant in the apical cytoplasm. Protein activity in these regions are higher because of the reconstruction of the peritrophic membrane. Due to the feeding of more protein in carnivorous species, shortness of the digestive tract and high cell height were observed. This is similar to that of Ecevit (11).

It is a fact that the knowledge of grasshopper histology and embryology will contribute to the development of pesticides to be used and more effective against the struggle with grasshoppers.

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