

## Investigation of Midgut's Ultrastructure of *Notonecta viridis* Decourt, 1909 and *Notonecta maculata* Fab., 1794 (Hemiptera: Notonectidae)

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### ABSTRACT

In this study, midgut's ultrastructure of the *Notonecta viridis* and *Notonecta maculata* was examined under transmission electron microscope (TEM). It was observed that midgut of the *Notonecta viridis* and *Notonecta maculata* are almost similar. Digestive canal is divided into three parts as foregut, midgut and hindgut. Midgut is wider and longer part than other parts of digestive canal. Midgut's heamasoel side is covered with muscular tissue and connective tissue and lumen side is covered with epithelial tissue. Epithelial layer consists of three different cells: Endocrine cells, regenerative cells and enterocytes cells. Endocrine cells possess secretory granules in the cytoplasm and they have the basement membrane folding in basal. Regenerative cells are small undifferentiated cells which are responsible for cellular regeneration. Enterocyte cells have many mitochondria and deep basal membrane folding in basal.

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### Key Words:

*Notonecta viridis*; *Notonecta maculata*; Midgut; Ultrastructure

### INTRODUCTION

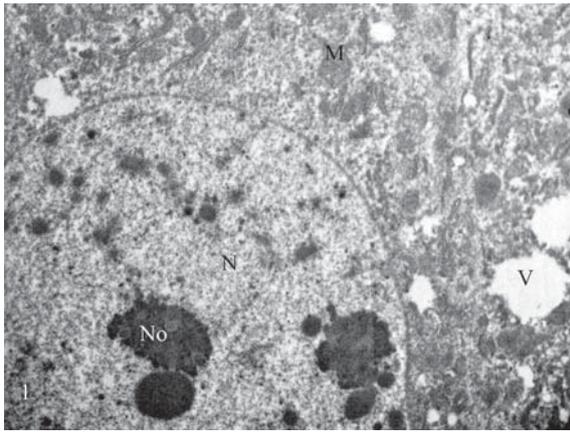
Apparently, since insects have so many species and perceived as harmful organisms, it has brought to mind the idea of avoidance of these organisms and fighting with them. Insects which we accept both harmful as well as beneficial even have important functions in maintaining the ecological balance [1]. Insect body is divided into three parts as head, thorax and abdomen. Head segments are completely fused and the boundaries of segments cannot be distinguished in adults [2].

In generally, insects get their nutrients by biting-chewing or sucking but internal parasite larvae get their nutrients through the body surface. Types of diet and food types of insects are as follows feeding with various parts of plants (herbivores), feeding with peat moss (saprophytes), feeding with small insects or other animals, feeding with fungi and bacteria, feeding with feces, carrion and blood, and those feeding with the filtrate.

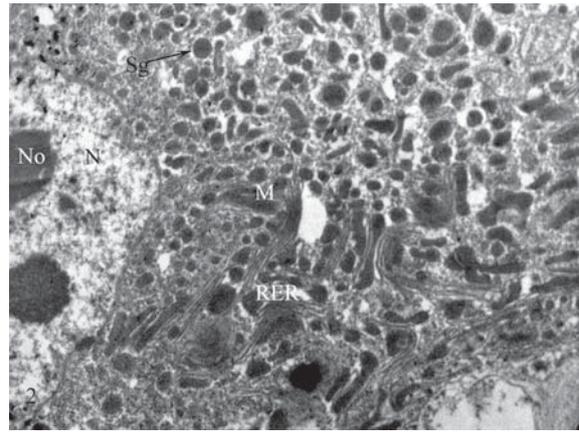
Digestive canal of insects is divided into three parts as front, middle and last intestine. Pre and post intestine is ectodermic and coated with chitin-cuticle but midgut is endoderm origin and not coated [3]. *Notonecta viridis* and *Notonecta maculata* (Hemiptera: Notonectida) are capable of feeding predators and this situation impede excessive reproduction of other insects in the aquatic environment. Because these species are fed everyday by eating 24-28 mosquito larvae, they help to provide the balance of food chain in the aquatic environment. So, by preventing excessive reproduction of mosquitoes, they have an important place as ecological for the reduction of the demand for insecticides [1].

### MATERIAL AND METHOD

Specimens were placed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 3 h at room temperature. After rinsing in phosphate buffer, the specimens were postfixed in 1% buffered osmium



**Figure 1.** Enterocyte cell in the midgut of *N. maculata*; **No:** Nucleolus, **M:** Mitochondria, **N:** Nucleus, **V:** Vacuol (TEM, x 6 000)



**Figure 2.** Enterocyte cell in the midgut of *N. viridis*. **No:** Nucleolus, **N:** Nucleus **Sg:** Secretory granules, **M:** Mitochondria, **RER:** Rough endoplasmic reticulum (TEM, x8 000)

tetroxide at pH 7.2 for 3 h at 4 °C. They were then dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate [4] and examined using a Zeiss Libra120 electron microscope.

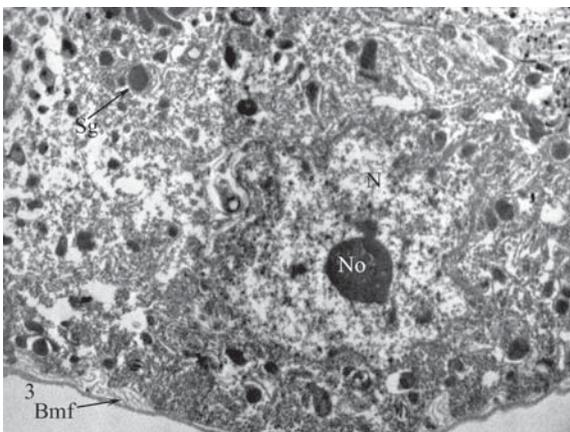
## RESULTS

The digestive canal of *N. maculata* and *N. viridis* consists of foregut, midgut and hindgut like other Hemiptera. Foregut consists of esophagus and partially enlarged proventriculus. Midgut found in the abdomen is wider and longer from other parts of the digestive canal. Hindgut has many folds in the abdomen. The total length of the digestive canal is 1.5 to 2 times than the length of insects. Morphologically, digestive canals of both species are seen similar. Midgut is surrounded by a single layer of epithelial tissue from inside and by a layer of muscle and connective tissue from outside. Midgut epithelium consists of three different cell types such as endocrine cells, enterocytes and regenerative cells. It is seen that they are almost similar to each other

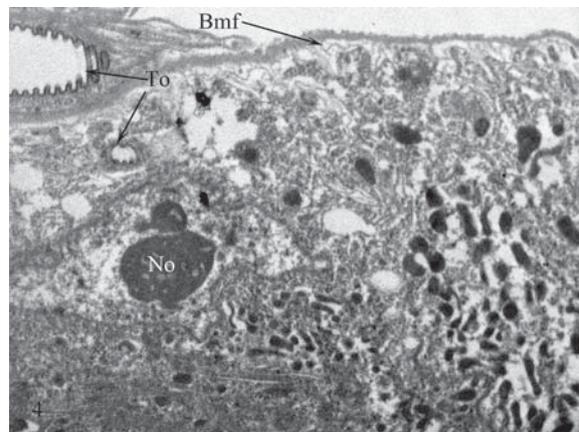
considered to the ultrastructure of epithelial cells of *N. viridis* and *N. Maculata*. A large and smooth nucleus and non-homogenously distribute chromatin in the nucleus are seen in the enterocytes cells of *N. maculata*. The same nucleus type, in a similar manner, is seen at *N. viridis*. However, more intense amounts of intracellular inclusions are seen in the enterocytes cells of *N. viridis* and the density of intracellular mitochondria is almost equal to each other (Figures 1 and 2).

Regenerative cells exist in epithelial tissue separately or in groups in basal position round or oval-shaped cells that are responsible for cellular regeneration contain rough endoplasmic reticulum and mitochondria which are not very dense within the cell. The chromatin shows a homogeneous dissemination and there are obvious nucleoli in nucleus. It is observed that basal membrane foldings extending into the cell in basal portion (Figures 3 and 4).

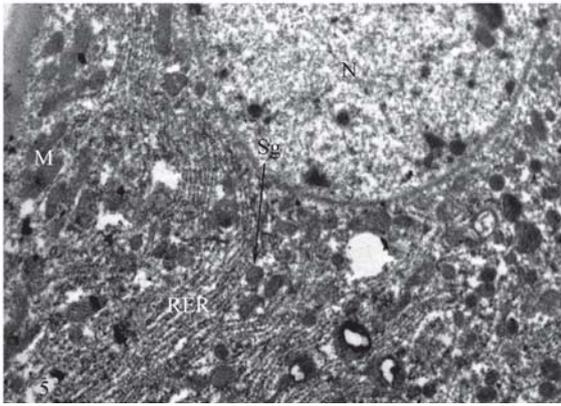
The most significant content is the secretory granule in endocrine cells of the intestine of these two species feeding



**Figure 3.** Regenerative cell in the midgut of *N. maculata* **N:** Nucleus, **Bmf:** Basal membrane folding, **No:** Nucleolus, **Sg:** Secretory granule (TEM, X6000)



**Figure 4.** Regeneratif cell in midgut of *Notonecta viridis*. **Bmf:** Basal membrane folding, **No:** Nucleolus, **To:** Tracheol (TEM, X 8 000).

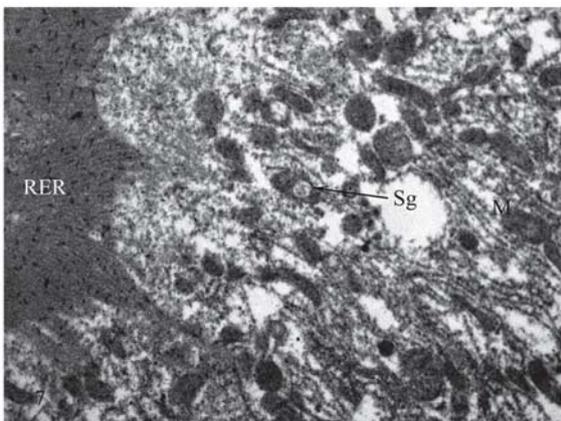


**Figure 5.** Endocrine cell in midgut of *N. maculata*. **Sg:** Secretory granule, **RER:** Rough endoplasmic reticulum, **M:** Mitochondria, **N:** Nucleus (TEM, X 8 000)

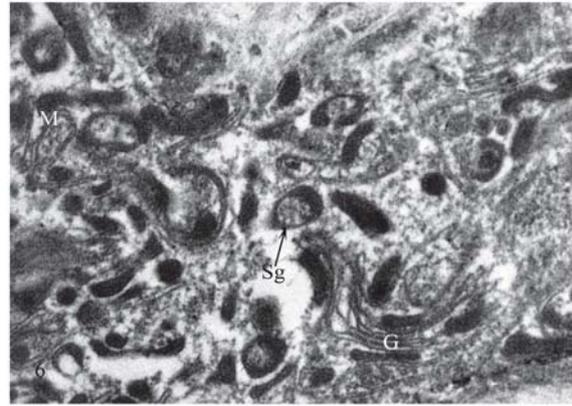
with animal foods. Furthermore, the cell is seen very rich in terms of mitochondria and rough endoplasmic reticulum (Figure 5 and 6). Vacuole-like structures and also Golgi apparatus are observed in the cells. Mitochondria and the density of rough endoplasmic reticulum are higher than other cell types.

It is observed that *N. viridis'* endocrine cells are much more intense endoplasmic reticulum (Figure 7). It is observed density of basal membrane foldings in basal portions of these cells and there are mitochondria between these basal membrane foldings (Figure 8).

The reason of both enterocytes and endocrine cells contain secretory granules in greater amounts is probably duo to their tasks related to digestive and secretory. Both enterocytes and endocrine cells contain secretory granules in greater amounts since probably making tasks are related to digestive and secretory. These secretory granules are spread approximately the same amount on each side of the cell.



**Figure 7.** Endocrine cell in midgut of *N. viridis*. **Sg:** Secretory granule, **RER:** Rough endoplasmic reticulum, **M:** Mitochondria (TEM, x10 000)



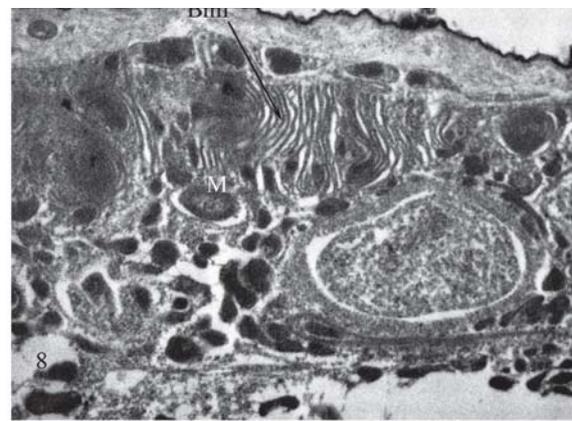
**Figure 6.** Endocrine cell in midgut of *N. maculata*. **M:** Mitochondria, **Sg:** Secretory granule, **G:** Golgi apparatus (TEM, X 16 000)

## DISCUSSION

The digestive canal of *N. viridis* and *N. maculata* contains similar structures as in many species of Hemiptera which are searched before. However, Barber et al. [5] reported that midgut consists of four parts including the first, second, third and fourth ventriculus and there are Malpighian tubules at the junction of the midgut and last intestine.

The midgut epithelium consists of regenerative cells, enterocyte cells and endocrine cells as reported by Demir and Suiçmez [6]. As stated in many studies [7-12], we have observed significantly larger nucleus, nucleolus and the RER cisternae in the cytoplasm of the midgut cells of both species.

Several authors [7, 8, 10, 14-18] reported the existence of enterocytes cells responsible for absorption which were observed in the midgut of *N. maculata* and *N. viridis*.



**Figure 8.** Endocrine cell in midgut of *N. viridis*. **M:** Mitochondria, **Bmf:** Basal membrane folding (TEM, x10 000)

The nucleus of *N. maculata* enterocyte cells are quite large and smooth and cover a large portion of the cell as indicated by Fialho et al., Hung et al. [8, 15]. Nucleus has a few nucleoli as stated by Rost-Roszkowska and Undrul [17] and nucleolus are seen significantly in the nucleus as stated by Gül et al. [14].

A large number of rough endoplasmic reticulum surrounded by mitochondria were observed in the cytoplasm of enterocyte cells in *N. maculata* and *N. viridis* as reported by authors [6-8, 11, 12, 15, 17, 19].

Glycogen granules and spherical crystals weren't able to mark which were observed before [7, 8]. Ferritin granules and large lysosomal bodies weren't detected either as mentioned by Taha et al. [20]. In enterocyte cells, Golgi apparatus couldn't be observed clearly in any other region of the midgut as indicated by Nardi et al. [19]. Transparent electron vesicles which were stated by Billen and Buschinger, Rost-Roszkowska and Undrul [16, 17] could not be observed in a very clear way in our study.

We observed the digestive cells and regenerative cells that can differentiate into endocrine cells in the study of both species we dealt, as stated by other authors [6, 7, 11, 12, 15, 19]. Hung et al. and Levy et al. supported that a large number of basal membrane infoldings, which are formed by invaginations made into the cells of the basal membrane of regenerative cells, are associated with mitochondria [11,15]. Rough Endoplasmic Reticulum and Smooth Endoplasmic Reticulum cisternae that were mentioned by Hung et al., Levy et al. and Nardi et al. [11, 15, 19] were also observed in the cells of both species we examined. Hung et al. indicated that there are no microvilli extensions in the apical parts of these cell types [15]. During the differentiation epithelium of midgut, it couldn't observe phosphate and Urospherite structures which contains carbonate chloride but non-uric acid as confirmed by Rost-Roszkowska and Undrul [17].

As Habibi et al. mentioned endocrine cells are shorter than the enterocyte cells and have no microvilli extensions in the apical parts as in enterocytes cells [13]. There are more secretory granules in endocrine cells than in other cells [6,12,14,21]. It suggests that these cells are specialized cells to make secretion. It is supported by Rost-Roszkowska et al. that very obvious basal membrane foldings are seen in the basal portion endocrine cells [21]. Rost-Roszkowska et al. and Demir and Suicmez reported that these cells are directly related to the synthesis and secretion functions since they contain well-developed rough endoplasmic reticulum and mitochondria [6, 21].

## CONCLUSIONS

*N. viridis* and *N. maculata* are aquatic insects and maintain their presence in the aquatic environment. These insects which meet their food from aquatic organisms also fulfill important functions in maintaining the ecological balance. As we mentioned earlier; these insect species consume the mosquito larvae as nutrient and this prevents their excessive proliferation and they can provide their population remain in balance in that region.

Thanks to these features, they have an important place to combat mosquitoes as an alternative way. Because these species are predators, they control the population of the species and also prevent their excessive proliferation. However, it's an inevitable reality that they are either directly or indirectly affected from the adverse changes in their environment. When evaluated with this aspect these species are also used as indicator organisms in the aquatic environment which they live. There are too many chemicals in soil and water as a result of so many agricultural activities. These chemicals are getting through to organisms in various ways. It will be impossible to sustain their existence over time as *N.viridis* and *N. maculata* are also negatively affected by these chemicals as in other organisms.

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