

Notes on the Gastric Caecum in *Ventocoris fischeri* (Herrich-Schaffer, 1851) (Heteroptera, Pentatomidae)

Damla Amutkan Mutlu¹, Irmak Polat², Hanife Gözüpek, Zekiye Suludere¹

¹Gazi University, Faculty of Science, Department of Biology, Ankara, 06500, TURKEY

²Çankırı Karatekin University, Faculty of Science, Department of Biology, Çankırı, 18100, TURKEY

E-mails: damlamutkan@gazi.edu.tr, irmakyilmaz@gazi.edu.tr, zekiyes@gazi.edu.tr

ORCID IDs: 0000-0002-4780-8520 (DAM), 0000-0001-7230-4589 (IP), 0000-0002-1207-5814 (ZS)

ABSTRACT: Gastric caecum is the region of the midgut in alimentary canal of insects. The main role of the gastric caecum in digestion is secreting the digestion enzymes. In this study, the gastric caecum of *Ventocoris fischeri* (Herrich-Schaffer, 1851) (Heteroptera, Pentatomidae) was investigated with using light microscope and scanning electron microscope. In *V. fischeri*, the gastric caecum is the last part of the midgut and has four longitudinal rows. The wall of each row has single layer cuboidal epithelium. The lumen of the gastric caecum contains numerous bacteria that have a role in helping digestion. The gastric caecum is connected to the alimentary canal at the region called as the pylorus.

KEYWORDS: Insect, alimentary canal, midgut, light microscope, scanning electron microscope.

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INTRODUCTION

Gastric caecum is highly specialized part of the midgut in insects but it generally is connected to the hindgut in the species belonging to Heteroptera suborder. The main duty of the gastric caecum of insects is secreting enzymes for helping digestion (Amutkan, 2012; Zucchi et al., 2012; Şimşek et al., 2020). The structure, position and number of the gastric caecum show diversity among different insect groups i.e. 2 in some Diptera larvae, 8 in Blattodea, many in Heteroptera and some Coleoptera (Amutkan, 2012; Chapman, 2013). The lumen of the gastric caecum in many insects is associated with different symbiont bacteria that

helping the digestion process (Amutkan, 2012; Zucchi et al., 2012; Şimşek et al., 2020).

Heteroptera suborder constitutes a widespread group of insect and includes over 40.000 identified species in the world.

These 40.000 species are classified under the 140 families with over 5.800 genus (Henry, 2009; Kiyak, 2019; Şimşek et al., 2020). In Turkey, about 1.520 species belonging to 40 families of Heteroptera suborder were identified until today (Önder et al., 2006; Küçükbasmacı & Kiyak, 2015; Kiyak, 2019; Şimşek et al., 2020).

Pentatomidae family is one of the families of the suborder Heteroptera with the highest number of species (about 5.000 identified species) all around the world.

Individuals of the Pentatomidae family are generally called as stinkbugs and show great diversity in feeding habits as phytophagous, zoophagous or omnivorous (Boyd et al., 2002; Parveen et al., 2015). The mouth parts structures can vary in consequence of the feeding habits.

Members of Pentatomidae family have piercing-sucking mouth parts that used for sucking the sap of crops, thus, most of them can cause a great damage on products of cultivated fields. However, this group contains some species that are used as biological control of harmful insects. Accordingly, Pentatomidae family has economical importance (Amutkan, 2012; Zucchi et al., 2012; Amutkan et al., 2015; Parveen et al., 2015; Şimşek et al., 2020).

Ventocoris is a genus which belongs to Pentatomidae family and is represented with twenty-four species, while this number is 8 in Turkey (Dursun & Fent, 2013). *Ventocoris fischeri* (Herrich-Schaeffer, 1851) is a species that is classified in genus *Ventocoris* and is a very widespread species both in Turkey and in the world.

V. fischeri is located in Afghanistan, Armenia, Azerbaijan, Egypt, France, Iran, Israel, Kazakhstan, Portugal, Sudan, Syria,

Tadzhikistan, Turkmenistan, and Uzbekistan.

Besides, it spreads in Amasya, Ankara, Antalya, Burdur, Çorum, Konya, Mersin, and Nevşehir in Turkey (Önder et al., 2006; Dursun & Fent, 2013). *V. fischeri* can generally be found in the cultivated areas but they are mostly seen on weeds instead of cereals or wheat (Schuh & Slater, 1995).

Although it is a very common species, there is no study on the structure of the digestive tract of this species in the literature. In our previously study, we described the salivary glands of *V. fischeri* (Amutkan Mutlu et al., 2019). The aim of this study is to reveal the detailed structure of the gastric caecum in *V. fischeri* in order to get more information about the biology of this stinkbug.

MATERIAL AND METHODS

The mature individuals of *V. fischeri* males and females were collected from the harvested crops and cultivated areas around Çağa Village and Sinanlı town in Ankara province in July 2018 and taken to the laboratory. The alimentary canals were dissected out and the gastric caecum was separated from the alimentary canal. After dissection process, the gastric caecum samples were prepared for light microscopic (LM) and scanning electron microscopic (SEM) examinations.

Light microscopic examinations

Some of the gastric caecum samples fixed in 10% formaldehyde for the LM examinations. After fixation, they were washed and dehydrated with the ascending series of ethyl alcohol. Following the dehydration step, the samples were blocked in paraffin. The sections with 5-6 µm thickness were cut from the paraffin blocks and the slide was stained with hematoxylin-eosin (H&E). Examinations were made under Olympus BX51 light microscope and photographed.

Scanning electron microscopic examinations

For the SEM examinations, some of the samples were dissected in 5% glutaraldehyde and washed with phosphate buffer afterward. Then, the samples were dehydrated and were dried with critical point dryer (Polaron CPD 7501). Dried samples were mounted on SEM stubs and coated with gold (Polaron SC 502). The examinations were applied with JEOL JSM 6060 SEM at 5-10 kV.

RESULTS AND DISCUSSION

The midgut is an essential part of the insect digestive system. It also plays critical roles in metabolism, immune response, and homeostasis of electrolytes, osmotic pressure, circulation, and other physiological regulation (Takeda, 2012).

Midgut cells are actively involved in the production and secretion of digestive enzymes and absorption of nutrients (Chapman, 2013).

In insects, the midgut is generally in the form of a simple tube. The gastric cecum varies in shape, number, and location among insect orders (Glasgow, 1914; Chapman, 2013). It has been reported that closed-ended sacs (gastric cecum or midgut cecum) are usually located at the anterior end of the midgut, however, they may also occur along the midgut or in the posterior region of the midgut (Li et al., 2018). While it is generally located at the posterior end of the midgut in Heteroptera species and form of 2-4 rows (Amutkan, 2012; Gangurde et al., 2019; Şimşek et al., 2020), it is mainly found in anterior part of the midgut in Orthoptera species and form of finger-like projections (Polat, 2016; Amutkan, 2020). It has been indicated that these sacs extend from the midgut and contain bacteria that helps to digestion (Amutkan, 2012). It has been stated that the gastric cecum expands the surface area in the digestive system and increases digestive enzyme secretion and nutrient absorption (Chapman, 2013; Li et al.,

2018; Jurenka, 2019).

The digestive system of *V. fischeri* composed of the foregut, the midgut and the hindgut, similar to the digestive tract of other Heteroptera species described previously (Hirose et al., 2006; Prado et al., 2006; Amutkan, 2012; Gangurde et al., 2019; Harris et al., 2019; Şimşek et al., 2020). The midgut is separated to four parts. These parts are called as the first, second, third and fourth ventriculus. The last region of the midgut is different from the other parts as surface. It is distinguished the presence of four longitudinal rows known as gastric caeca.

Although this structure consists of four longitudinal rows in *V. fischeri* (Figures 1, 2) and many Heteropteran insects, this has two longitudinal rows in some Heteroptera species such as *Anasa tristis* (Hemiptera, Coreidae), *Largus cinctus* (Hemiptera, Largidae) and *Largus californicus* (Hemiptera, Largidae) (Steinhaus et al., 1956; Gordon et al., 2016). In spite of that, there is no gastric caeca in some insect as *Graptostethus scrvus* (Hemiptera, Lygaeidae) and *Sphex flavipennis* Fabricius, 1793 (Hymenoptera, Sphecidae) (Glasgow, 1914; Kurup, 1964; Demir & Suiçmez, 2011). It is indicated that the presence or absence of gastric caecum in Heteroptera species has taxonomic and phylogenetic significance (Gangurde et al., 2019). It is also stated that morphological differences in the structure vary considerably according to the development degree of the families to which the species belong (Glasgow, 1914; Chapman, 2013).

The outer surface of the gastric caeca in *V. fischeri* is quite smooth and has a slightly knottily (Figures 1, 2). It is also encircled by trachea (Figures 1, 3-5).

In SEM investigation and the cross sections of light microscope, it is observed that the gastric caecum has numerous canal shape structure (Figures 2-4). Each caecum consists of a thin monolayer cuboidal epithelium with presence of round shape nucleus

(Figures 4-6). It is also seen that the lumen of gastric caeca and the cytoplasm in cells are full of with specific bacteria that help to digestion (Figures 4-8). While some of these rode-shaped bacteria have generally thick (Figure 7), some of them are thin (Figure 8). It has been noticed that bacteria are dividing (Figures 6, 7).

The association between species of Hemiptera order and bacterial symbionts in their digestive system has been of interest to entomologists and micro-biologists long since (Glasgow, 1914; Prado et al., 2006). Although bacteria have been observed to be associated with the gastric caeca in digestive system, these bacteria have been first identified in 1882 (Glasgow, 1914). Phylogenetic placement of the species is easily conclusive by identifying of the bacteria associated with gastric caecum in digestive system of Heteroptera species

(Prado et al., 2006).

The gastric caecum is connected to the alimentary canal with the structure which is known as the pylorus in *V. fischeri* (Figures 9-10). It is surrounded by single-layer epithelium (Figures 11-12). The nuclei of the cells are round in shape and located at the basal site of the cell (Figures 11-12). It is observed that the granules in different shape are found in the lumen (Figures 13).

With this histological and morphological studied, we revealed the structure of the gastric caecum in *V. fischeri*. When all these results are compared with the previous studies in the literature, it was observed that the general structure of *V. fischeri*'s gastric caecum was similar to the species belonging to the Heteroptera order.

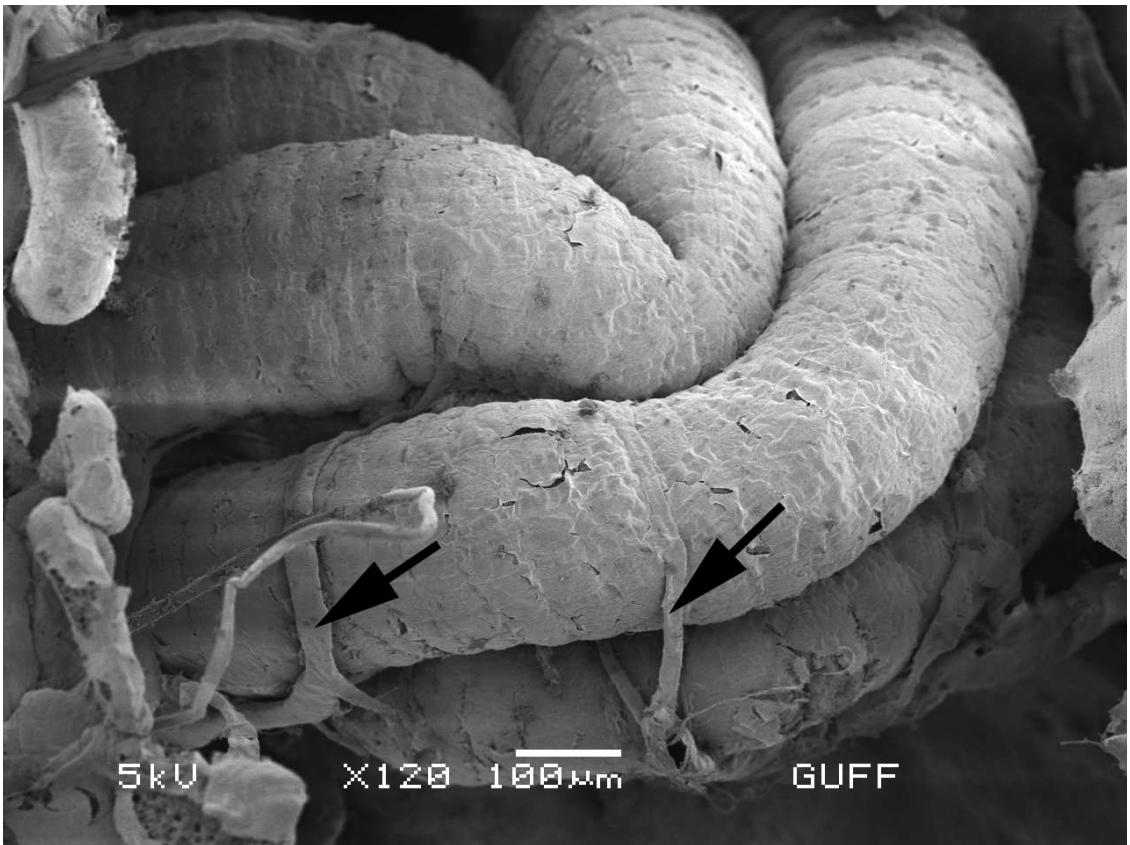


Figure 1. Outer surface of the gastric caecum in *Ventocoris fischeri*. Trachea (→) (SEM).

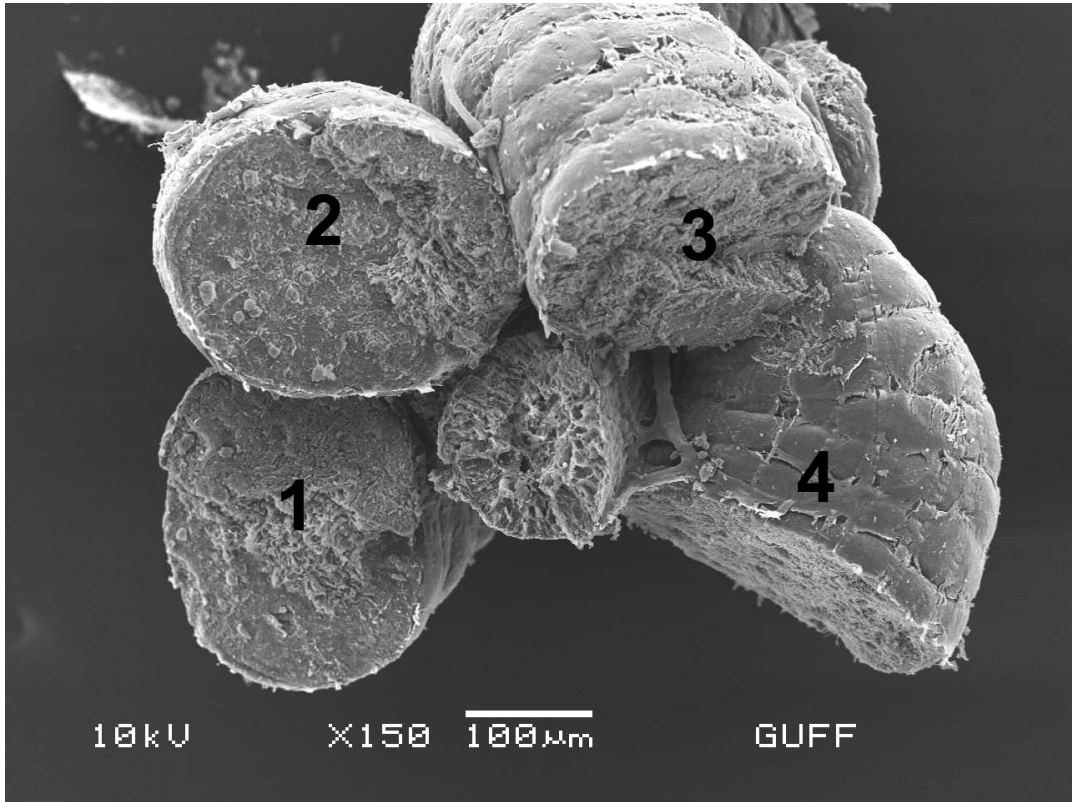


Figure 2. Four longitudinal rows in cross section of the gastric caecum (SEM).

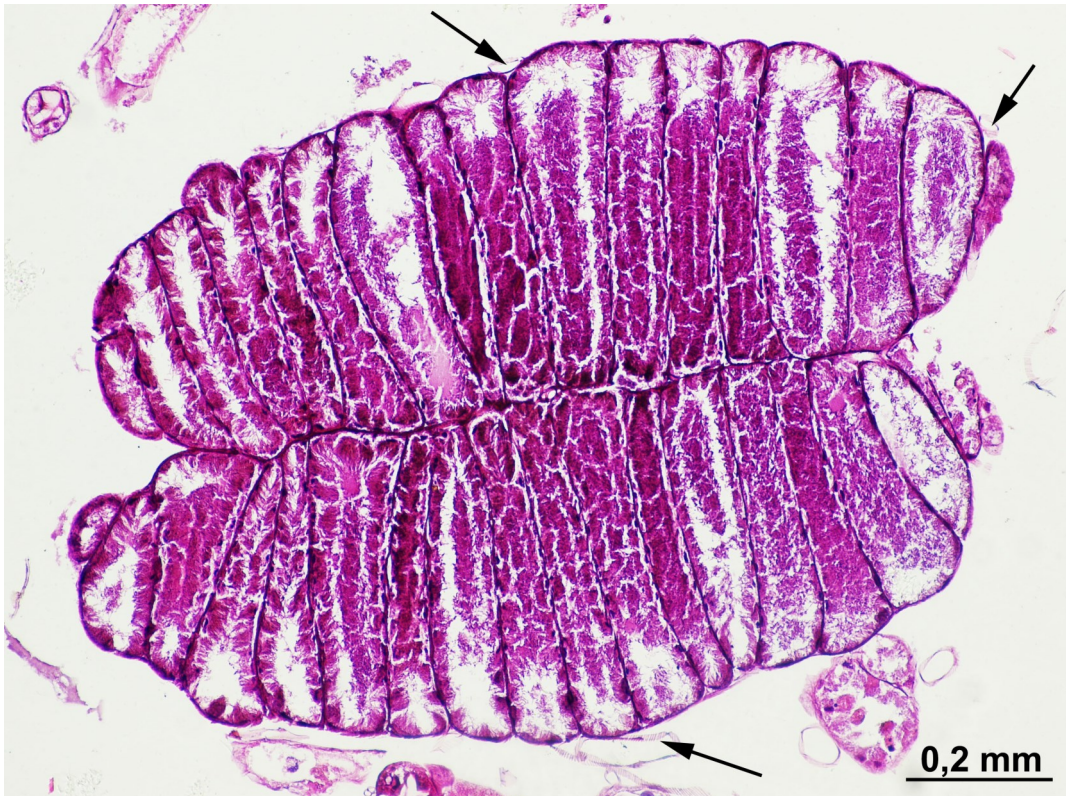


Figure 3. Two of four longitudinal rows in cross section of the gastric caecum. Trachea (→) (H&E, LM).

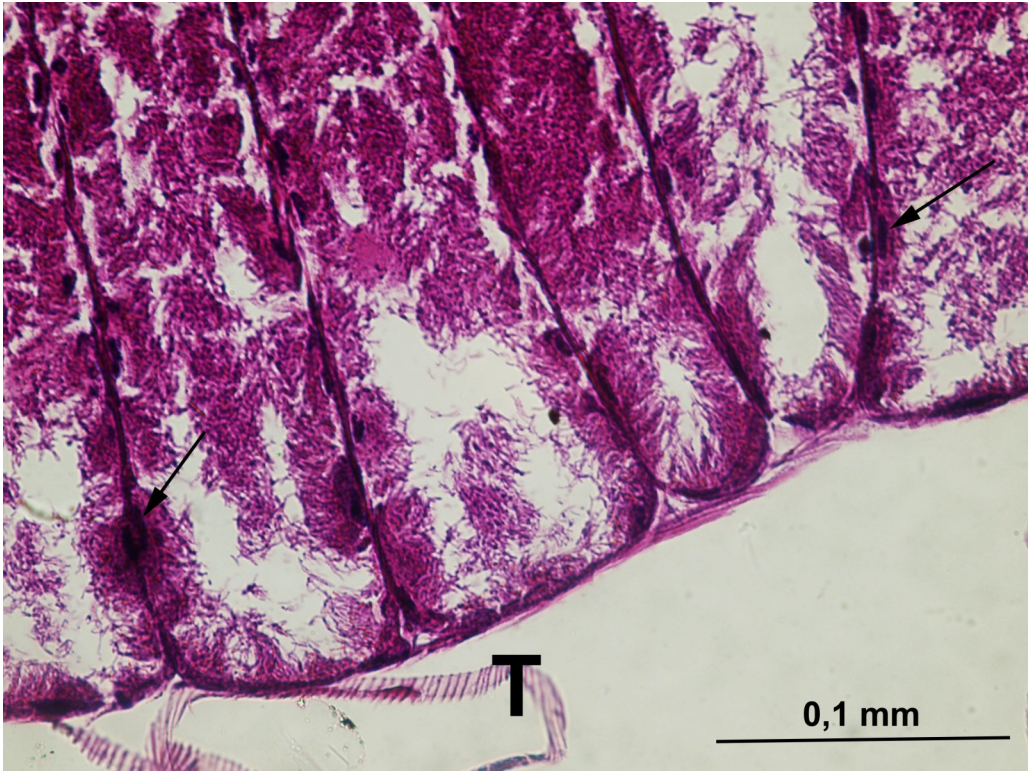


Figure 4. Cross section of the gastric caecum. Trachea (T), nucleus (→) (H&E, LM).

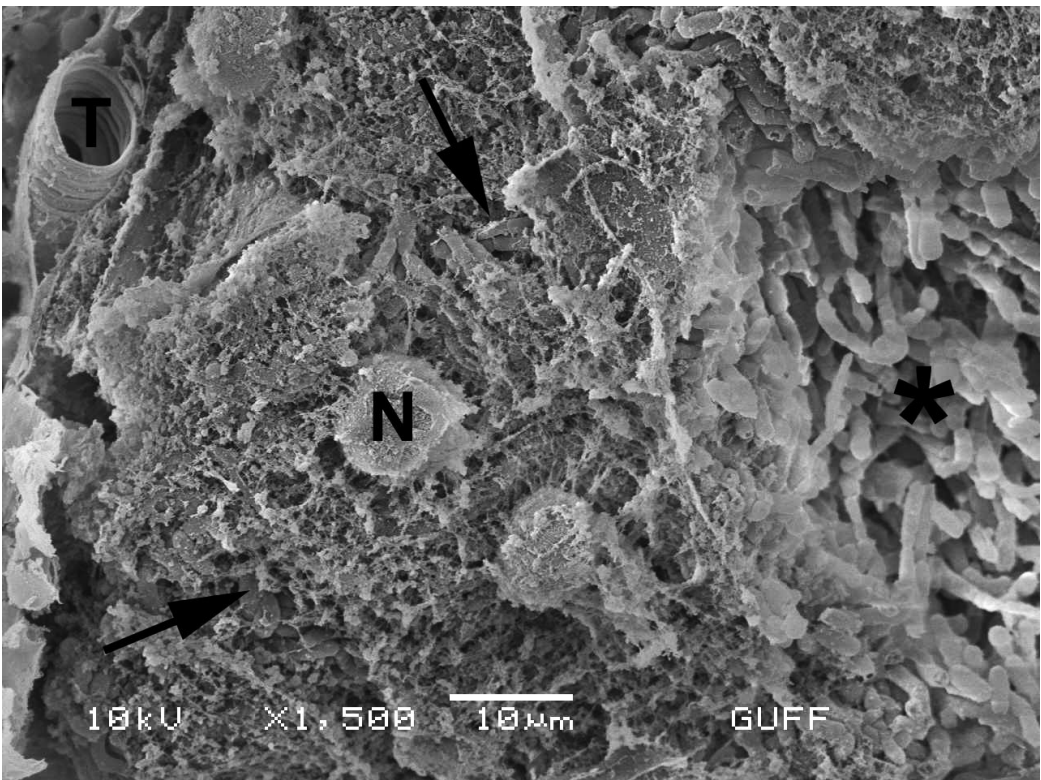


Figure 5. Cross section of the gastric caecum. Trachea (T), nucleus (N), bacteria in cytoplasm of the cell (→), bacteria in the lumen (*) (SEM).

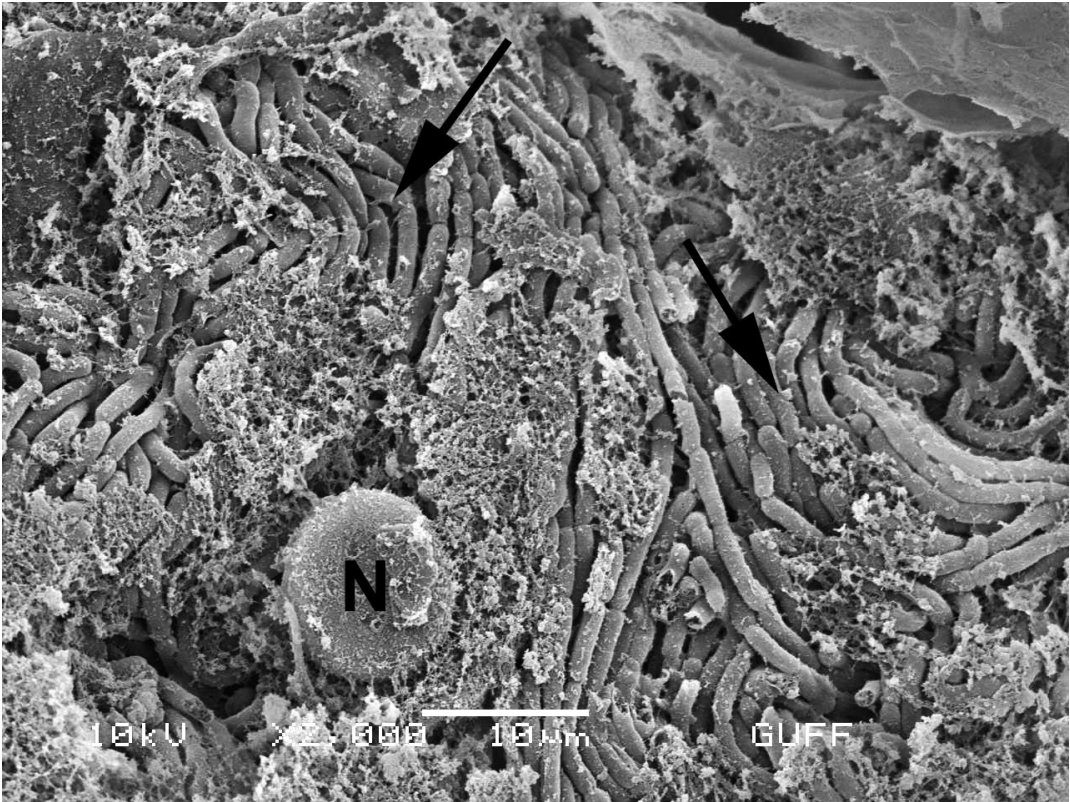


Figure 6. Cross section of the gastric caecum. Nucleus (N), bacteria in cytoplasm of the cell (→) (SEM).

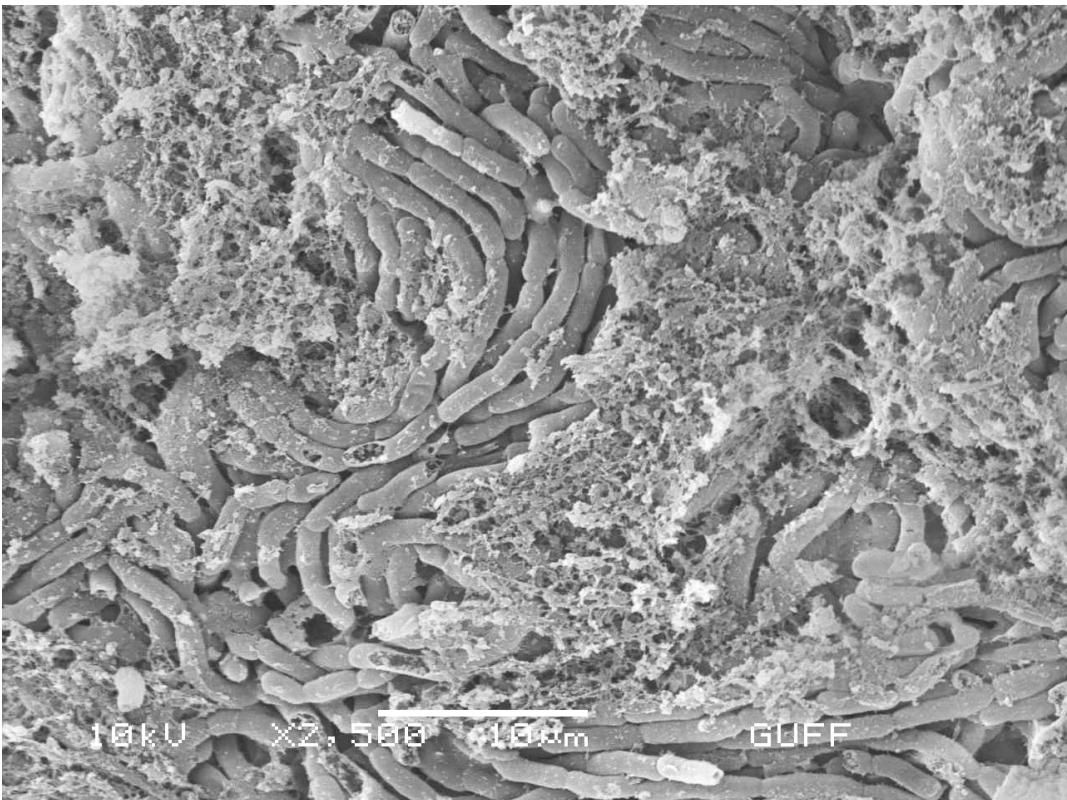


Figure 7. Overall appearance of bacteria (thick shape) in cytoplasm of the cell (SEM).

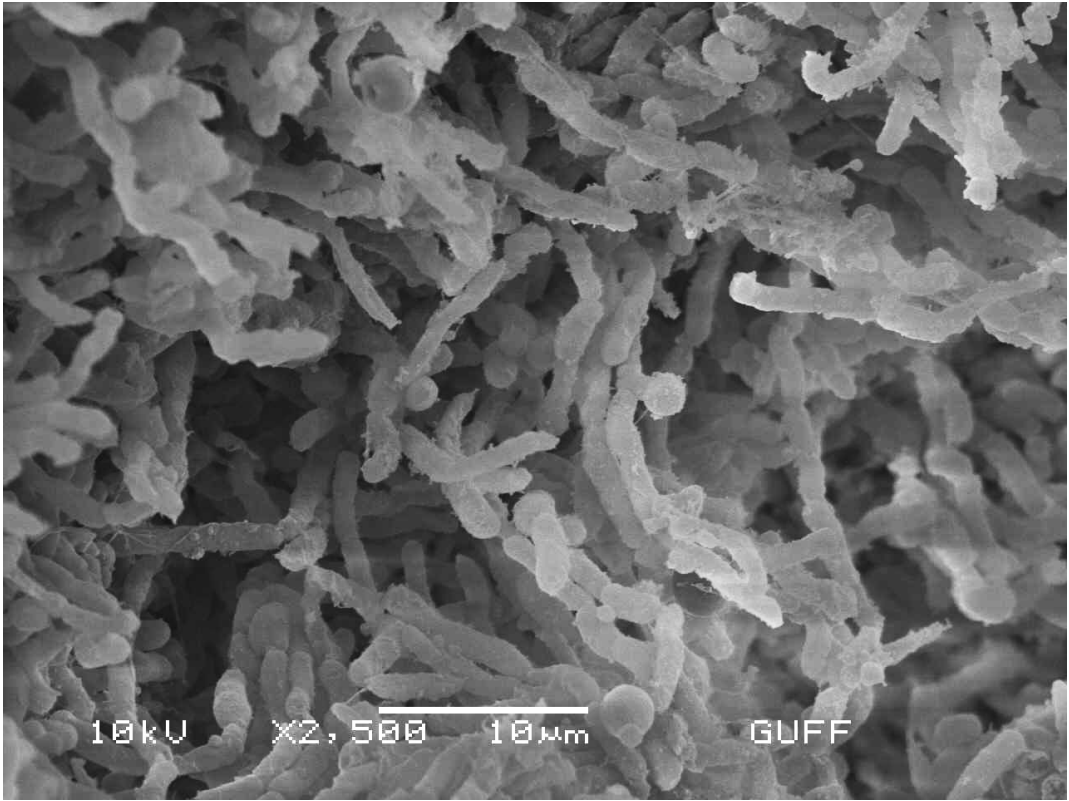


Figure 8. Overall appearance of bacteria (thin shape) in cytoplasm of the cell (SEM).

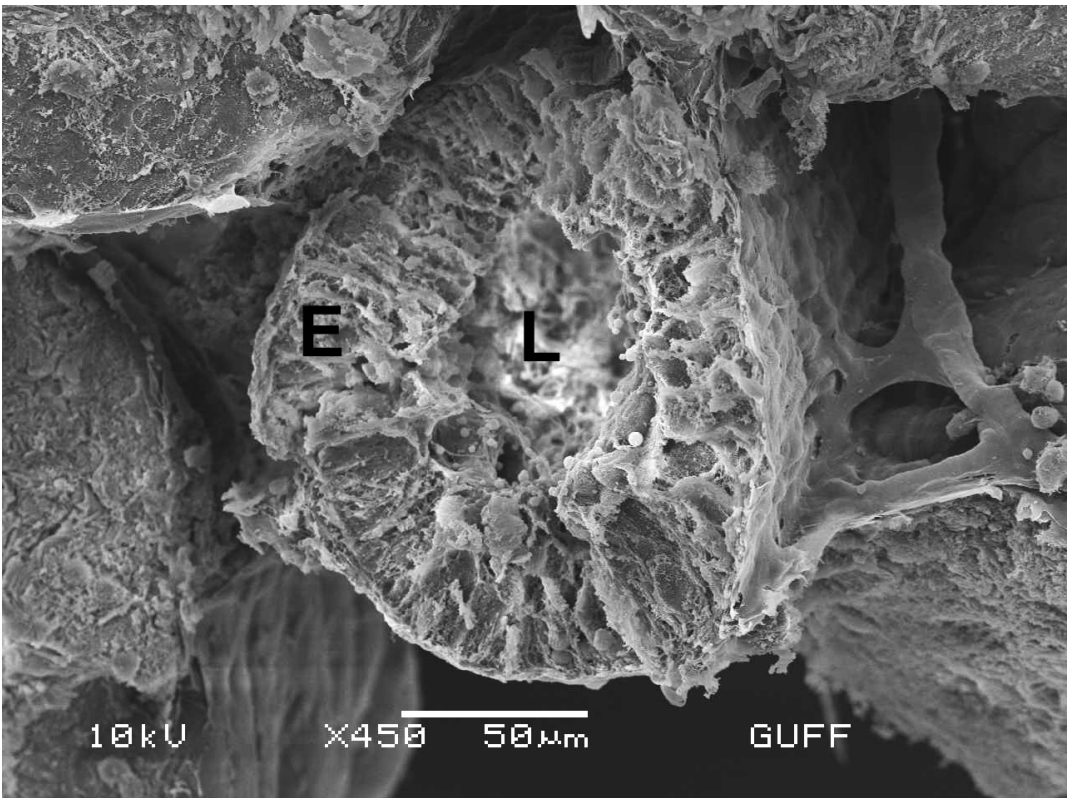


Figure 9. Overall appearance of the pylorus. Lumen (L), epithelium (E) (SEM).

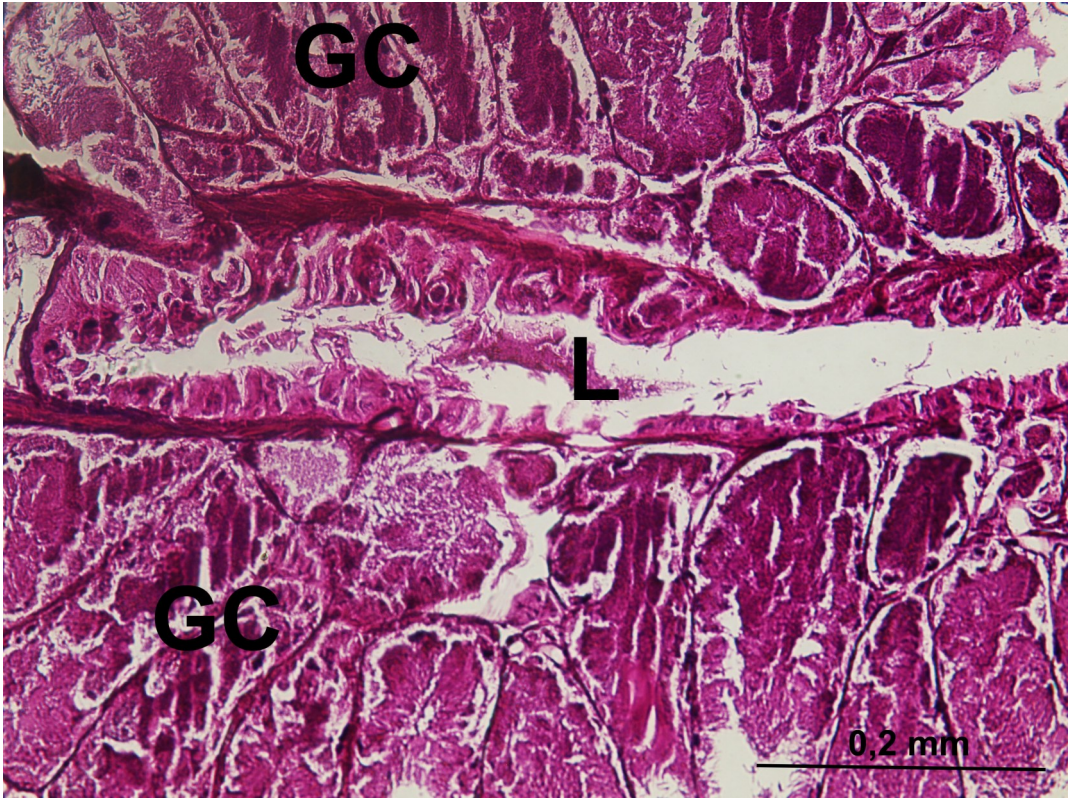


Figure 10. The cross section of the pylorus. Lumen (L), gastric caecum (GC) (H&E, LM).

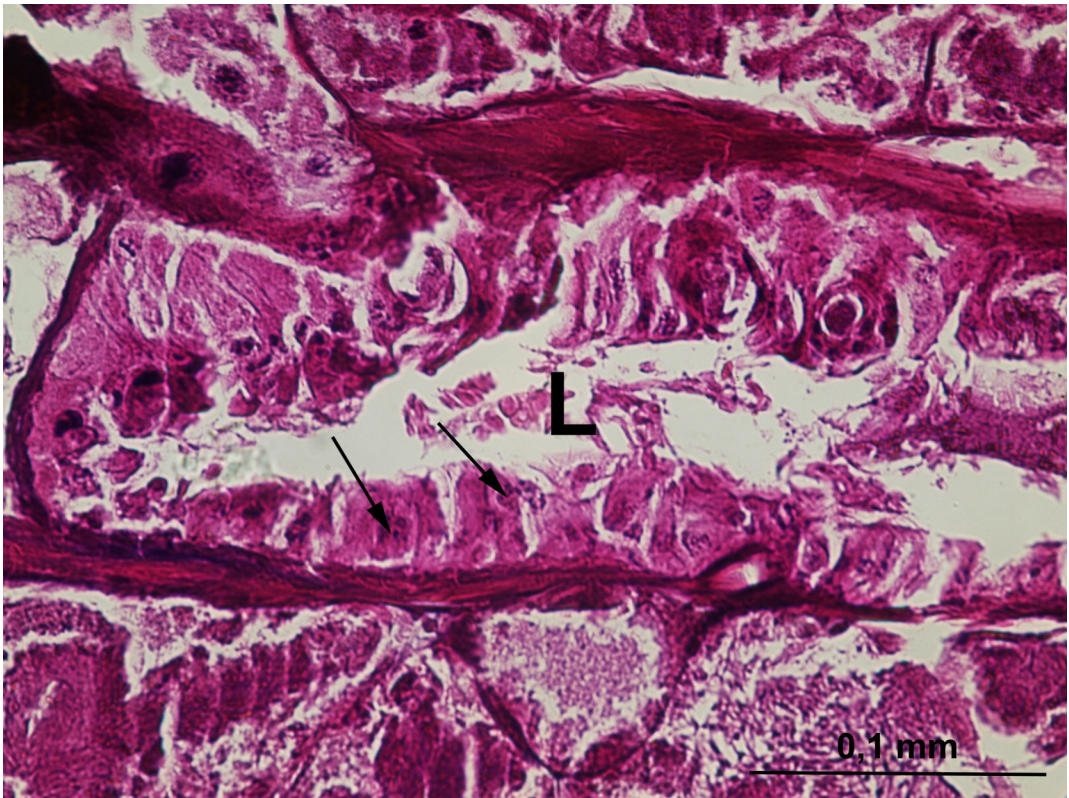


Figure 11. The cross section of the pylorus. Lumen (L), nucleus (→) (H&E, LM).

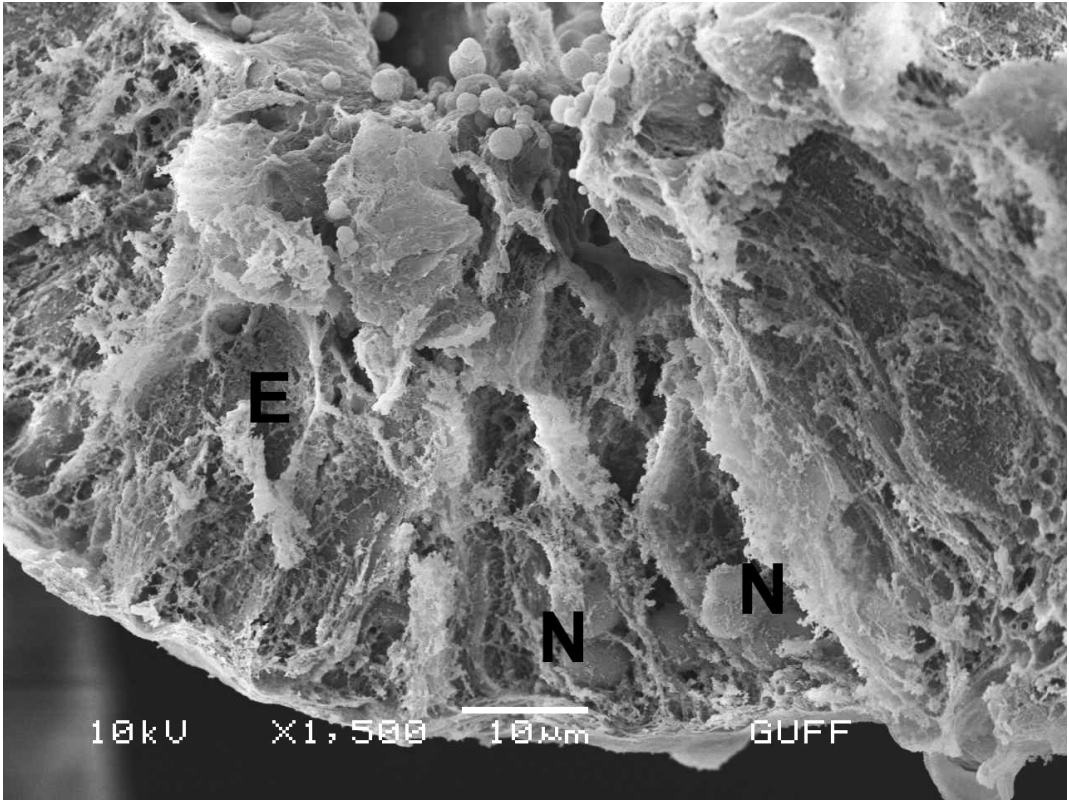


Figure 12. The cross section of the pylorus. Epithelium (E), nucleus (N) (SEM).

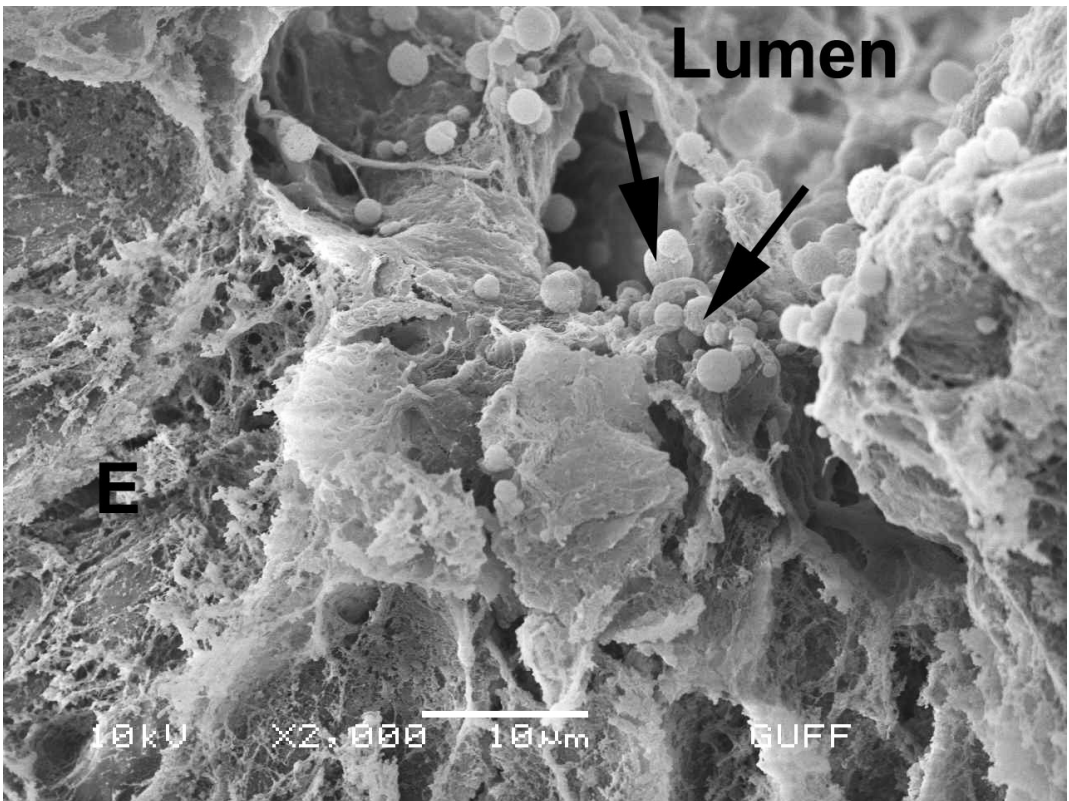


Figure 13. The cross section of the pylorus. Epithelium (E), granules (→) (SEM).

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