

## The morphological and histological features of gastric caecum and pylorus of brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855) (Hemiptera, Heteroptera, Pentatomidae)

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**ABSTRACT:** The gastric caecum and the pylorus of *Halyomorpha halys* (Stål, 1855) (Hemiptera, Pentatomidae) was examined morphologically and histologically with stereomicroscopic, light microscopic, and scanning electron microscopic (SEM) techniques in this study. *H. halys* is a polyphagous species that feeds mainly on plants of the Rosaceae and Fabaceae families and agricultural areas and can cause massive damages on them. Three

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distinct areas comprise *H. halys*'s digestive tract. The pylorus is the first section of the hindgut, and the gastric caecum of *H. halys* is seen at the posterior end of the second region known as the midgut. In comparison to other insect species in the order Hemiptera and other insect groups, it can be easily stated that their structure is generally close to many species belonging to this order.

**KEYWORDS:** Alimentary canal, bacteria, digestive canal, hindgut, midgut, *Halyomorpha halys*

## INTRODUCTION

In recent years, the fast pace of globalization has led to a significant escalation in invasive species outbreaks affecting native biodiversity around the globe. It is no doubt that with the increase in international trade, there are many more exotic introductions ultimately lead to the establishment of foreign species. This situation may have developed accidental and intentional, but ultimately it was a problem that could lead to great economic losses. In fact, biological invasions are now second only to habitat destruction as the main factor contributing to the current decline in biodiversity (Keane and Crawley, 2002; Liu et al., 2011; Zhu et al., 2012).

*Halyomorpha halys* Stål (Hemiptera: Pentatomidae) (syn. *H. mista*) is one such invasive species and it is also referred to as the brown marmorated stink bug (BMSB) which is strong flier in many references. Although it is known that BMSB prefers to feed mainly on plants of the Rosaceae and Fabaceae families, it is a very polyphagous species that consumes around 300 different kinds of plants (Hoffman 1931; Lee et al. 2013; Bariselli et al., 2016; Bergmann et al. 2016; Horwood et al., 2019). *H. halys* is a fruit-piercing stink bug, causing wilting and reduced yield. This massive damage can be observed on many plants such as vegetables, orchard crops, ornamentals, grapes, nursery crops, row crops, and other small fruits. The damage caused by this insect has major economic consequences, as products with reduced yields and damaged products cannot be accepted by the markets. Adult BMSB, which are unable to reproduce, also cause secondary damage, such as the damage they cause to buildings by overwintering inside them and the foul

odours they emit when disturbed. For all these reasons it poses a major biosecurity risk (Watanabe et al. 1978, 1994; Nielsen et al. 2008a; Toyama et al., 2011; Zhu et al., 2012; Rice et al. 2014; Kriticos et al., 2017; Horwood et al., 2019).

Although *H. halys* originates from north-eastern Asia, it has also been detected in America, Europe, and Oceania, so much so that *H. halys* has been recorded in many continents and countries around the world (Horwood et al., 2019). *H. halys* is originated from China, the North and South Korea, Japan, and Taiwan in Asia. *H. halys* has invaded large areas of North America such as the USA and Canada (Bariselli et al., 2016) and Sydney (Australia) and New Zealand in Oceania. In Europe, Spain, Austria, Germany, Hungary, Bulgaria, Switzerland, France, Italy, Liechtenstein, Greece, Serbia, Romania, and Turkey are the countries where this species has been reported. Abkhazia, Georgia and Russia are other regions where this invasive species has been seen recently (Hoffman 1931; Hoebeke and Carter 2003; Rabitsch, 2008; Nielsen et al., 2008a, 2008b; Wermelinger et al. 2008; Aldrich et al., 2009; Arnold 2009; Jones and Lambin, 2009; Nielsen and Hamilton, 2009a, 2009b; Harris, 2010; Wyniger and Kment, 2010; Heckmann 2012; Zhu et al., 2012; Callot and Brua 2013; Milonas and Partsinevelos 2014; Vêtek et al. 2014; Cesari et al. 2015; Macavei et al. 2015; Šeat 2015; Bariselli et al., 2016; Dioli et al. 2016; Gapon 2016; Mityushev 2016; Simov 2016; Kriticos et al., 2017).

This study's primary goal is to identify the histological and morphological structure of gastric caecum and pylorus of the alimentary canal of the widespread species, *H. halys*, that causes so much

damage. For this purpose, scanning electron microscopy and light microscopy were used to examine the structure of these organs.

## MATERIALS AND METHODS

### Light microscopy (LM) methods

Adult male and female specimens of *H. halys* were taken in Ankara. For light microscope studies, the gastric caecum and pylorus of ten individuals from each sexes were dissected. Dissections were done with stereomicroscope (Leica EZ4D). The tissues were fixed in formaldehyde (24 h) and underwent a series of ethanol dehydration (70%, 80%, 90% and 100%) and paraffin was used to block the tissues. The 6-7  $\mu$  thick sections were cut by using a Thermo Scientific Finesse 325 Microtome and were stained with Hemotoxylin-Eosin routine staining protocol. The slides were examined (Leica DM500 light microscope) and images were taken in Çankırı Karatekin University, Faculty of Science, Zoology Laboratory.

### SEM methods

For SEM studies, gastric caecum and pylorus of alimentary canals of *H. halys* were fixed in glutaraldehyde (5%, pH 7.2, with PB), underwent a (ascending) series of ethanol dehydration, and were dried (with Polaron CPD 7501). Subsequently, the samples were loaded onto SEM stubs, which were then coated with Au (with Polaron SC 502). Next, at the Prof. Dr. Zekiye Suludere Electron Microscope Center at Gazi University, the stubs were analyzed (with JEOL JSM 6060 LV SEM, 10-15 kV) and pictures were acquired.

## RESULTS

### Gross morphology of the alimentary canal

The foregut, midgut, and hindgut make up *H. halys*'s alimentary canal (Figure 1). When the midgut is longer than the other two portions of the alimentary canal, the foregut and hindgut are relatively short.

The anterior side of the foregut is linked to the salivary glands. The anterior midgut (M1), median midgut (M2), posterior midgut (M3), and gastric caecum (M4) are the four separate regions that make up the midgut. At the pylorus, four Malpighian tubules are exposed to the alimentary canal. The pylorus and rectum are the two sections of the hindgut (Figures 2 and 3).

### Gastric Caecum

*H. halys* individuals have four rows of gastric caecum in their digestive system. Each row of gastric caecum is gnarled structure arranged transversal axis and encircle a tube (Figure 4). The caecum is connected to the hindgut's pylorus area. (Figure 5). In the images from the cross section, there are four gastric caeca and a pylorus in the middle of them. The light microscope images show that each part of gastric caecum looks like flat sacs and has a thin wall with a single layered epithelium (Figure 6). Each gastric caecum is fulfilled with numerous microorganisms and mostly bacteria in the cells and lumen (Figures 7 and 8). It has been observed that the gastric caecum is associated with tracheas on its outer side (Figure 4).

### Pylorus

The pylorus is the first region of the hindgut. It is associated with four Malpighian tubules in *H. halys* individuals. It can be said that the Malpighian tubules are connected to the alimentary canal at this point of the hindgut (Figure 3).

The secretion produced by the epithelial cells of the Malpighian tubules meets with food residue in the lumen of the alimentary canal in pylorus. The cross sections of the pylorus show that the monolayered cylindrical epithelium and large lumen (Figure 9).

## DISCUSSION

In insects, the caecum is where the majority of the digestive enzymes are

created. The digestion process in insects is carried out by the function of gastric caecum enzymes and, in addition, with the help of symbiont bacteria living in the caecum. In fact, many studies have shown that the gastric caecum lumen is almost completely covered with bacteria (Glasgow, 1914; Chapman, 1998; Ferreira et al., 1999). The formation of caecum in many insects is very different from one order to another in terms of morphology, number, size, and position, while the histological structures are very similar. The structures of gastric caeca differ greatly depending on the degree of development of the families in which they are found. The digestive tract of some species does not have a gastric caecum. *Graptostethus scrvus* (Hemiptera, Lygaeidae), *Sphex flavipennis* Fabricius, 1793 (Hymenoptera, Sphecidae), and *Lygaeus trivittatus* (Hemiptera, Lygaeidae) are some of such species without gastric caecum (Glasgow, 1914; Kurup, 1964; Demir & Suiçmez, 2011; Gangurde et al., 2019). In insects belonging to the order Heteroptera, the midgut is generally divided into four regions. Gastric caecum is generally seen as the fourth region. As in *H. halys* examined in this study, the midgut in *Halys dentatus* (Hemiptera, Pentatomidae) has 4 regions and the last region is called gastric caecum (Gangurde et al., 2019; Şimşek et al., 2020).

Numerous microorganisms, particularly bacteria, are present in the lumen of the gastric caecum of *H. halys* like many other species that were examined by

various researchers before such as *Leptocorisa chinensis* Dallas, 1852 (Hemiptera, Alydidae), *Nezara viridula* (L.) (Heteroptera, Pentatomidae), *Riptortus clavatus* (Thunberg, 1783) (Hemiptera, Alydidae) *Eurydema rugosa* Motschulsky (Heteroptera: Pentatomidae) and *Eurydema dominulus* (Scopoli) (Heteroptera: Pentatomidae). These bacteria in the gastric caecum live symbiotically with the host organism, aiding in the digestion of nutrients or producing antibiotic barriers against pathogens (West, 1980; Kikuchi et al., 2005, 2012; Hirose et al., 2006; Tada et al., 2011; Zucchi et al., 2012).

The hindgut, which is the last part of the digestive tract in insects, shows variations in different insect groups, as in other parts. The hindgut is divided into 3 parts in some insect species. These 3 parts, which are called ileum, colon and rectum in some, can be seen as ileum, rectal caecum and rectum in some species such as *Abedus ovatus* (Stål) (Hemiptera, Belostomatidae). In some species, such as *Ceroplastes japonicus* (Green) (Hemiptera: Coccoidea), the hindgut is divided into two: pylorus and rectum as in *H. halys*. In *Lepyronia coleopterata* (L.) the 2 hindgut layers are called ileum and rectum (Goverdhan et al., 1981; Xie et al., 2011; Zhong et al., 2013).

When *H. halys*'s pylorus and gastric caecum features were contrasted with those of other species belonging to the Hemiptera order, it was observed that it was quite similar to other species in terms of number, location and structure.

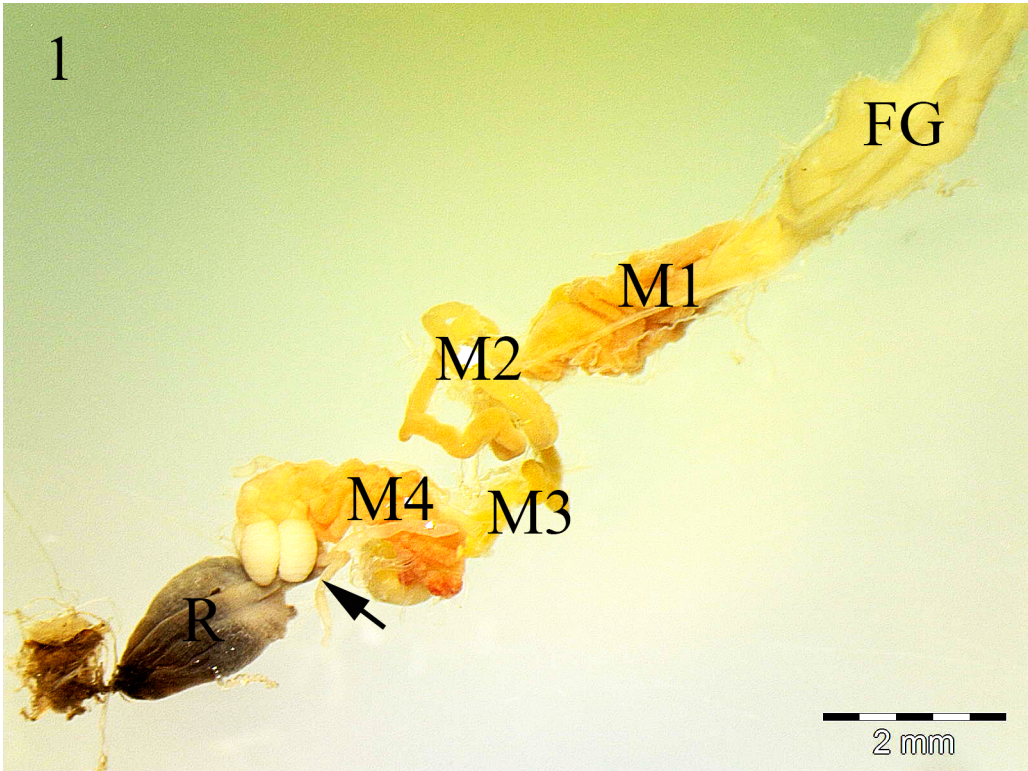
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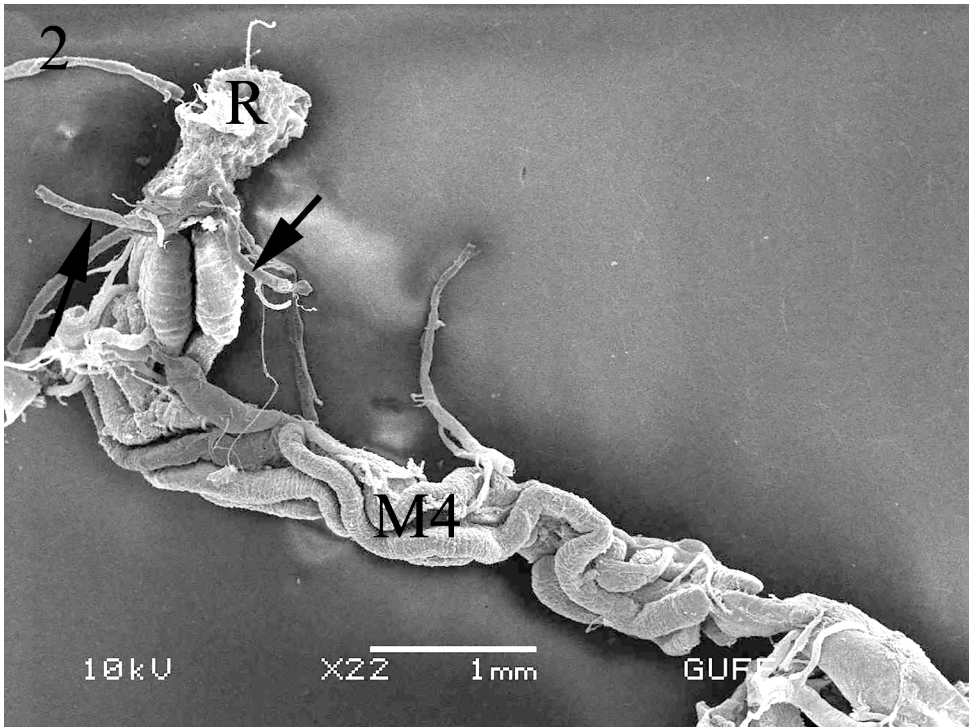
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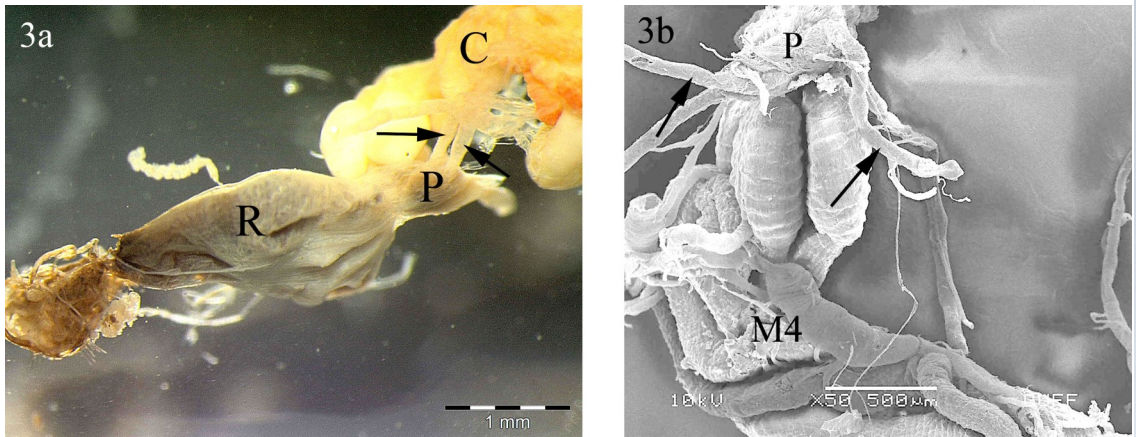


**Figure 1.** Stereomicroscope image of the alimentary canal in *H. halys*. FG: foregut, M1: anterior midgut, M2: median midgut, M3: posterior midgut, M4: gastric caecum, arrow: Malpighian tubule, R: rectum.

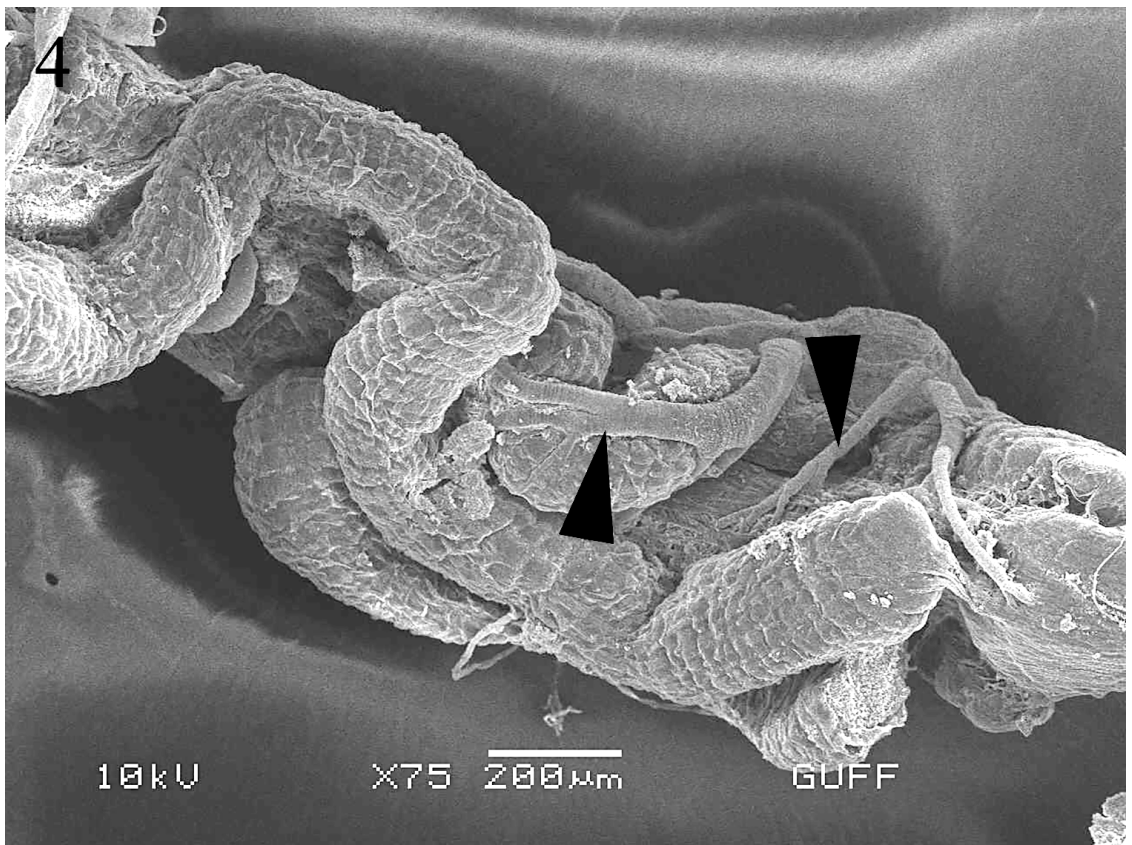


**Figure 2.** SEM image of the outer surface of gastric caecum (M4), rectum (R), and Malpighian tubules (arrows).





**Figure 3.** The connection junction of gastric caecum (M4), pylorus (P) and Malpighian tubules (arrows). a. Stereomicroscope image, b. SEM image (R: rectum).



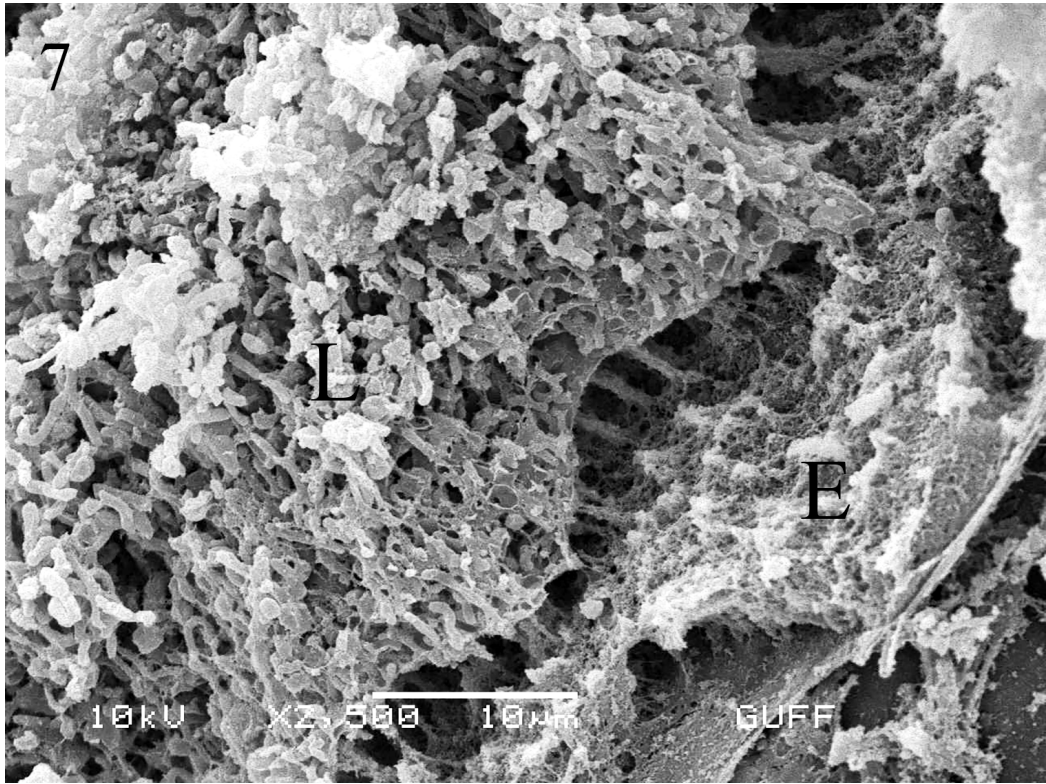
**Figure 4.** SEM image of the outer surface of gastric caecum associated with trachea (arrowheads).



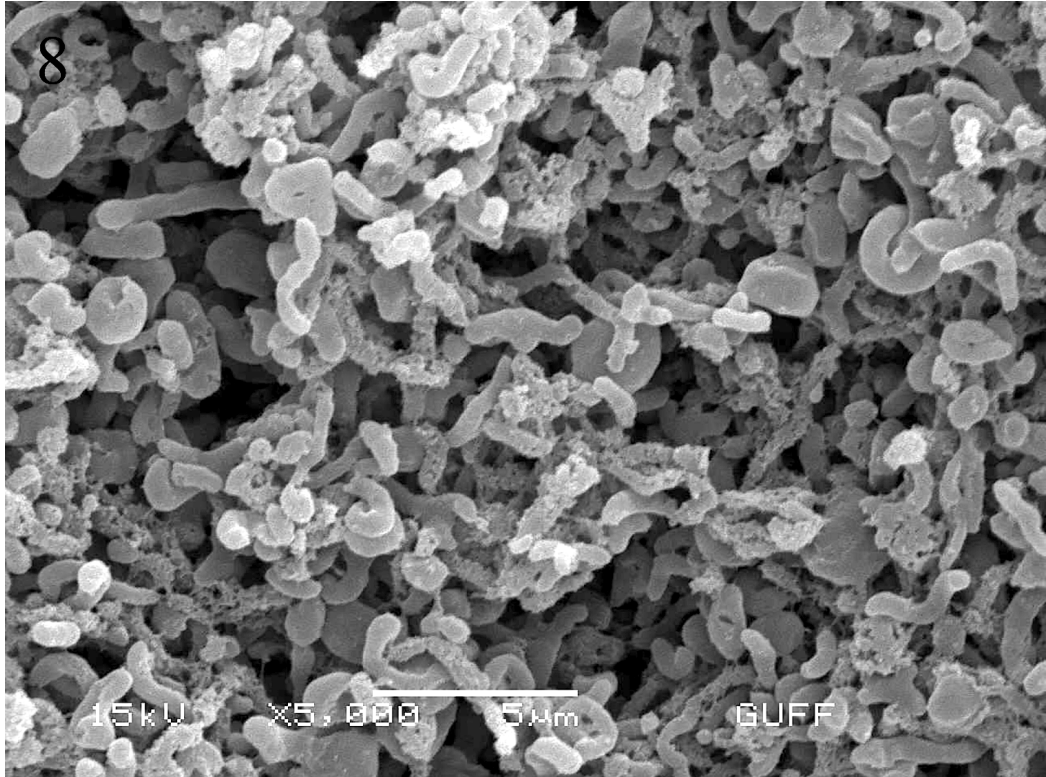
**Figure 5.** Light microscopic image of the cross section of gastric caecum (C) and pylorus (P). (Magnification: X100).



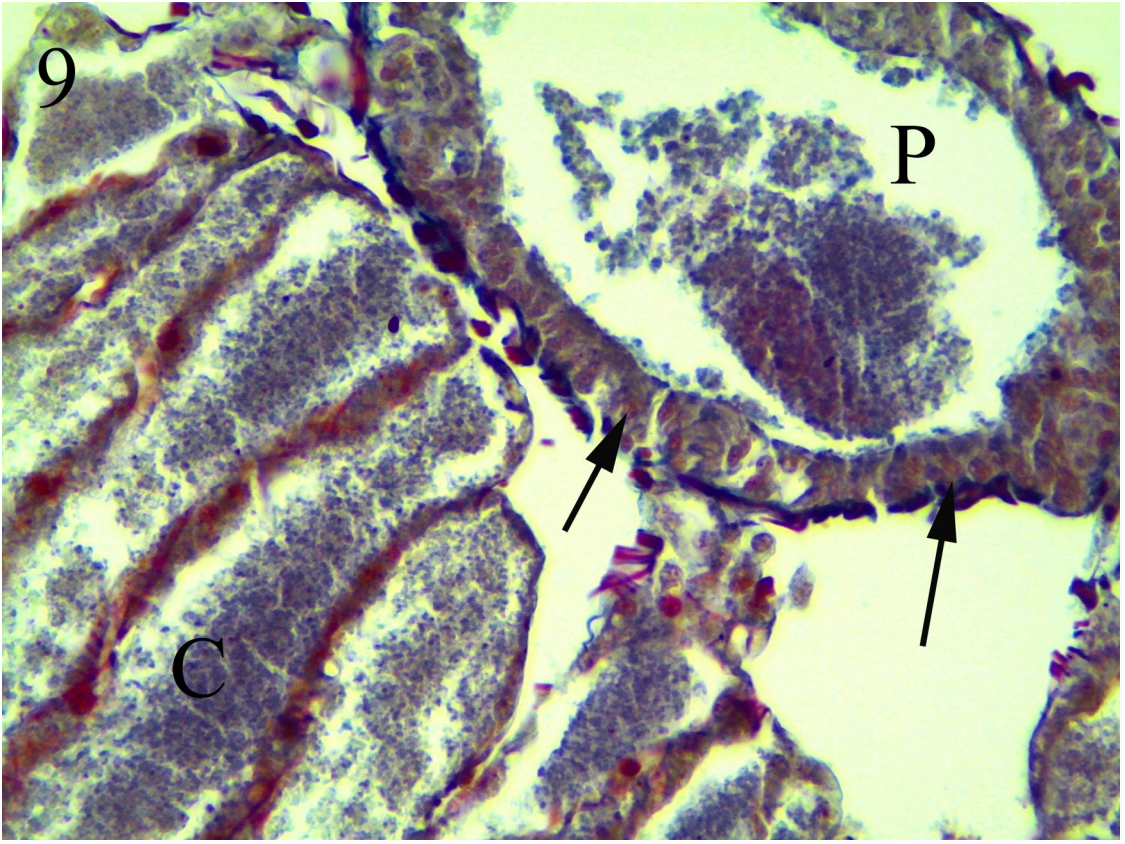
**Figure 6.** Light microscopic image of the cross section of gastric caecum. Arrows: nucleus of epithelial cells, asterisks: bacteria in the lumen, P: pylorus. (Magnification: X4100).



**Figure 7.** SEM image of the epithelial layer (E) of the gastric caecum and lumen (L) full of bacteria.



**Figure 8.** SEM image of the bacteria in the lumen of the gastric caecum.



**Figure 9.** The light microscopic image of the cross section of the pylorus (P). Arrows: nucleus of the epithelial cells, C: gastric caecum. (Magnification: X4100).