The Anatomy and Ultrastructure of the Digestive Tract and Salivary Glands of *Hishimonus lamellatus* (Hemiptera: Cicadellidae)

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Abstract

In recent years, we found that *Hishimonus lamellatus* Cai et Kuoh is a potential vector of jujube witches’-broom phytoplasma. However, little is known about the anatomy and histology of this leafhopper. Here, we examined histology and ultrastructure of the digestive system of *H. lamellatus*, both by dissecting and by semi- and ultrathin sectioning techniques. We found that the *H. lamellatus* digestive tract consists of an esophagus, a filter chamber, a conical midgut and midgut loop, Malpighian tubules, an ileum, and a rectum. Furthermore, both the basal region of the filter chamber epithelium and the apical surface of the midgut epithelium have developed microvilli. We also identify the perimicrovillar membrane, which ensheaths the microvilli of midgut loop enterocyte, and the flame-like luminal membrane, which covers the microvilli of the conical midgut epithelium. In addition, *H. lamellatus* has the principal and accessory salivary glands. Our observations also showed that the endoplasmic reticulum, mitochondria, and secretory granules were all highly abundant in the secretory cells of the principal salivary glands, while the accessory glands consist of only one ovate or elbow-like acinus. We also briefly contrast the structure of the gut of *H. lamellatus* with those of other leafhopper species. These results intend to offer help for the future study on the histological and subcellular levels of phytopathogen–leafhopper relationships, including transmission barriers and the binding sites of pathogens and other microorganisms within their leafhopper vectors.

Key words: leafhopper, digestive system, Malpighian tubule, ultrastructure, histology

Leafhoppers are herbivorous insects belong to the family to the old suborder Auchenorrhyncha. More than 25,000 species of leafhoppers are known worldwide (Dietrich 1997), of which over 1,200 are present in China (Li et al. 2012). Some of the leafhoppers must be mesophyll feeders and hence do not tap into plant vasculature to suck plant sap, thereby directly harming plants. Moreover, many leafhoppers are responsible for the transmission of plant pathogens—including viruses and bacteria—thus causing serious losses of production in the agriculture and forestry industries (Nault and Ammar 1989, Bové et al. 2003). To date, 118 species of leafhopper are known vectors (Weintraub and Wilson 2010). In East Asia, the pathogen of rice dwarf disease is spread by leafhoppers, which is the reason for the sharp decline in rice production every year (Ruan et al. 1985, Sivamani et al. 1999). Furthermore, mulberry dwarf disease phytoplasma, which is transmitted by *Hishimonus sellatus* (Hemiptera: Cicadellidae), has caused a severe decline of mulberry tree (Kawakita et al. 2000, Mitsuhashi et al. 2002).

Jujube witches’-broom (JWB) phytoplasma is known to be transmitted by leafhoppers, and the resulting disease results in economic losses that reach hundreds of millions of dollars annually (Liu et al. 2010). Early studies suggested that the transmission of the JWB phytoplasma occurred mainly by insect vectors such as *H. sellatus* (La and Woo 1980, Sun et al. 1988). *Hishimonus lamellatus* was first reported in Huolu County, Hebei Province, China (Cai et al. 1995). Both leafhopper species were found to be carrying JWB phytoplasma in vivo, but vector competence of *H. lamellatus* was not empirically tested (Hao et al. 2015).

Phytoplasmas, the single-celled prokaryotes, are parasitic on the phloem of plants. Insects that feed on phloem can acquire and transmit phytoplasmas (Lee et al. 2000, Weintraub and Beanland 2006), and it is generally believed that phytoplasmas circulating in insects need to pass through the midgut and salivary glands before transmission to healthy plants via interface and salivary secretions during feeding (Weintraub et al. 2004, Weintraub and Beanland 2006, Ammar et al. 2011).

To date, few researchers have conducted detailed analyses of the digestive systems of leafhopper vectors. Gil-Fernandez and Black...
observed the general structure of the digestive tract of Agallia constricta Van Duze (Hemiptera: Cicadellidae) (Gil-Fernandez and Black 1965). In addition, Lindsay and Marshall (1980) described the morphology and ultrastructure of the Euonyma distincta Signoret (Hemiptera: Euryemelidae) filter chamber (Lindsay and Marshall 1980), Cheung and Purcell (1993) revealed the ultrastructure of the digestive system of Euscelidius variagatus (Kirschbaum) (Hemiptera: Cicadellidae) (Cheung and Purcell 1993), and Wayadande et al. (1997) compared the general morphology of the digestive tracts of Circulifer tenellus (Hemiptera: Cicadellidae) and Dalbulus maidis (Hemiptera: Cicadellidae) (Wayadande et al. 1997). More recently, Zhang et al. (2012) observed the ultrastructure of the digestive tract of Psammotettix striatus (Linnaeus) (Hemiptera: Cicadellidae) (Zhang et al. 2012), and Utiyama et al. (2016) studied similar features in Bucephalogonia xanthophis (Hemiptera: Cicadellidae) (Utiyama et al. 2016). Taken together, the results of these analyses suggest that there are important differences in the morphology and ultrastructure of the digestive tracts and salivary glands of different leafhopper species (Sogawa et al. 2016). Globally, there are 41 species of Hishimonus, of which there are 17 Hishimonus in China (Li and Wang 2004). However, little information is currently available regarding the structural characteristics of the digestive systems of Hishimonus insects. Actually, there are few research on the histology and ultrastructure of the digestive tract and salivary glands of H. lamellatus.

Therefore, the purpose of this study was to characterize the structure of the digestive system of H. lamellatus using optical and transmission electron microscopy (TEM), and to understand the ultrastructural characteristics of the digestive tract, the Malpighian tubule (MT) system, and the salivary glands.

Materials and Methods

Leafhopper Rearing

Hishimonus lamellatus individuals were raised in the insect breeding room of the Beijing Key Laboratory of New Technology in Agricultural Application. Leafhoppers were originally collected in Liucun, Chaping District, Beijing in August 2010. A laboratory population was established in the insect breeding room and identified by Professor Cai Ping. The H. lamellatus population was reared on jujube (Ziziphus jujuba Mill (Rhamnaceae: Rhamnaceae)) seedlings and placed in a 40-cm-high cylindrical transparent plastic worm cage, which was sealed with 40 × 50 mesh gauze. The feeding temperature was maintained at 25 ± 1°C, the relative humidity ranged between 50 and 70%, and the photoperiod was 16 L:8 D (Hao et al. 2015).

Sample Preparation for Light Microscopy

Hishimonus lamellatus samples were chilled at −20°C for 20 min and then each sample was then placed on a grooved glass slide (SAIL BRAND, 7103 Single Concave). Under a stereoscopic microscope (Motic, K Series), the wings and feet of leafhoppers were removed with forceps (Dumont, 0208-5-p), and the side line of the abdomen stalk was cut with scissors. A drop of phosphate buffer solution was added dropwise to this cut, and an anatomical needle was used to extract the digestive tract from abdominal segments 1–7. The salivary glands, located at the top of the head, were gently separated using forceps. The salivary glands were dissected and stained with toluidine blue solution and photographed using a stereomicroscope (Zeiss StoREO Discovery, V20) (Gil-Fernandez and Black 1965, Sogawa 1965). About 80 leafhopper individuals were examined by light microscopy.

Semi-Thin Section Sample Preparation for Light Microscopy

The selected adult H. lamellatus individuals, anesthetized with ether, were placed on single concave slides. We then added 2.5% glutaraldehyde fixative and quickly dissected the digestive tract of the leafhopper using tweezers. Next, the digestive tract was then placed in a centrifuge tube containing 1.5 ml of glutaraldehyde fixative. The digestive tract was fixed for 40 h in the dark at room temperature. Next, fixed tissue were washed with phosphate-buffered saline (PBS) for 3 h. Afterward, we added 1% osmium tetroxide to the digestive tracts for 90 min before rinsing with phosphate buffer. Then, the samples were washed with PBS for 30 min. The samples were dehydrated in a series of ethanol concentrations of 30, 50, 70, 80, 90, and 100% for 6 min, respectively, and again in that of 100% ethanol for 30 min. Samples were then infiltrated with a 1:2 mixture of ethanol and epoxy resin 618 for 2 h and pure epoxy resin 618 for 12 h. The samples were transferred to plastic flat embedding mold (Electronic Microscopy Sciences) and polymerized for 24 h at 37°C, 12 h at 45°C, and 48 h at 60°C. The embedded block was trimmed to the appropriate size and the sample was cut into 2-μm-ultrathin sections with a glass knife of Leica UC6 ultratimn slicer. The sections were placed in a saturated solution of NaOH in absolute ethanol for 3 min. The samples were infiltrated in a series of ethanol concentrations of 100, 95, 80, 70, 50, and 35% for 5 min. Slides were immersed in a staining jar containing 2% hydrochloric acid for 5 min, and then in a staining jar containing distilled water for 2 min. The samples were stained in Harris’s hematoxylin for 20 min, and washed in tap water. Slides were immersed in a staining jar containing acid alcohol (0.5:99 v/v) differentiation solution for 30 s, and then in a staining jar containing tap water for 15 min, and counterstained in 1% aqueous cosin for 2 min. Slides were immersed in a staining jar containing tap water for 5 min. The samples were dehydrated in a series of ethanol concentrations of 80 and 95% for 1 min and twice in 100% for 1 min. The samples were sealed with a drop of neutral balsam, capped with a cover slip, and then kept in drying oven. The treated samples then were observed with a Zeiss microscope Imager A1 (Aparicio and Marsden 1969).

Sample Preparation for TEM

To prepare samples for analysis using TEM, we used the same method as described in ‘Semi-Thin Section Sample Preparation for Light Microscopy’. At least five embedded blocks were made for each sample to be observed. The ultrathin resin sections (60 nm) were cut with an ultramicrotome (Leica UC6) using a glass knife, transferred to copper grids, and stained with 2% (w/v) uranyl acetate for 15 min (in dark), and then washed in distilled water. The sections were stained with lead citrate for 20 min, and then washed in distilled water again. The sections were kept in a desiccator (Ghanim et al. 2016, Ammar et al. 2017). Then ultrathin sections were examined at 80 kV using an Hitachi H-7500 transmission electron microscope at the electron microscope laboratory of the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences. About 30 leafhopper individuals were examined by TEM.

Results

Histology and Morphology Features of the Digestive Tract

The foregut, midgut, and hindgut (Fig. 1) are the three major parts of the digestive tract of adult H. lamellatus.
Foregut

The esophagus is a narrow, slender tube. The esophagus is approximately 50 μm in diameter and is translucent or pale white in color (Fig. 1).

Filter Chamber

The filter chamber (FC) has a half-moon shape, a diameter of 300–400 μm, and is light milky white in color. The FC is composed of the anterior segment of the midgut, the posterior segment of the midgut, the base of the Malpighian tube, and the basal hindgut. It is also connected to the conical midgut (CM).

Midgut

The midgut is divided into a CM and a midgut loop (ML). The spherical or long vesicular CM is about 1,200 μm long and 700 μm wide. The surface of the CM is translucent and sometimes contains white particulate matter. The apical of the CM, which has a thick basement membrane, rapidly collapses into the ML. The ML has a diameter of about 400–600 μm and is only slightly smaller than the diameter of the rest of the midgut (Fig. 1). Its surface is rough and its color is yellowish. The basement membrane is relatively thin, and the columnar epithelium cells (CECs) found there are large and are located near the basement membrane (Fig. 2).

Hindgut

The hindgut consists of an elongated tubular ileum (Fig. 1) and an enlarged rectum (RE; see Fig. 1). The ileum is connected to the rectum through four MTs (Fig. 1). Its surface is smooth, translucent, and is not easily stained with toluidine blue; its diameter is approximately 100 microns. The ileal apical swells into the rectum, and the end of the ileum sac is open to the anal tube.

Ultrastructure of the Digestive Tract

Filter Chamber and Conical Midgut

The FC has a thin membrane known as the peritoneal membrane (PM), which is adjacent to the longitudinal muscles (LMU). Cells of these tissues include large numbers of mitochondria, which distributed around developed muscle fibers (Fig. 3C). The circular muscles (CMU) lie on the inside of the LMU surround the basement membrane. The basement membrane has channels, formed by infolding (IF), which gives it a network-like and highly developed appearance. The intima is highly specialized into microvilli (MV) which are dense and regularly arranged. Many mitochondria were also observed in the MV-containing cells. Furthermore, many well-developed MV extend into the lumen (L) (Fig. 3D). Moreover, there are also a large number of secretory vesicles (SVs) in the cytoplasm of many of these cells in these tissues (Fig. 3A and B), and many cells also contain a highly developed rough endoplasmic reticulum (RER) connecting the SVs and the basement membrane (Fig. 3B). In many cases, there are also distinct septal desmosomes (SDs) between the cells (Fig. 3D).

The basement membrane of the CM was found to be thinner than that of the FC, and we also found that the basement membrane has one or more IFs. Mitochondria are present between the basement membrane and the IF (Fig. 3E). The intima is highly specialized into dense and regularly arranged MV (Fig. 3E and F). In addition, its uniform and well-aligned flame-like luminal membrane (FLM) is covered in MV (Fig. 3).

Midgut Loop

The midgut's PM is relatively thick (Fig. 4A and B) and is wrapped with developed LMU (Fig. 4B). There is a tracheole (TR) distributed between the PM and the LMU (Fig. 4B), and the LMU are found adjacent to the CMU (Fig. 4A). The basement membrane has channels formed by IF (Fig. 4A), and these are network-like and highly developed; in addition, there are a large number of mitochondria distributed therein (Fig. 4A). Many well-developed MV extend into the lumen of the midgut (Fig. 4A, C, and D), and these MV are generally covered with a perimicrovillar membrane (PMM) (Fig. 4A and C). Moreover, many mitochondria were observed near the developing MV (Fig. 4D). SVs in the cytoplasm (Fig. 4A) were found to differ in size and shape. We also found unknown inclusions present in the SVs (Fig. 4C), and there were SDs between the cells (Fig. 4A). In addition,
many lysosomes (LY) were observed in the cytoplasm of ML cells. These cells also show internal organelle fragments (Fig. 4C).

Ileum
The nucleus of ileal cells is relatively large. N1 has a distinct heterochromatin, whereas N2 is not obvious (Fig. 5A). The ileum PM is thin, and is adjacent to the developed circular muscle (Fig. 5B and C). The cytoplasm of the cells of the ileum PM is filled with SVs and mitochondria that are circular, oval, or clavate. We also identified a developed RER around mitochondria (Fig. 5C). An elliptical bacterial-like structure is present in the ileum cytoplasm of 50% leafhopper individuals (Fig. 5C).

Malpighian Tubule
The cells of the MT contained many SVs (Fig. 6A) in which a large number of brochosomes (BRs) were found. The cell showed the widespread RER (Fig. 6B). Mitochondria were located near SVs (Fig. 6C). The outer wall of each BR was honeycomb-shaped. BRs were observed: Some had high central electron density and appeared dark (Fig. 6C; white arrow); while other BRs were centrally transparent (BR2), embedded (BR1), or showed multiple small cavities (BR3) (Fig. 6C). This may be mitochondria at different developmental stages. SVs with low electron density were scattered around the nuclei (Fig. 6A). Sparse MV were observed on the outside of the intima (Fig. 6D).

Morphology of Salivary Glands
The salivary glands of *H. lamellatus* are located in the head cavity. The salivary gland complex consists of pairs of principal glands (PGs) and accessory glands (AGs) (Fig. 7).

Principal Gland
The vesicular PG is a major part of the salivary glands (Fig. 7A). The PG includes both an anterior lobe (AL) and posterior lobe (PL) (Fig. 7B). The distal and proximal regions of the AL consist of small, closely packed acini that have a smooth surface. In addition, we identified five larger acini in the middle region that are loosely arranged. The PL consists of about 10 acini that are divided into two types: the first type is a group of about five relatively small acini that are closely arranged in a petal shape and have a rough surface. The second type refers to about five larger acini present in a loose pattern around the periphery of the petal-like acinus (Fig. 7B). The acini of the PL are slightly larger than the acini of the middle region of the AL. Finally, the PGs are connected via the lateral salivary ducts which converge to form the common salivary duct (Fig. 7B).

Accessory Glands
The AGs (Fig. 7A) found in the salivary gland complex were rod- or elliptically-shaped. These are connected to the PG by a short accessory duct. AGs are simply constructed and have only one acinus, which is similar in structure to the acini of the PL. AGs are free on both sides of PG.

Ultrastructure of the PG
The nucleus of secretory cells contained a variety of heterochromatin (Fig. 8A), and was generally located in the basement membrane.
region, as were the TRs (Fig. 8B). Unknown inclusions were also present in the salivary duct (Fig. 8C, see asterisk [*]). The atrium is lined with a cuticle, which is surrounded by IFs of the apical plasma membrane (Fig. 8C).

We observed the presence of many secretory granules (SGs) within the cells (Fig. 9A). There are differences between the SGs, SG1 has a transparent core and filaments are attached to its inner edge, and the core of SG2 is opaque and has no filaments (Fig. 9A). We also identified dispersed SVs that have MV-like protrusions along the inner edge of the core (Fig. 9B). In the salivary gland cells, significant muscles were observed (Fig. 9C). We observed the presence of rod-shaped bacteria-like structures in the cytoplasm of salivary gland cells (Fig. 9D).

**Discussion**

**The Ultrastructure and Function of the Tubular Midgut in *H. lamellatus***

The ML of *H. lamellatus* is divided into two parts. In the front is the cone, which is connected to the FC. The tubular midgut is also connected to the FC, thus forming the ‘midgut ring’. The presence of this structure is consistent with previous reports of other leafhoppers (Tsai and Perrier 1996, Zhong et al. 2014). Our results also confirmed that the epithelial cells of the tubular midgut of the digestive tract of *H. lamellatus* were present in the shape of a column, and that the endoplasmic reticulum and SVs were abundant in the cells. We found many well-organized and developed brush-like borders of MV, which play an important role in the absorption of digested nutrients including carbohydrates, amino acids, and water.

The midguts of most insects have a peritrophic matrix, which is mainly used to protect midgut cells from pathogens and food particles, and has a compartmentalization effect on the midgut (Terra 1990). However, there is no peritrophic membrane in the midgut of Hemiptera insects. Instead, a layer of lipoprotein membranes covered with MV tips is present, termed the PMM (Terra 1988). Here, we identified an obvious PMM in the tubular midgut of *H. lamellatus*. Its morphology is similar to that found in the midgut cells of *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae), *Mahanarva posticata* (Hemiptera: Cercopidae), and *Cicadella viridis* (Hemiptera: Cicadellidae) (Fonseca et al. 2010, Zhong et al. 2014). The PMM and MV form a closed space, i.e., the perimicrovillar space that mediates the digestion and absorption of nutrients in the midgut (Terra 1990).
The salivary glands of Cicadellidae insects are composed of the PG, the AG, and its salivary duct. Importantly, the morphology of the principal and AGs are significantly different (Ammar et al. 1985). Our observations revealed that the salivary glands of *H. lamellatus*, including both the principal and AGs, are lightly cream-colored or translucent. The former is bulky, has a more complex structure, and consists of an aggregate body containing many acini. In contrast, the latter is a short rod-shaped tubular gland with a structure similar to that of salivary gland of *H. sellatus* (Sōgawa 1965). However, the salivary glands of *H. lamellatus* are morphologically distinct from those found in other leafhoppers, such as *P. stratus* (L.), where the AGs are more slender than those of *H. lamellatus* (Zhang et al. 2012). Moreover, the AGs of *C. tenellus* and *D. maidis* are both relatively large (Wayadande et al. 1997), and the PGs of *Nephotettix cincticeps* (Hemiptera: Cicadellidae) and *Chlorita flavescens* (Hemiptera: Cicadellidae) have a small number of acini and a relatively simple structure (Sōgawa 1965).

We also observed that the PG is composed of two parts: an AL and a PL. The AL is itself divided in two parts, i.e., the head and tail portions. The ALs of *H. sellatus* are quite different from those found in *Ggraminella nigrifrons* (Hemiptera: Cicadellidae) or *D. maidis* (Sōgawa 1965, Tsai and Perrier 1993). Moreover, as mentioned by Sōgawa (1965), the size, number, and shape of the ALs vary from species to species in the Cicadellidae. The PL consists of approximately 13 acini arranged in a multilayered petal-like shape similar to the shape and structure of the principal salivary glands of the subfamily Deltacephalinae (Sōgawa 1965). The principal salivary gland is a complex gland that contains at least two secretory systems, one that secretes precursors of the salivary sheath and another that produces water saliva that contains several enzymes (Sōgawa 1965). The AGs secrete mucus and phenolic enzymes which, together with proteins secreted by the principal salivary gland, constitute the salivary sheath (Miles 1964, 1965). In general, the salivary sheath of the leafhoppers contains lipids and neutral mucus substances (Sōgawa 1965, 1967). Saliva secreted by leafhoppers can damage host plants due to the presence of toxins and anticoagulants, and saliva containing pathogenic microorganisms may transmit them during mouthpart penetration (Raine and Forbes 1969, Sauer 1977). A variety of plant pathogens have been reported in the salivary glands of the leafhoppers (Ghanim and Medina 2007, Ammar et al. 2009), and the phytoplasma associated with mulberry dwarf disease has been observed in the salivary glands of *H. sellatus* and *Hishimonoides sellatiformis* (Hemiptera: Cicadellidae) (Kawakita et al. 2000). In addition, we used molecular data to show that the JWB phytoplasma was present in some leafhoppers (Hao et al. 2015). Moreover, recent studies of the relationship between microorganisms and their insect vectors have suggested that the primary glands are beneficial to the survival of microorganisms, since they are very sensitive to nutritional quality in the intracellular environment (Crotti et al. 2009, 2010). Kwaik (1996) found that the vesicular RER and its SVs may provide a rich environment replete with essential nutrients for...
microorganisms. This is thought to be due to the fact that there is a lot of RER, mitochondria, and numerous SGs within leafhopper PG cells (Kwaik 1996). Here, we found rod-shaped or oval microorganisms in the PGs of the leafhoppers, although these glands are known to contain phytopathogens in other leafhopper species (Gonzalez 2016). Although to date there has been no detailed investigation of the saliva of H. lamellatus, our histological and ultrastructure results suggest that the salivary glands of H. lamellatus play an important role in the transmission of plant pathogens (Reis et al. 2003).

The Structure of the Hindgut of the Leafhopper and Its Microorganisms

Ultrastructural observations showed that cells of the ileum (hindgut) of H. lamellatus possessed apical IFs associated with SVs as well as an abnormal abundance of mitochondria. These IFs were delicately formed by the invaginations of apical plasma membrane. Similar IFs have been described in the intestines of the leafhoppers P. striatus (L.) and Cic. viridis (Zhang et al. 2012, Zhong et al. 2014), and the IFs were found to increase the contact surface with food in the luminal space. In addition, we identified oval and short rod-shaped microorganisms embedded within the epithelial cells of the hindgut wall in H. lamellatus. These microorganisms are likely to be prokaryotic microbes (i.e., bacteria), and resemble those found in the intestines of other leafhopper vectors including Cic. viridis and A. constricta (Van Duzee) (Gil-Fernandez and Black 1965, Zhong et al. 2014). The microbes present in insect intestines and gut cells are probably involved in digestion processes (Caetano et al. 2009), and may participate in many different metabolic pathways. Thus, the presence of microorganisms is thought to be generally beneficial to host insects (Ishikawa 2003). These microorganisms were observed to be surrounded by the endoplasmic reticulum, mitochondria, and cytoplasm of the host cells, which again is likely beneficial for leafhoppers digestion and essential nutrient uptake (Ishikawa 2003, Salehi et al. 2007). Because the leafhoppers feed on phloem sap, which contains few amino acids, they are likely to rely on microbes to supply certain nutrients that are lacking in their food. However, more research is needed to explore the identity and characteristics of these microbes.

Structure and Function of FC and Cone Segment

Our results also showed that the muscles of the FC of H. lamellatus were well developed. The MV of the epithelial cells of the FC were tubular, uniform in length, and regularly arranged. Many insects in the Cicadomorpha that feed on plant juices have developed LMU, which power the contraction of the FC, allowing a large amount of water to move directly from the anterior midgut into the rear end of the midgut and the MT. This in turn concentrates sap in the xylem and phloem before absorption, and the FC then acts as a waterway to the lumen; the inner ileum thereby facilitates the absorption of water (Lindsay and Marshall 1980). The inner ileum is deeply deepened and forms IFs associated with mitochondria and openings of the basal lamina. In addition, the RER around the mitochondria is clearly visible, as is the apical membrane of the epithelial cells, which is tightly arranged into MV. The mitochondria at the base of the MV of enterocytes provide a large amount of energy for transport, and we also identified a large number of SVs in the cells, which play an important role in the absorption, storage, and secretion of metabolites (Silva et al. 2004). Thus, the absorption of nutrients and ions occurs in the CM and the anterior midgut (Cheung and Marshall 1973). In addition, the MV are covered with a filamentous membrane complex similar to the FLM found in the CM of the leafhopper B. xanthophis. This membrane complex originates from intestinal epithelial cells, and does not secrete product directly. The MV tips of the enterocytes are often constricted, thereby forming a membrane that projects into the midgut lumen and remains associated with MV (Utiyama et al. 2016). However, unlike the PMM, they may be anchored during intestinal digestion. Enzymes such as cathepsin and a-glucosidase prevent excetration and binding to amino acids, thereby constricting and concentrating them at the absorption site and enhance to effect of absorption (Cristofoletti et al. 2003). In the end, further studies are needed to explore the relationship between this FLM complex and intestinal microorganisms.

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