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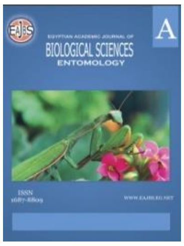

Ultrastructure of Midgut of *Scrabeus sacer* (Coleoptera:Scrabaeidae)

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ABSTRACT

The midgut of the adult *Scrabeus sacer* was divided into three parts, anterior, middle and posterior midgut. In the anterior part of the midgut, the apical part showed microvilli long and slender. These microvilli appear open in other parts. Numerous organelles appeared, well-developed nucleus mitochondria, rough endoplasmic reticulum. A thin basement membrane was observed and well-developed basal labyrinth. Regenerative cells were observed and well-developed circular muscle fibers. Tracheae and mitochondria were also observed. In the median portion of the midgut, well-developed long and slender microvilli were observed. Polymorphic mitochondria, well-developed nucleus, rough endoplasmic reticulum and other organelles were coming out of the cell to the lumen through the apical part of the middle midgut. A basement membrane, well-developed basal labyrinth, circular muscles, tracheae and vacuoles were observed in the basal part of the median midgut. The rear part of the midgut showed a well-developed brush border of microvilli. Polymorphic mitochondria, rods of bacteria, nucleus and other organelles are projecting in the lumen of the cell. Lateral cell membranes and numerous crescent shape mitochondria were observed in the posterior midgut. Gap junctions were observed in the posterior midgut. A well-developed basal labyrinth was observed enclosing mitochondria and tracheae. Regenerative cells are obvious in the basal part of the posterior region. The rough endoplasmic reticulum is not restricted to a defined part of the cell. No peritrophic membrane was observed throughout the midgut of the adult beetle *Scrabeus* sacer. Multivesicular bodies were observed in different parts of the midgut.

INTRODUCTION

Coleoptera is one of the largest order of insects with about 370,000 insect species described worldwide. Dung beetles are a major insect group (Coleoptera: Scarabaeidae) distributed globally except Antarctica with a high number of diversities comprising nearly 6,200 species and almost 267 genera (Tarasov & Génier 2015). These species are coprophagous in nature which live freely in soil and mostly feed on both wet and dry dung materials of herbivorous mammals. The undigested excreta of mammals are utilized as

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food and nesting material throughout their life cycle; hence, they possess many ecologically beneficial functions. It is believed that the quality and development of the larvae and adult depends on the quality and quantity of the brood prepared by beetles (Halffter & Edmonds 1982). The dung beetles play a vital role in nutrient recycling by decaying organic matter and developing soil aeration (Brown *et al.*,2010; Manning *et al.*, 2016) thereby, reducing the greenhouse gas fluxes (Penttilä *et al.*, 2013 & Slade *et al.*, 2016). It also enhances plant growth and grain production (Bornemissza *et al.,* 1970; Koyama *et al.,* 2003; Holter & Scholtz 2007). They increase the above-ground biomass by means of burying dung (Bang *et al.,* 2005). Secondary seed dispersal (Andresen & Feer 2005; Nichols *et al.,* 2008) and suppressing gastrointestinal parasites, etc. As an insect group, they possess an incredible innate immune system and hold multiple defense strategies such as possessing an epithelial barrier, synthesizing novel peptide-like compounds against pathogenic microbes and releasing reactive oxygen species to protect them from the pathogenic environment (Buchon *et al.*,2009.

The alimentary canal of insects is composed of three main parts: the foregut, the midgut, and the hindgut. The foregut is primarily involved in receiving, transporting and the initial digestion of food, whereas the midgut participates in food digestion and nutrient absorption (Johnson and Rabosky, 2000). The present study investigates the fine structure of different portions of the midgut of the dung beetle *Scrabeus sacer*

MATERIALS AND METHODS

Collection and Dissection of Insects:

Dung beetles were collected from Kafr EL-Sheikh, Egypt, dissected and the alimentary canal was separated and divided into three portions.

Transmission Electron Microscopy (TEM):

For TEM, the midguts of adult *Scrabaeus sacer* were washed thoroughly with PBS, and then fixed in 2.5% glutaraldehyde buffer overnight. After being washed in buffer twice, samples were post-fixed in 1% OsO4 and washed in buffer once more. The preparations were subsequently dehydrated in a standard ethanol series and embedded in EPON. Ultrathin sections were cut with a glass knife, stained with uranyl acetate and lead citrate, and photographed with the ZEISS EM 10 electron microscope (Germany).

RESULTS

The midgut of the adult *Scrabeus sacer* was divided into three parts, anterior, middle and posterior midgut.

In the anterior part of the mid gut, the apical part showed microvilli long and slender. These microvilli appear open in other parts. Numerous organelles appeared, well-developed nucleus mitochondria, rough endoplasmic reticulum (Plate 1 a&b). A thin basement membrane was observed and well-developed basal labyrinth. Regenerative cells were observed and well-developed circular muscle fibers. Tracheae and mitochondria were also observed (Plate 2 c&d). In the median portion of the midgut,well-developed long and slender microvilli were observed. Polymorphic mitochondria, well-developed nucleus, rough endoplasmic reticulum and other organelles were coming out of the cell to the lumen through the apical part of the middle midgut (Plate 4 a,b,c&d). A basement membrane, well-developed basal labyrinth, circular muscles, tracheae and vacuoles were observed in the basal part of the median midgut (Plate 5 a,b,c&d).

The rear part of the midgut showed a well-developed brush border of microvilli. Polymorphic mitochondria, rods of bacteria, nucleus and other organelles are projecting in the lumen of the cell (Plate 6 a,b,c&d). Lateral cell membranes and numerous crescent shape mitochondria were observed in the posterior midgut (Plate 7 c&d). Gap junctions were observed in the posterior midgut (Plate 8 c). A well-developed basal labyrinth was observed enclosing mitochondria and tracheae (Plate 9 a&b). Regenerative cells are obvious in the basal part of the posterior region. The rough endoplasmic reticulum is not restricted to a defined part of the cell. No peritrophic membrane was observed throughout the midgut of the adult beetle *Scrabeus* sacer. Multivesicular bodies were observed in different parts of the midgut (Plate 5 d; Plate 8 d).

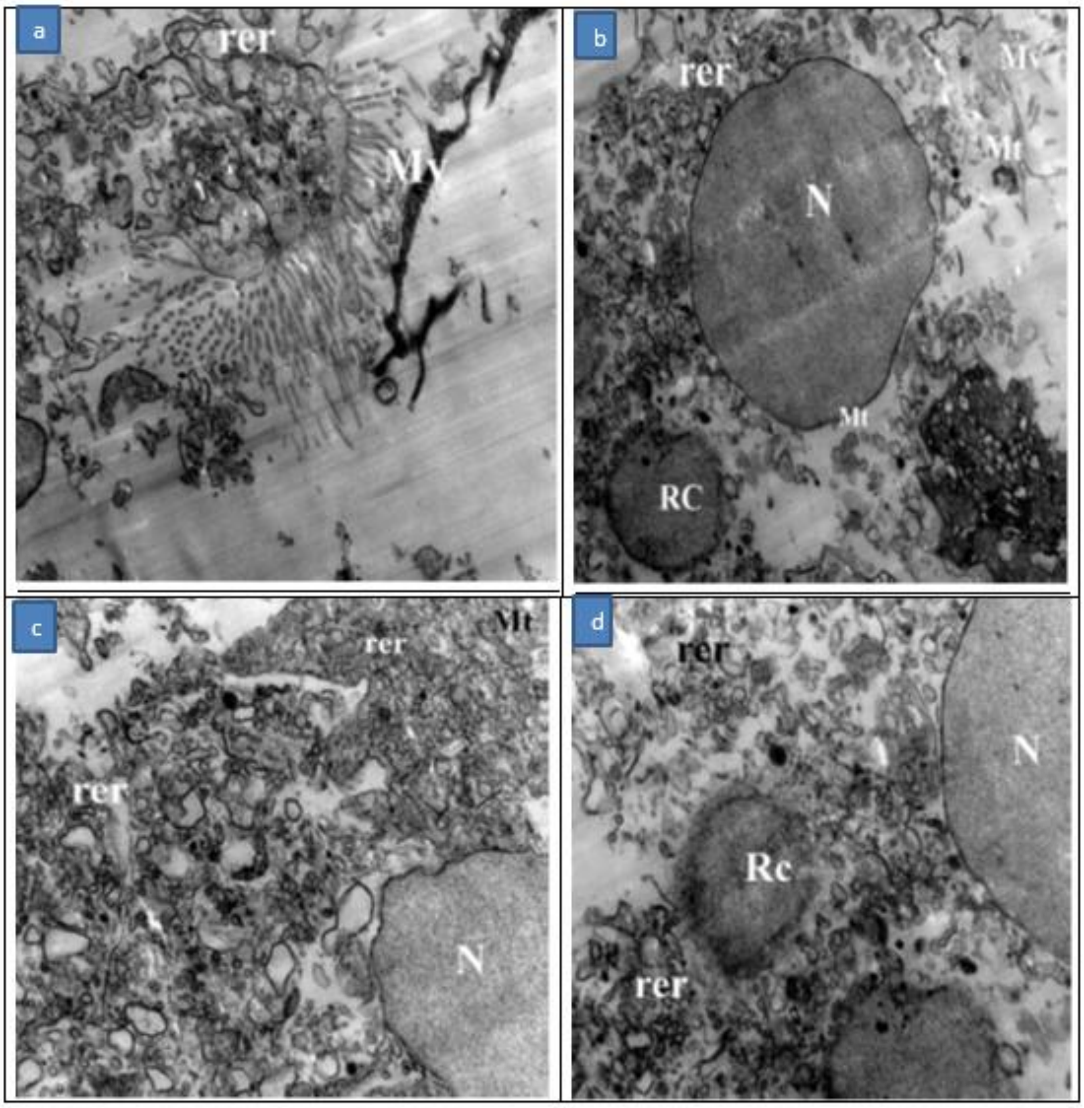

Plate 1: Electron micrograph of the anterior mid-gut epithelium of adult *Scarabaeus sacer* showing:

a: Microvilli (mv) and rough endoplasmic reticulum (rer). Magnification (x 8000).

b: Microvilli (mv), lumen (lu), rough endoplasmic reticulum (rer) , nucleus (N) and regenerative cell (Rc). Magnification (x 8000).

c: Rough endoplasmic reticulum (rer), nucleus (N) and mitochondria (Mt). Magnification (x 12000).

d: Rough endoplasmic reticulum (rer) , nucleus (N) and regenerative cell (Rc). Magnification (x 12000).

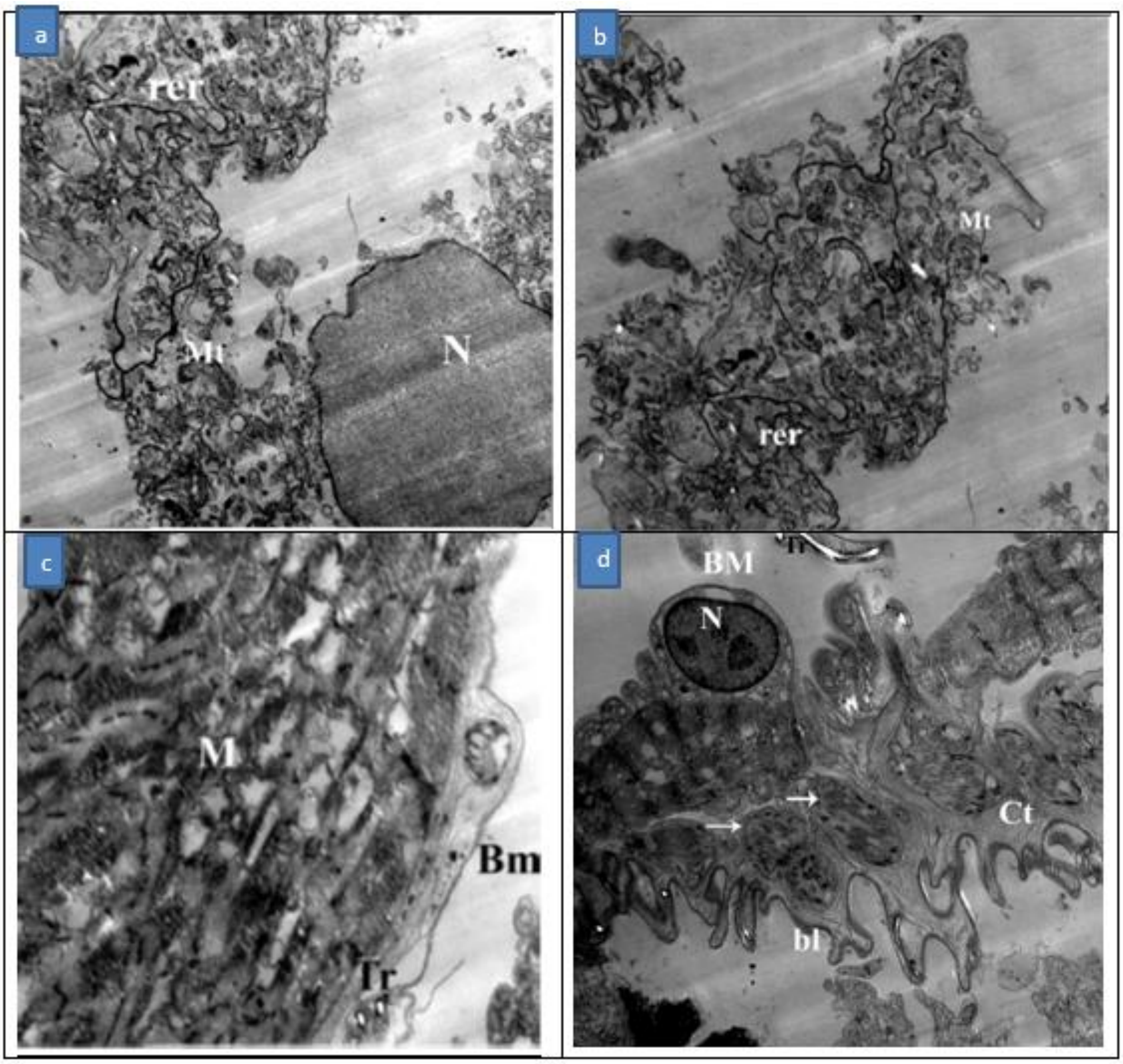

Plate2: Electron micrograph of the anterior mid-gut epithelium of adult *Scarabaeus sacer* showing:

a: Rough endoplasmic reticulum (rer), nucleus (N) and mitochondria (Mt). Magnification (x 12000).

b: Rough endoplasmic reticulum (rer) and mitochondria (Mt). Magnification (x 8000).

c: Muscle (M), trachae (Tr) and basement membrane (Bm). Magnification (x 8000).

d: Muscle (M), trachae (Tr), connective tissue (Ct). basement membrane (Bm) and basal labyrinth (bl). Magnification (x 5000).

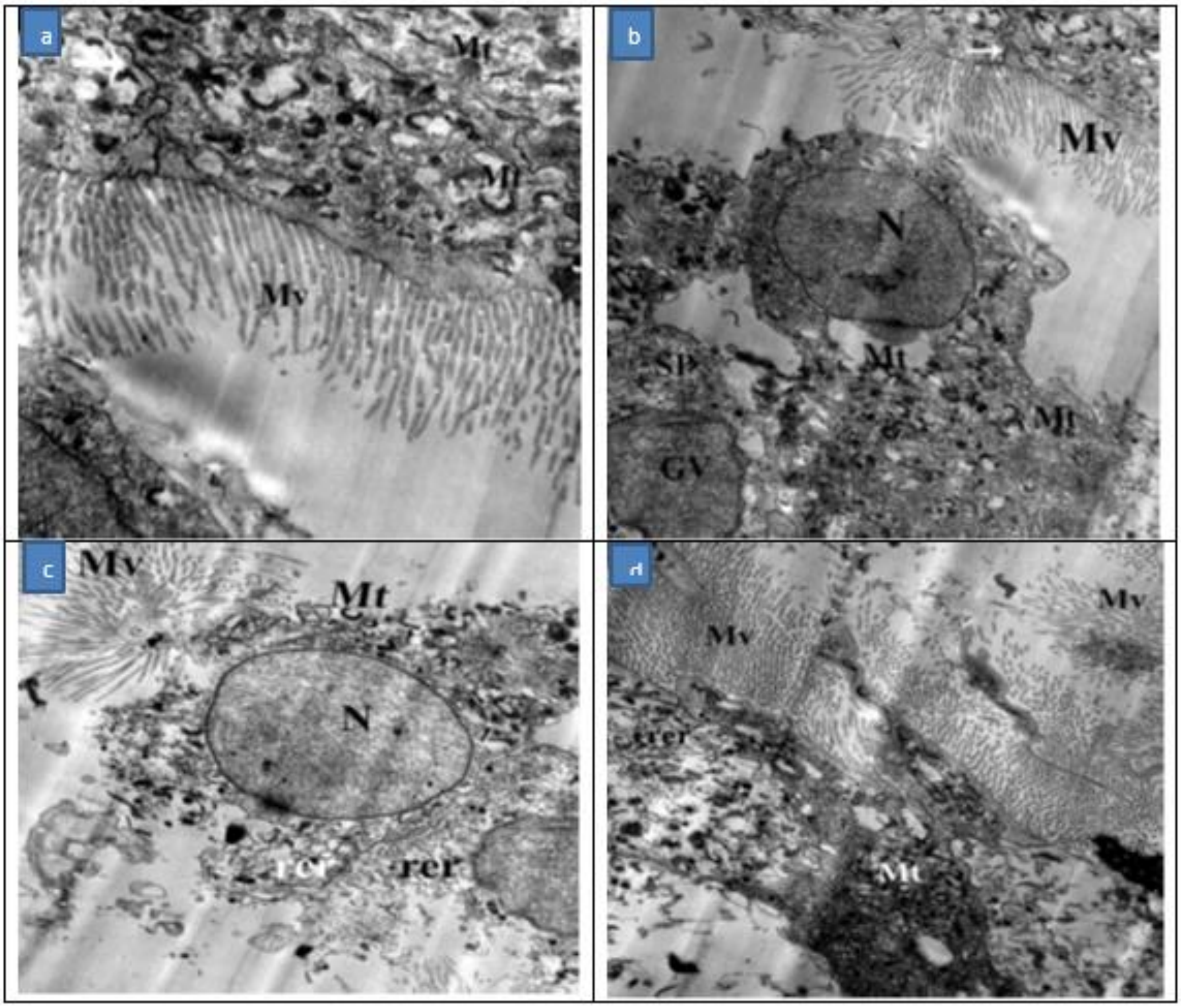

Plate 3: Electron micrograph of the middle midgut epithelium of adult *Scarabaeus sacer* showing:

a: Microvilli (Mv), mitochondria (Mt) and rough endoplasmic reticulum (arrow). Magnification (x 12000).

b: Microvilli (Mv), mitochondria (Mt), nucleus (N), rough endoplasmic reticulum (arrow), granulated vesicle (GV) and secretory product (SP). Magnification (x 6000).

c: Microvilli (Mv), mitochondria (Mt), nucleus (N) and rough endoplasmic reticulum (rer). Magnification (6000).

d: Microvilli (Mv), mitochondria (Mt) and rough endoplasmic reticulum (rer). Magnification (x 6000).

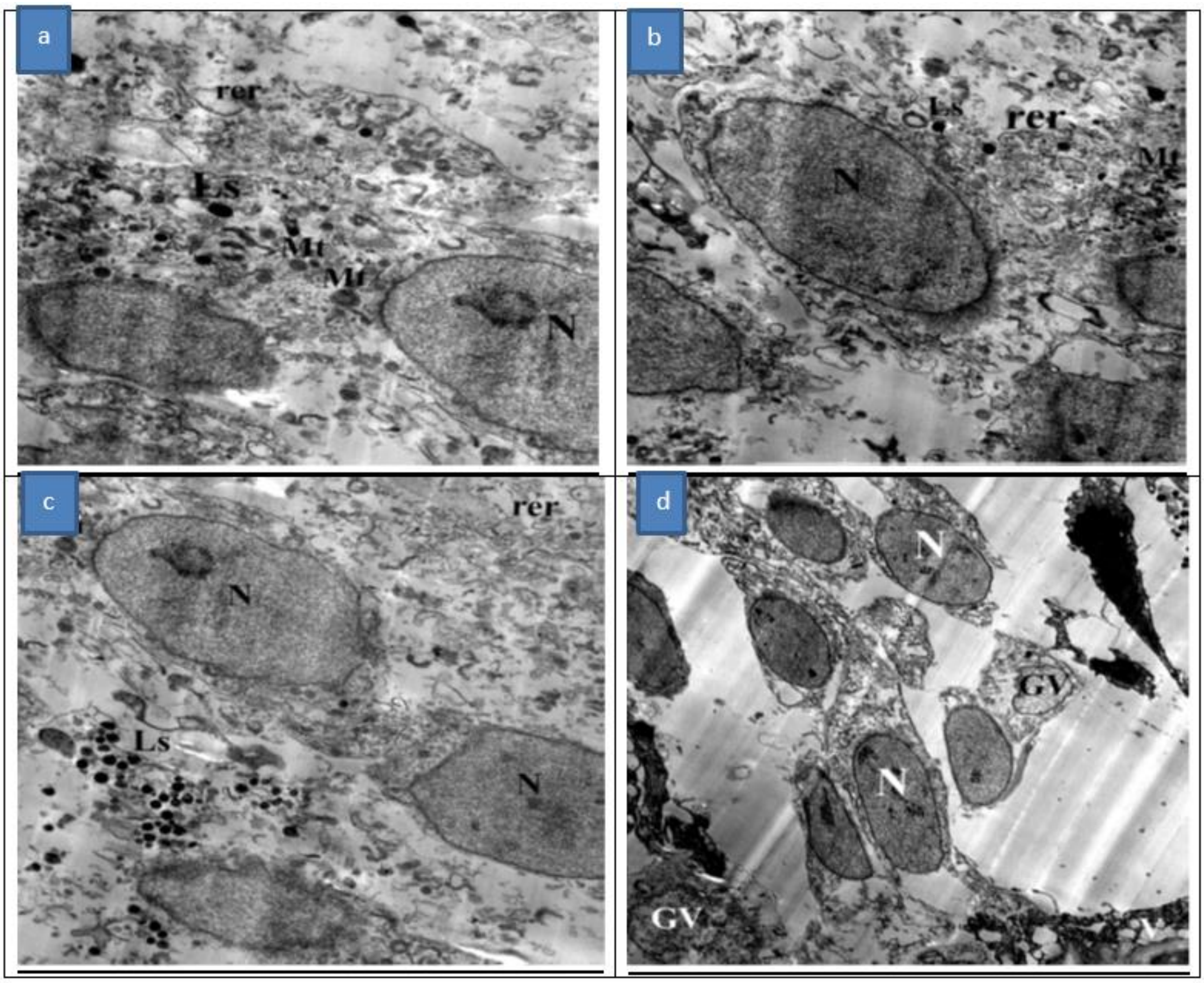

Plate 4: Electron micrograph of the middle midgut epithelium of adult *Scarabaeus sacer* showing:

a: Lysosome (Ls), mitochondria (Mt), nucleus (N)and rough endoplasmic reticulum (rer). Magnification (x 10000).

b: Lysosome (Ls), mitochondria (Mt), nucleus (N)and rough endoplasmic reticulum (rer). Magnification (x 8000).

c: Lysosome (Ls), mitochondria (Mt), nucleus (N)and rough endoplasmic reticulum (rer). Magnification (x 8000).

d: Nucleus (N) and granulated vesicle (GV). Magnification (x 4000).

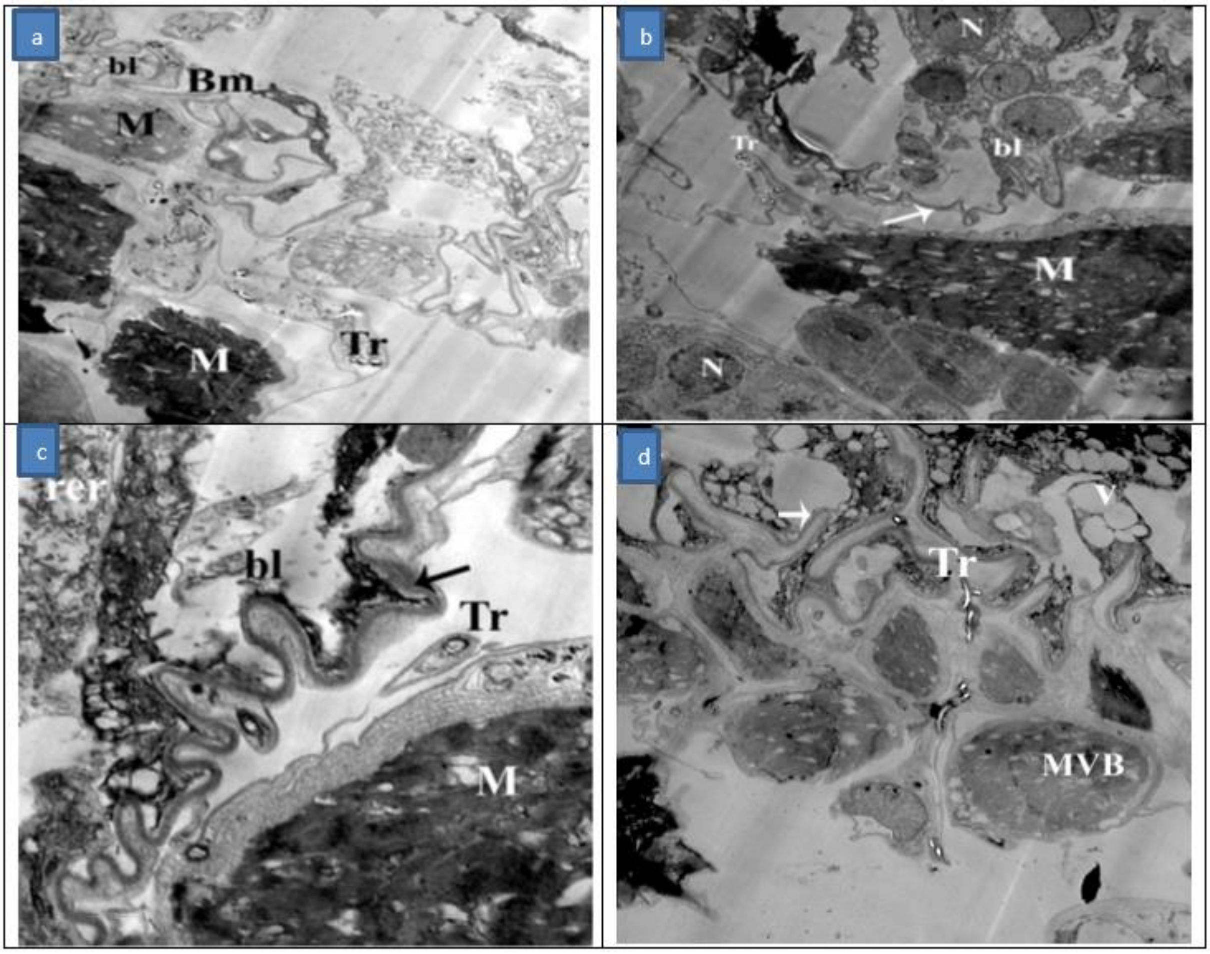

Plate 5: Electron micrograph of the middle midgut epithelium of adult *Scarabaeus sacer* showing:

a: Basement membrane (Bm), basal labyrinth (bl), muscle (M) and trachae (Tr). Magnification (x 4000).

b: Basement membrane (arrow), basal labyrinth (bl), muscle (M),trachae (Tr) and nucleus (N). Magnification (x 3000).

c: Basement membrane (arrow), basal labyrinth (bl), muscle (M),trachae (Tr) and rough endoplasmic reticulum (rer). Magnification (x 10000).

d: Basement membrane (arrow), muscle (M),trachae (Tr) and vacuole (V). Magnification (x 5000).

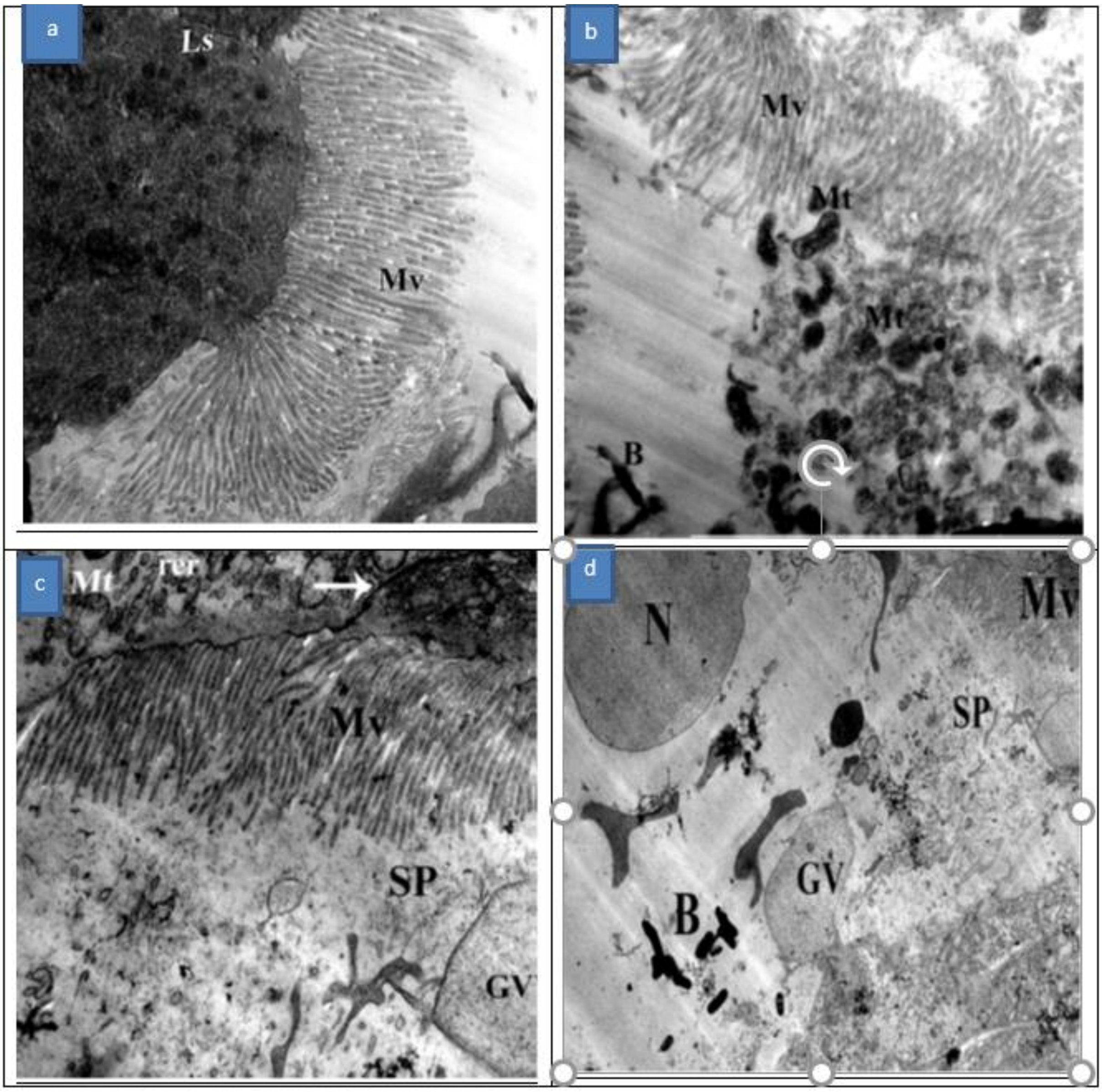

Plate 6: Electron micrograph of the posterior midgut epithelium of adult *Scarabaeus sacer* showing:

a: Microvilli (Mv) and lysosome (Ls). Magnification (x 10000).

b: Microvilli (Mv), mitochondria (Mt) and bacteria (B). Magnification (x 10000).

c: Microvilli (Mv), rough endoplasmic reticulum (rer), mitochondria (Mt), granulated vesicle (GV), secretory product (SP) and cell junction (arrow). Magnification (x 10000).

d: Microvilli (Mv), nucleus (N), granulated vesicle (GV), secretory product (SP) and bacteria (B). Magnification (x 3000).

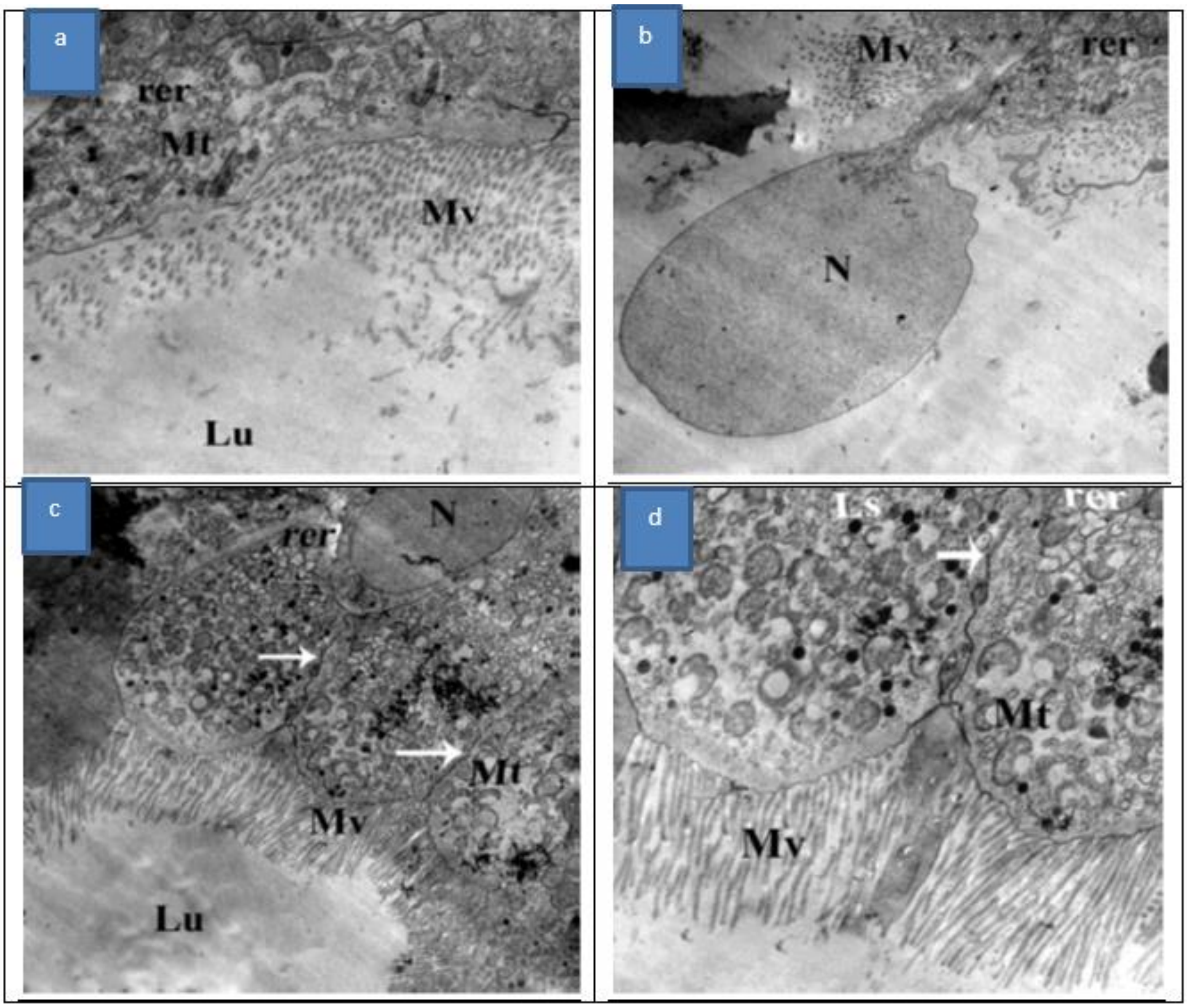

Plate 7: Electron micrograph of the posterior midgut epithelium of adult *Scarabaeus sacer* showing:

a: Microvilli (Mv), mitochondria (Mt), rough endoplasmic reticulum and lumen (Lu). Magnification (x 10000).

b: Microvilli (Mv), nucleus (N) and rough endoplasmic reticulum. Magnification (x 5000).

c: Microvilli (Mv), nucleus (N), rough endoplasmic reticulum (rer), mitochondria (Mt) lumen (Lu) and cell junction (arrow). Magnification (x 5000).

d: Microvilli (Mv), rough endoplasmic reticulum (rer), mitochondria (Mt), lysosome (Ls) and cell junction (arrow). Magnification (x 10000).

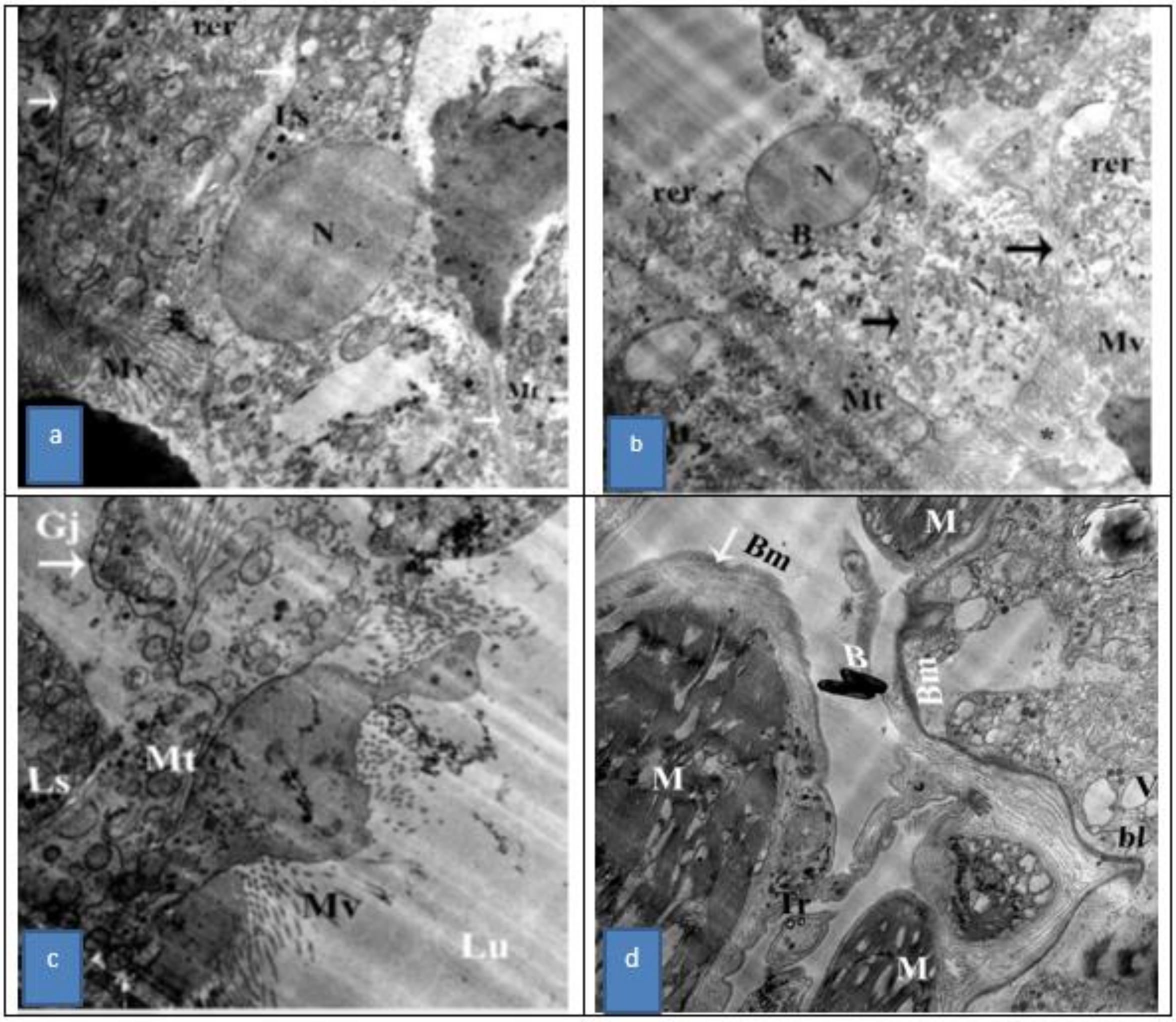

Plate 8: Electron micrograph of the posterior midgut epithelium of adult *Scarabaeus sacer* showing:

a: Nucleus (N), mitochondria (Mt), secretory product (SP), and cell junction (arrow). Magnification (x 5000).

b: Nucleus (N), rough endoplasmic reticulum (rer), mitochondria (Mt), muscle (M) and cell junction (arrow). Magnification (x 6000).

c: Microvilli (Mv), mitochondria (Mt), gap junction (Gj arrow), lysosome (Ls) and lumen (Lu). Magnification (x 6000).

d: Basement membrane (BM), basal labyrinth (bl), bacteria (B) and muscle (M). Magnification (x 6000).

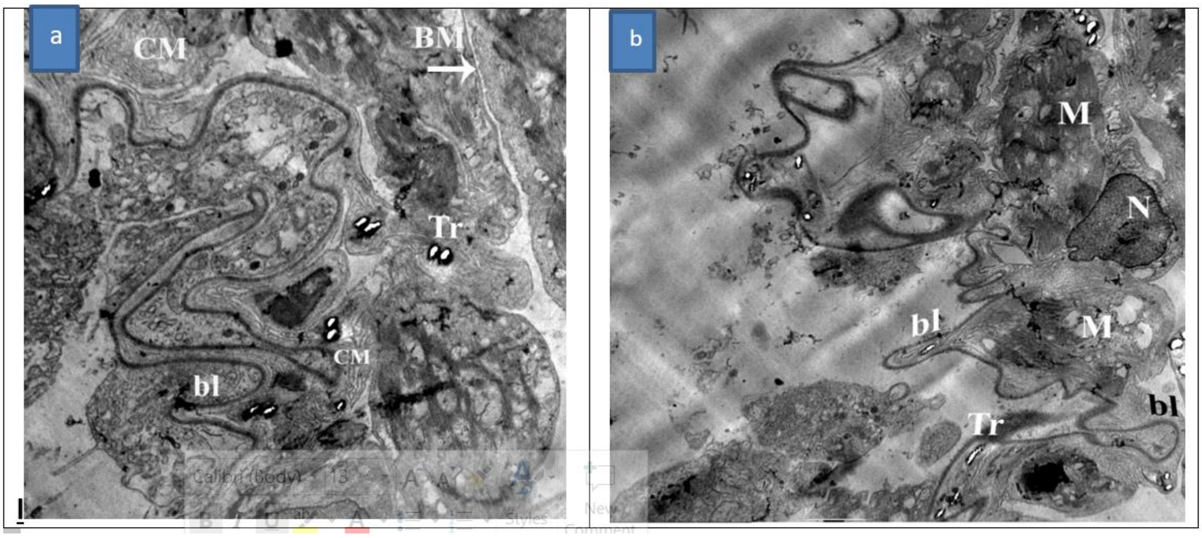

Plate 9: Electron micrograph of the hind-gut epithelium of adult *Scarabaeus sacer* showing:

a: Basement membrane (BM), basal labyrinth (bl), nucleus (N), muscle (M) and trachae (Tr). Magnification (x 5000).

b: Basement membrane (BM), basal labyrinth (bl), cell junction (Cj arrow), muscle (M) and trachae (Tr). Magnification (x 8000).

DISCUSSION

The three portions of the midgut in the present study showed long slender microvilli. Microvilli dramatically increase the apical membrane area of a cell for enzyme secretion and absorption of digested products, and in *Scrabeus sacer* longer microvilli were found in the posterior midgut than in the other three regions of the midgut. Therefore, high rates of absorption likely occur in the posterior midgut (Ke Li *et al.*, 2018). MVBs are a special type of late endosome that primarily separates and delivers proteins to lysosomes for degradation. MVBs regulate the secretory process by overproducing secretory granules (Lederberg 2000). Regenerative cells were observed while no peritrophic membrane was observed throughout the midgut of adult beetle *Scrabeus* sacer. The continuous regeneration of the midgut epithelium through division and differentiation of regenerative cells during the adult stage in response to external factors appears to be a common process in Coleopterans. In these insects, larval regenerative cells differentiate into pupal/adult midgut epithelium at metamorphosis (Parthasarathy and Palli 2008), while some of them retain the status of their regenerative cell and become adult regenerative cells organized in regenerative crypts (Nardi *et al.,* 2010). A comparative study that considered 18 coleopteran species demonstrated a correlation among the presence and density of regenerative cells in the adult midgut epithelium, the feeding habits of the beetle, the presence of the PM, and the turnover of the mature cells (Nardi and Bee 2012). Thus, few regenerative cells are present in those species that have a PM as a protective barrier of the midgut epithelium, while in species lacking peritrophic membrane, the need to continuously replace midgut cells requires the presence of a high number of regenerative cells. Adult beetles that rarely, if ever, feed, do not have a peritrophic membrane, nor regenerative cells, in their midgut epithelium (Silvia Caccia *et al.*, 2019).

A well-developed basal labyrinth was observed enclosing mitochondria and tracheae in the midgut of adult beetle *Scrabaeus* sacer. The basal portion of the midgut epithelial cells from *Dendroctonus micans*, *D.pseudotsugae, D. terebrans*, and *D. valens* exhibits basal labyrinth (Ange´ lica silva-Olivares *et al.*,2003).

This aspect is common to the other insects and may be attributed to the transport of components (Lehane and Billingsley, 1996). In addition, when the membranes of the basal labyrinth become separated, they appear to function in ion and water transport out of the lumen (Billingsley, 1990). This exposes to the haemolymph a large surface area of membrane containing integral sodium pumps (Macvicker, 1993). Also, an association of numerous mitochondria across the basal labyrinth supports this role (Claudia *et al*., 2001). The presence of rough endoplasmic reticulum in the cells of both regions of the midgut indicates that a large amount of proteins is synthesized in their interior. These could be destined for the membrane,for secretion, or as constituents of certain organelles, including the Golgi complex, lysosomes, and other cytoplasmic vesicles (Geneser 2000). In the present study, the mitochondria were numerous and polymorphic in all parts of the midgut, which is necessary energy for the transport of substances through the membrane (Hecker *et al*.,1971, Houk, 1977). Also, microbes appeared in the midgut of adult *Scrabeus sacer*.

Insects are the most widespread animal group having more diversity and density in their intestinal microbial population. Generally, polyphagous insects show more microbe diversity than monophagous insects (Brune & Friedrich 2000; Suh 2003; Mrazek 2008; Priya 2012; Schauer *et al.,* 2012). Scarab beetles mostly feed on dung, decaying wood, or other plant materials, therefore, need strong intestinal materials to digest the tough diet. The basic layout of the insect gut allows many alterations, reflecting variations according to specialized niches and feeding habits, with a lot of these specializations evolving for the residence of intestine microorganisms in distinct gut compartments (Engel *et al.*, 2014). The surprising fact is that the insect gut contains 10 times more microbial cells than its host cell number (Lederberg 2000; Krishnan *et al.,* 2014). The key role of gut microbes in the gut and how they influenced the insect immune system. In general, scarab beetles spend their whole lives in the soil containing dung and other undigested waste materials. Dung contains pathogenic microbes along with dung material from the gastrointestinal tract of mammals. Many metabolites comprising tiny organic molecules produced by gut microbes may help stimulate the host cell immune system (Nicholson et al., 2012). Apart from the insect's excellent defensive strategies, it is evidently proven by many researchers that the gut microbiome also produces many smart molecules that stimulate host immunity at the time of a pathogenic attack.

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