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Morphological Study of the Digestive Tract of the Mud Crab (*Hemigrapsus penicillatus* De Haan) and the Symbiotic Crab (*Pinnotheres cyclinus* Shen)

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The influence of eating habits and food type on the ultrastructural characteristics of the digestive tracts was studied under the scanning and transmission electron microscopes in two crustacean decapods (*Hemigrapsus penicillatus* De Haan; mud crab, *Pinnotheres cyclinus* Shen; symbiotic crab). The relative ratio of the length of midgut versus hindgut was 1:1 in the mud crab, but 4:1 in the symbiotic crab. Observation through the scanning electron microscope revealed that the midguts of both species have densely-arranged longitudinal mucosal folds with a smooth surface. In the hindgut of the mud crab, mucosal folds were longitudinally oriented, clusters of two to five spines were observed on the cuticular surface, and the length of the spine in the distal hindgut was longer than that in the proximal portion. In the symbiotic crab, the mucosal folds were irregulary arranged, and numerous rudimentary spinal structures were noted on the cuticular surface. Through observation of a transmission electron microscope, the epithelial cells of the midgut in both species had numerous microvilli, but the length of the microvilli was slightly longer in the mud crab than in the symbiotic crab. The central layer of the basement membrane and the muscular layer of the midgut were more developed in the mud crab than in the symbiotic crab. The thickness of the cuticular layer over the hindgut surface in the mud crab was about 4 times than that of the symbiotic crab.

Although crustaceans (arthropoda) possess a wide variety of complex digestive systems, in most cases the gut is composed of an interconnected mouth, foregut, midgut, hindgut which leads to the anus, and appendicular glands (Bliss, 1983). Of the digestive tract, the foregut and hindgut are developed from the ectoderm during embryological development and the cuticular layer is formed on the epithelial surface, while the midgut which arises from the endoderm possesses no such cuticular layer (Erri Babu et al., 1982). The foregut is defined as the portion from the esophagus to the stomach. The stomach, which usually functions to store food material, can be further divided into three components; a region composed of chitinous teeth to crush food particles for mechanical digestion, a portion responsible for chemical digestion, and a filtering segment. Of the digested food that travels through the stomach, liquid matter composed of micromolecules is passed on to the hepatopancreas and the remaining unfiltered particles which possess large macromolecules are passed on to the midgut (Stainer et al., 1968). The midgut which is connected to the stomach, functions along with the anterior midgut caeca to digest and absorb, but depending on the species of decapod, the midgut and its accessory organs differ drastically in structure (Smith, 1978). The portion of the hindgut connected to the midgut forms one posterior midgut caeca and on the epithelial cells lining the inner surface of the intestine are groups of spines oriented towards the anus to prevent food particles from backward motion (Leake, 1975).

Those species belonging to the decapod possess extremely clean and straight digestive tracts which function in absorption and osmotic control, this characteristic also holds true for crustaceans as well (Talbot et al., 1972; Chu, 1987). In terms of structural research on the crustacean midgut, clarification of the midgut at an anatomical level and classification of the cell types comprising the midgut epithelium on the ultrastructural level differ according to the species (Hallberg and Hirche, 1980; Sullivan and Bisalputra, 1980; Miyawaki et al., 1985; Perkins, 1994). The hindgut of arthropods functions not only to transport food particles, but to control osmotic pressure as well by mediating the

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delivery and absorbance of water and ions (Edson and Vinson, 1977; Sullivan and Bisalputra, 1980).

This research focused on the influence of eating habits and food type on the structural characteristics of the digestive system of organisms. Mud crabs inhabit muddy seashores and ingest mud because of the food matter mixed within. Symbiotic crabs carry on a mutually beneficial relationship with the clam by acquiring food particles from the filtration of seawater which flows in through the clam's inhalent siphon. In this study, the difference in the morphological characteristics of the digestive tract between these two species was analyzed.

Materials and methods

Materials

The mud crab (decapod, crustacean, arthropoda) caught under rocks during low tide in the muddy seashores of Jakyak Island, Inchon (Korea) and the symbiotic crab (decapod, crustacean, arthropoda) found in the mantle cavity of the clam were collected in Gimjae, Jeonbuk (Korea). These specimens were collected from May to August. No discrimination of sex was made; ten symbiotic and 15 mud crabs were used.

Methods

The mud crab and symbiotic crab were dissected using a dissection microscope. After confirmation of the digestive tract, it was transfered into 3.4% NaCl solution, and excised from the organism. In preparation for scanning electron microscopic observation, the extracted digestive tract was cut longitudinally and stretched out length-wise on a cork plate, affixed with bamboo pins. The inner surface of the intestine was cleaned with 3.4% NaCl solution. The tissue was immediately prefixed for 3 hours with a 2.5% glutaraldehyde-2% paraformaldehyde mixture in a phosphate buffer (pH 7.2). After being postfixed with 2% osmium tetroxide (in 0.1 M phosphate buffer, pH 7.2) for one hour, the tissue was dehydrated with alcohol. After dehydration, the tissue was then dried in a critical point dryer (Polaron 300) which required liquid CO_2. Finally an Ion Sputter (JFC-1100) was used to cover the intestinal surface with gold and observation was carried out with a scanning electron microscope (JSM-35C).

For transmission electron microscopic observation, the midgut, hindgut and rectum portions were divided under a dissection microscope. Fixation and dehydration were performed in a similar manner as described above. The dehydrated tissue was embedded in an Epon 812 mixture and polymerized in a Polymerizer (Reichert-Jung) for 72 h at 60℃. The polymerized tissue blocks were sectioned to 1 μm thickness and stained with 1% toluidine blue. After confirmation of the region we intended to observe, the ultrathin specimens were prepared using a LKB-ultratome and stained with uranyl acetate and lead citrate. Lastly, transmission electron microscope a (JEM-1200EX) was used for observation.

Results

Scanning electron microscopic observation

The comparative lengths of the midgut and hindgut of the mud crab and the symbiotic crab were very different. In the case of the mud crab, the lengths were nearly equal, whereas the ratio in the symbiotic crab was 4:1 in favor of the midgut (not shown). The midgut had longitudinal and smooth surfaced mucosal folds in both species, with the mud crab exhibiting more densely packed folds (Fig. 1A and B). The mucosal folds of the mud crab hindgut were aligned in a dense longitudinal pattern. From the luminal surface of the hindgut connected to the midgut, groups of 2-5 spines were observed (Fig. 1C). Closer to the rectum, the number of spines comprising these groups decreased to 1-2, but the length of each spine increased to a size approximately 8 times longer than those of spines at the front of the hindgut (Fig. 1E). The ends of the spines were split into many ends (Fig. 1E inset). The width of the longitudinal mucosal folds of the hindgut were wider in the closer proximity to the lower portion of the intestine and exhibited a more irregular pattern. Folds could not be observed in the rectum (Fig. 1G). There were less spines in the rectum compared to the hindgut (Fig. 1G).

In the symbiotic crab, the luminal surface of the hindgut had irregular folds and the surface, in contrast to the mud crab which had extremely short vestigial traces of spines, gathered in groups of 30-40. There was no noticeable change in the number and structure of the spines in the entire length of the hindgut (Fig. 1D and F). In the rectum, longitudinal mucosal folds, along with irregular latitudinal folds and any traces of spines were hardly observed (Fig. 1H).

Transmission electron microscopic observation

The epithelial cells of the midgut in the mud crab were columnar in shape and the nucleus was either spherical- or oval-shaped. Also, mitochondria, and lysosomes containing small vesicles and various concentric structures were observed to spread out throughout the cytoplasm (Fig. 2A). The epithelial cells of the midgut possessed many microvilli and the lateral membranes of adjacent epithelial cells were comparatively smooth (Fig. 2A). The basement membrane lay in between the midgut epithelial cells and the striated muscle layer of the midgut. This basement membrane took up a large region and was further composed of a central layer, exhibiting a severe wave-like structure and outer layers on flanking each side. Especially the central layer exhibited various electron densities (Fig. 2C). The epithelial cells of the mud crab hindgut were arranged in

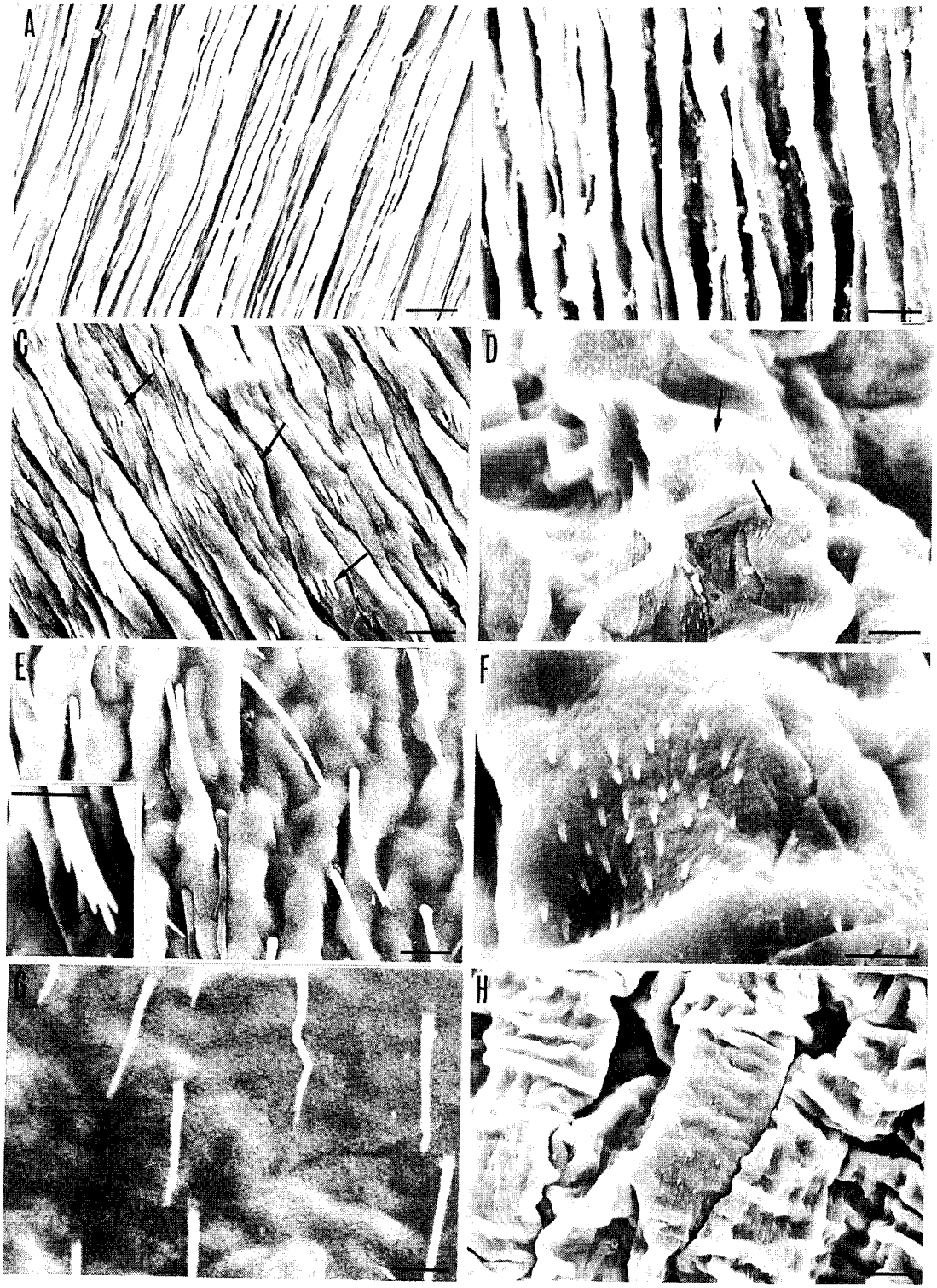

Fig. 1. Scanning electron micrographs of the intestines of the mud crab (*Hemigrapsus penicillatus* De Haan) (A, C, E, G) and symbiotic crab (*Pinnotheres cyclinus* Shen) (B, D, F, H). Midgut portions have numerous longitudinal foldings of the mucosa (A, B). Clusters of prominent spines (arrows) are observed on the longitudinal foldings of the mud crab hindgut (C). Tiny spines (arrows) are noted on the irregular foldings of the symbiotic crab hindgut (D). A higher magnification of Fig. D (F). Numerous slender spines which split into several branches (arrowheads) are noted on the distal portion of the mud crab hindgut (E, inset). Spines are also distributed on the rectal surface of the mud crab (G). The irregular longitudinal foldings are shown at the rectum of the symbiotic crab(H). Scale bars=1 μm (inset of E, F) and 2 μm (A, B, C, D, E, G, H).

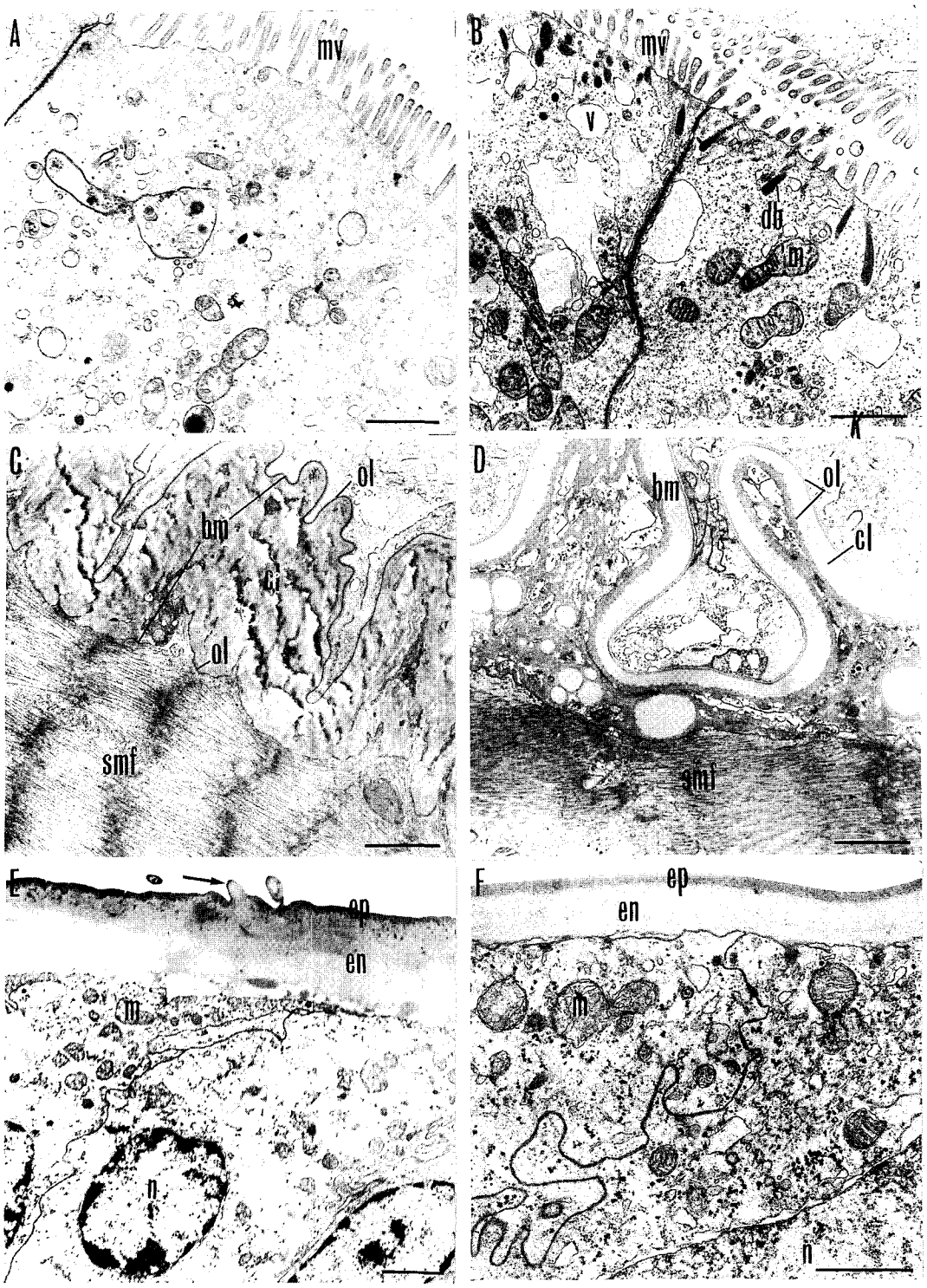

Fig. 2. Transmission electron micrographs of the mud crab (A, C, E) and the symbiotic crab (B, D, F) intestine. Numerous microvilli (mv) on the surface membrane are noted in the epithelial cells of the mud crab midgut (A). In the midgut of the symbiotic crab, epithelial cells with numerous microvilli (mv) on the luminal surface are columnar in shape and contain well-developed organelles such as mitochondria (m), vesicles (v), and dense bodies (db) (B). Basement membrane (bm) and striated muscle fiber (smf) beneath the mud crab midgut epithelium show peculiar structure composed of thick central (cl) and thin outer layers (ol) (C). The prominent basement membrane (bm) consists of the electron-luscent central (cl) and the electron-dense outer layers (ol) in the symbiotic crab midgut (D). In the both species, cuticular layers of the hindgut epithelial cells consist of epicuticle (ep) and endocuticle (en) well developed (E, F). Especially the subcuticular space and spines (arrow) are noted in the mud crab hindgut (E). n, nucleus. Scale bars=1 μm (F) and 2 μm (A-E).

a columnar pattern and the nucleus was either spherical or oval in shape. The heterochromatin were usually distributed in the periphery of the nucleus. The lateral membranes of adjacent cells showed interdigitation (Fig. 2E). The luminal surface of the hindgut epithelium were covered by two cuticular layers, an epicuticle and an endocuticle. Protruding spines could also be observed (Fig. 2E). Also, the cuticular layer of the mud crab hindgut was 6 times thicker than that of the symbiotic crab and the subcuticular space was wide (Fig. 2E and F). In the apical portions of the epithelium, there were many cytoplasmic protrusions of the cell membrane (Fig. 2E).

The epithelial cells of the symbiotic crab midgut were columnar and many microvilli were observed over the luminal surface (Fig. 2B). And a spherical- or oval-shaped nucleus was positioned in the center of the cell. There were many mitochondria at the supranuclear cytoplasm, along with large vesicles and ribosomes (Fig. 2B). The lateral membrane of the midgut epithelial cells had hardly any wave-like patterns and desmosomes and gap junctions were also observed. The basement membrane of the symbiotic crab midgut was comprised of 3 layers, including a central layer with an evenly distributed medium-level electron density, and 2 outer layers on each side which contributed to a wave-like structure (Fig. 2D). In between the basement membrane and the striated muscle layer was a connective tissue layer. Two cuticular layers covered the luminar surface of the symbiotic crab hindgut epithelial cells. A subcuticular space was nearly absent and the cell membrane of the cuticular layer was slippery. Mitochondria were present in the cytoplasm above the nucleus in the hindgut epithelial cells, glycogen particles were evenly distributed throughout the entire cytoplasm and the lateral membrane between adjacent cells formed a severe wave-like structure (Fig. 2F).

Discussion

Organisms belonging to the decapod (crustacean, arthropoda) are filter feeders and possess a special apparatus in the foregut, which allows filtration of food, midgut and hindgut. In addition, the secretion of digestive enzymes, digestion and absorption are controlled by digestive organs such as the hepatopancreas and midgut caeca. Crustaceans possessing this basic digestive structure have additional structural differences in the living environment, food type and eating habits according to the species. In the case of crustaceans, research on the differences in midgut structure showed that the front portions of the midgut of species with carnivorous eating habits, hyperiid, are enlarged into a digestive chamber (Sheader and Evans, 1975). The midgut in Harpacticoid of copepod is separated into two regions by a sphinctor (Sullivan and Bisalputra, 1980); the midgut of Daphnia and Calanoids is divided into three sections (Hallberg and Hirche, 1980). The midgut of decapod and isopod were extremely short or nonexistent (Pike, 1947; Moon, 1988) while that of Homarus is known to be quite long (Baker and Gibson, 1977).

In this research, the midguts of both the mud crab and symbiotic crab were long, straight tubular structures, with the midgut of the symbiotic crab being longer. This contradicts previous findings (Pike, 1947; Moon, 1988) that decapod possess either no or an extremely short midgut and shows that depending on the species and eating habits, the midgut structure varies drastically.

In the arthropoda, the primary function of the midgut is in digestion and absorption and thus according, the midgut secretes digestive juices and absorbs the digested nutrients before transporting them to hemolymphs. In order to mediate and fascillitate this process, the midgut possesses many folds and microvilli cover the luminar epithelial surface (Quaglia et al., 1976; Arnaud et al., 1980). These densely packed microvilli covering the luminar surface and the mucosal folds lining the length of the midgut in the mud crab and symbiotic crab aid in digestion and absoprtion.

Factor (1981) and Miyawaki et al. (1985) researched the basement membrane, which is the special structure of the various crustacean midgut (*Menippe merceuraria, Homarus americanus, Caridina denticulata*). The basement membrane consisted of 3 layers, including the central layer and two outer layers on each side. Depending on the species, the material making up the central layer was either fine granules or loose reticular formations of a fibrous material. In terms of this characteristic of the midgut basement membrane structure, it had also been reported that insects also possess this trait (Hess and Pinnock, 1975; Bayon and Francois, 1976). Factor (1981) found that the midgut in the decapod differs from the foregut and hindgut in embryological development and structure. The foregut and hindgut possess a cuticular layer which provides strength to the structure of the tissue lying beneath and during eating, provides for the increase of intestinal wall elasticity. The midgut possesses no such cuticular layer but has a specialized basal membrane which functions in a similar manner as the cuticular layer by providing strength and elasticity during food digestion. Also, Nylund et al. (1992) revealed upon study of *Lepeophtherirus salmonis* of copepod, that contractions of the midgut wall muscles function not only to mix the contents of the intestine but also to secrete digestive enzymes and absorb nutrients along the digestive tract. The analysis of the mud crab and symbiotic crab revealed a basement membrane structure in the midgut which was similar to the observations of Factor (1981). However, compared to the symbiotic crab, the basal membrane and muscular layer of the mud crab were much thicker. This led us to believe that the mud crab's diet which consists of hard food matter mixed with the mud allows the midgut to have more mechanical strength.

The digestive duct of the symbiotic crab, which digests matter caught in the filtration apparatus of the clam might cause the strength and structure of the midgut to be comparatively weaker. Also, the hindgut of the mud crab has many spines and in the case of the symbiotic crab, these spines are non-functional traces of what once may have been working structures. Our findings suggested that this difference could be related to the state and condition of the fecal matter of these two species. Mud crab's fecal matter is harder and larger in volume because the spines oriented toward the anus compress the fecal matter and prevent the matter from backward flow. Symbiotic crab's excrement consists of many components and is semi-motile, suggesting that excretion is accomplished through muscle contraction rather than through a system designed to prevent backward motion. This fact is consistent with previous reports (Schmitz and Schultz, 1969; Moon, 1988; Yeun, 1988) that the main function of the hindgut is to concentrate and excrete fecal material and the spines on the epithelial surface of the hindgut inhibit backward flow.

The majority of the foreguts and hindguts in crustacean, especially in terms of developmental biology, is derived from the ectoderm and consists of a cuticular layer on the luminal surface while the midgut is developed from the endoderm and lacks this layer (Chapman, 1969; Bliss, 1983). The results of our observations show that the midguts in the mud crab and symbiotic crab lack a cuticular layer. While the hindgut epithelial surface has a cuticular layer, it was much thicker in the mud crab. This complements the afore mentioned fact that, depending on the eating habits, the epithelial surface of the hindgut in the mud crab comes in contact with harder excretory matter and this cuticular layer functions to shield the intestinal wall from tissue damage during excrement formation.

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