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The Importance of Cuticle and Chitin Formation in Some Parts of *Octopus vulgaris*

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ABSTRACT

Both chitin and cuticles are insoluble substances essential for providing structural support to organs and skeletal systems, particularly in loose tissue. In cephalopods, they are primarily responsible for forming beaks and covering the entire stomach in adult animals. The beaks of cephalopods are critical organs for cutting food into small pieces during feeding. The upper beaks have a distinct characteristic: their inner surface is hard and contains growth lines, which can be used to estimate the age of the animals and determine seasonality, with broader and harder lines forming during the summer. The beaks, composed of chitinous structures, are affected by heat during the summer, as are all chitinous structures. Both the upper and lower beaks have a sugar structure mixed with chitin during the animals' growth, giving them a shiny appearance under an electron microscope. Age determination by growth lines is an accurate method, especially in cultured animals, as the growth lines precisely indicate age and seasonality throughout the life cycle. The stomach of *Octopus vulgaris* has a thick and robust wall, and in adult animals, a cuticle layer (first recorded in this study) was observed through anatomical examination. This cuticle inside the stomach is clear and elastic, serving to protect the stomach during the consumption of hard prey. The second stomach contains goblet cells that secrete carbohydrates and mix saliva with stomach secretions to aid in digestion. These goblet cells have a unique, elongated shape and are fewer in number compared to those in the esophagus of the same specimens. Finally, the beaks are the primary organs for cutting food during feeding. The buccal mass, which houses the beaks, can be easily removed by hand during dissection due to the presence of large invaginations created by pressure when the octopus captures hard prey.

INTRODUCTION

Chitin is an insoluble substance that forms the upper and lower beaks, originating from specialized cells genetically. It first appears in the phylum Coelenterata, specifically in corals, as a structure composed of a collection of cells. As animals evolved, the presence of chitin decreased in higher animals within the animal kingdom **(Tracey, 1955; Roberts *et al*., 1992; Lefkaditou & Bekas, 2004; Muzzarelli, 2013; Abd Elrheem, 2023; Nurutdinova *et al.*, 2024)**. The digestive system of cephalopods has evolved to contain two stomachs, although one has disappeared in higher animals, replaced by an accessory gland through the course of evolution. In cephalopods, the digestive system includes a true stomach (first stomach) where food is ground and true digestion occurs, and a second stomach, which secretes various digestive enzymes and melanin **(Abd Elrheem, 2022, 2024)**.

The nomenclature of beaks is often inaccurate due to their chitinous structure, as they are primarily protein-based. The term was established by Clarke in 1962, referring to animals that possess true upper and lower jaws **(Clarke, 1962; Lefkaditou & Bekas, 2004; Duarte *et al.*, 2023; Roscian *et al.*, 2023; Staaf, 2023)**. The buccal mass of *Octopus vulgaris* serves as the primary organ in the feeding process. Beaks vary significantly between species, undergoing large modifications in shape. These differences are used in taxonomy and age determination, making beaks crucial indicators for age estimation **(Hernández-López *et al.*, 2001; Lefkaditou & Bekas, 2004)**.

The growth of an animal is reflected in the growth of its upper and lower beaks, with new growth lines being added as the beaks develop. Beaks grow and become more robust with age, with younger animals having clearer beaks, while adult beaks are darker in color **(Phuynoi *et al.*, 2021; Lopes & Junior, 2022; Souquet *et al.*, 2023)**. The pigmentation and coloration of both beaks and the body, as well as the production of ink, increase with age. The upper beaks of octopuses take on the melanin color of the organism, darkening as the animal ages.

Within the buccal mass, several important structures are present, such as the mandibular gland, which secretes carbohydrates, and the salivary papilla, which injects venom into large prey during feeding. Additionally, there are pairs of upper and lower jaws enclosed by rounded lips **(Uyeno & Kier, 2005; Ramachandiran *et al.*, 2020; Abd Elrheem, 2022)**. The mandibular muscles support the beaks in opening and catching hard prey during feeding, as well as assisting in the function of the buccal mass region **(Roscian *et al.*, 2023; Souquet *et al.*, 2023; Wang & Fang, 2023)**. The upper beaks, which are smaller than the lower ones, are connected by muscles of articulation inside the buccal mass, which also contains a movable radula that helps cut the prey. Beccublasts, layers added by the animals as they grow, can be analyzed to estimate the accurate age of the organism **(Hernández-López *et al.*, 2001; Souquet *et al.*, 2023)**.

The buccal mass is composed of rounded organs containing chitinous beaks and is attached to the anterior salivary gland, which lies at the base of the buccal mass. This gland has a canal that connects to the buccal cavity (upper and lower beaks) and is directly connected to the salivary papilla **(Abd Elrheem, 2022; Verhoeff, 2023)**. The first stomach in cephalopods, known as the true stomach, has a thick muscular wall to grind food and contains goblet cells that aid in digestion **(Abd Elrheem, 2022; Xuemei *et al.*, 2023)**. In adult species, a clear cuticle protects the stomach from hard shells, while the stomach's wide wall is lined with cuticle layers attached to the inner side above the second anatomical layer. The true stomach secretes multiple enzymes for digestion, and the emulsified food then passes to the ileum, which is connected to the stomach.

The development and growth of the buccal mass and beaks continue throughout the organism's life. This occurs through the addition of new muscle layers to damaged ones, resulting in solidified muscles. The lower and upper jaws grow by adding new layers, leading to a larger buccal mass more suitable for the body size of the animal. The lower jaw's wings are embedded inside the resting buccal mass, positioned for optimal function and strength. The upper jaw's lateral wall is wide, and its circular shape provides strength for its primary function.

The chemical composition of beak pigmentation may be a useful method for estimating the age of animals in the family Octopodidae, as pigmentation in the beaks increases with age. The buccal mass itself varies between genera, being more massive in species like *Nautilus* where the mouth opening is smaller compared to octopuses, which develop a larger buccal mass as they grow **(Nixon & Mangold, 1996; Messenger & Young, 1999; Uyeno & Kier, 2005)**.

The study aimed to shed light on the importance of cuticles in the stomach, providing the first evidence of their significance in octopuses. Additionally, it seeks to measure the amount of pigmentation in beak cuticles as an accurate method for age estimation.

MATERIALS AND METHODS

1. Sample collection

Six samples of *Octopus vulgaris* were collected from one site on the western coast of the Red Sea in January during the winter season of 2022. This site is located 17km South of Safaga City (latitude 26° 38' N longitude 33° 59' E). The collection site is on rocky shores, and samples were collected from the intertidal zone at the time of low tide. The collection was conducted by using the hand and then packing it up. One specimen was collected and put in plastic containers containing Seawater. Specimens were narcotic by adding menthol crystal (El-Naser Chemicals Company in Egypt) to the water surface of the jar and waiting until relaxation. Specimens dissected in the field to get the studied organs were fixed and put in Bouin's solution for 24 hours for histological preparations.

2. Histological studies

Scanning electron microscope

For scanning electron microscope (SEM) studies, the skin of *Octopus vulgaris* was fixed in a mixture of three volumes of 4% glutaraldehyde and one volume of 1% osmium tetroxide. They were dehydrated in a graded series of alcohol, critical point dried, gold-coated, and monitored under a JEOL 5300 scanning electron microscope at an operating voltage ranging from 10- 30v.

Histopathological study

Organs sectioned were cut off from the body and placed into Bouin’s solution in seawater for 24 hours. Fixed parts were then passed to the graded series of alcohol from 100%. They were cleared in toluene three times each for 5 minutes then embedded in paraffin wax. Sectioning was made by microtome at 5-7µm thickness. Sections were stained with the following stains:

- Harris hematoxylin and eosin combination **(H&E) (Harris, 1900)**.

The slides were dehydrated through an ascending series of ethanol after staining. They were then passed through xylene to a mounting medium and covered with coverslips.

RESULTS

The buccal mass in cephalopods is a rounded structure that contains several important organs, including the complete mouth, which consists of true chitinous jaws (both lower and upper) and circular lips that close or open the mouth during feeding. The term "true mouth" is the correct nomenclature, and its main structure is composed of chitin, which can be easily removed from the animal upon observation. In contrast, the beaks have a more robust and stable structure, similar to those of birds (Aves), and are not easily removable (Fig. 1A, B).

The mouth is a chitinous structure consisting of key components: the lower and upper jaws, each shaped for specific functions. The upper jaw is rounded and rests in a stable position during feeding, becoming more fixed and stable over time (Fig. 1B). The most important part of the upper jaw is the lateral wall, which is folded inside the buccal mass and supported by the mandibular muscle located within the buccal mass. The second key structure is the rostrum tip, which the animal uses to penetrate hard shells; it is thickened and has a small surface area at the tip. The third structure is the hood, a thick section in both the upper and lower jaws that provides additional strength and support (Fig. 2A, B).

The upper beak's structure is composed of chitin and mucopolysaccharides, giving it a shiny, rough surface that aids in cutting prey, as seen under electron micrographs (Fig. 2A, B). The upper beak or jaw also contains microstructures called age growth lines, which can be used to accurately estimate age by analyzing these layers, which are added as the animal ages (indicated by red arrows). The growth lines, which are microstructure layers (M.L.), reflect seasonal changes rather than daily ones, with more prominent structures forming during the winter when chitin thickens and growth increases during the summer (Fig. 3C, D). These microstructures help estimate seasonal age, not daily, as daily growth lines would be more numerous and narrower, as observed under a scanning micrograph. Chemical analysis of beak pigments may provide a more accurate age estimation than growth lines, which form continuously as the animal ages.

The lower jaw, or beak, is also rounded but has long lateral walls and wings embedded beneath the upper beak, providing additional force during the cutting process. This is further supported by the jaw angle (J.A.), which ensures an optimal closure during prey bites. The wing fold is a critical structure, free from the upper beak, and serves as a point of stability and pressure during cutting, becoming as massive as the hood and integrating into its structure (Fig. 4A, B, C).

The first true stomach is where the buccal mass jaws grind the cut meal; in cephalopods, the stomach generally has two cuticle layers. The first layer, visible to the naked eye (S.C.L), appears in adult animals and serves a protective function against foreign or undigested materials, which may decompose in this layer (Fig. 5A, B). Under light examination with H&E staining, the wide wall appears as a thick layer of connective tissue, followed by the lamina epithelialis, which contains goblet cells (G.C.) among secretory cells that secrete mucus to facilitate the passage of the partially digested meal to the second stomach (digestive gland appendix). This second stomach contains secretory cells that produce enzymes, aiding in digestion alongside the true stomach. The second microanatomical layer, visible under a light microscope (C), surrounds the stomach wall for protection, taking up a large area (Fig. 5A, B).

The lamina propria (L.P.) forms wide layers in all specimens and is present in all contractile organs, aiding in pushing the partially digested meal. The lamina epithelialis (L.E.) is a single, continuous layer lining the secretory organs throughout the animal kingdom (Fig. 6A, B).

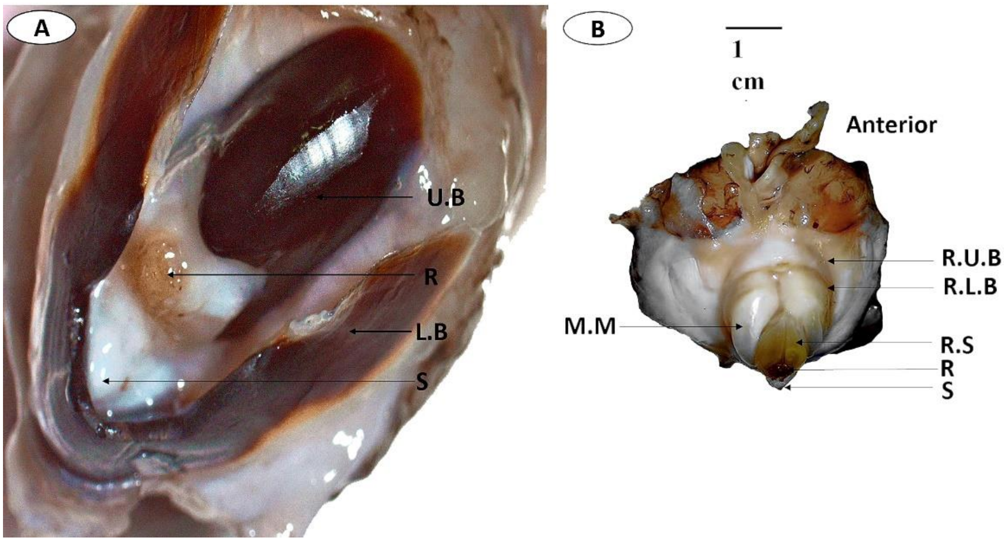

Fig. 1. The completely buccal mass of *Octopus vulgaris* (Vertical view) showing: **(A)** U.B (Upper beak), R (Radula in normal size), L.B (Lower beak), and S (Salivary papilla); **(B)**: R.U.B (Resting of the upper beak), R.L.B (Resting of the lower beak), and M.M (Mandibular)

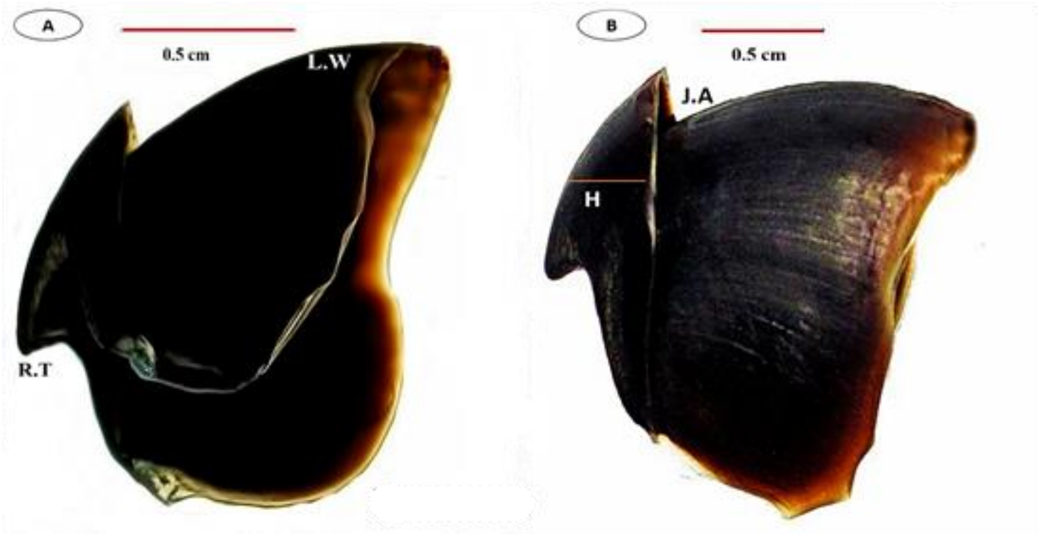

Fig. 2. Upper beaks of *Octopus vulgaris* showing: **(A)** R.T (Rostrum tip), L.W (Lateral wall) and **(B)** H (Hood region), J.A (Jaw angle)

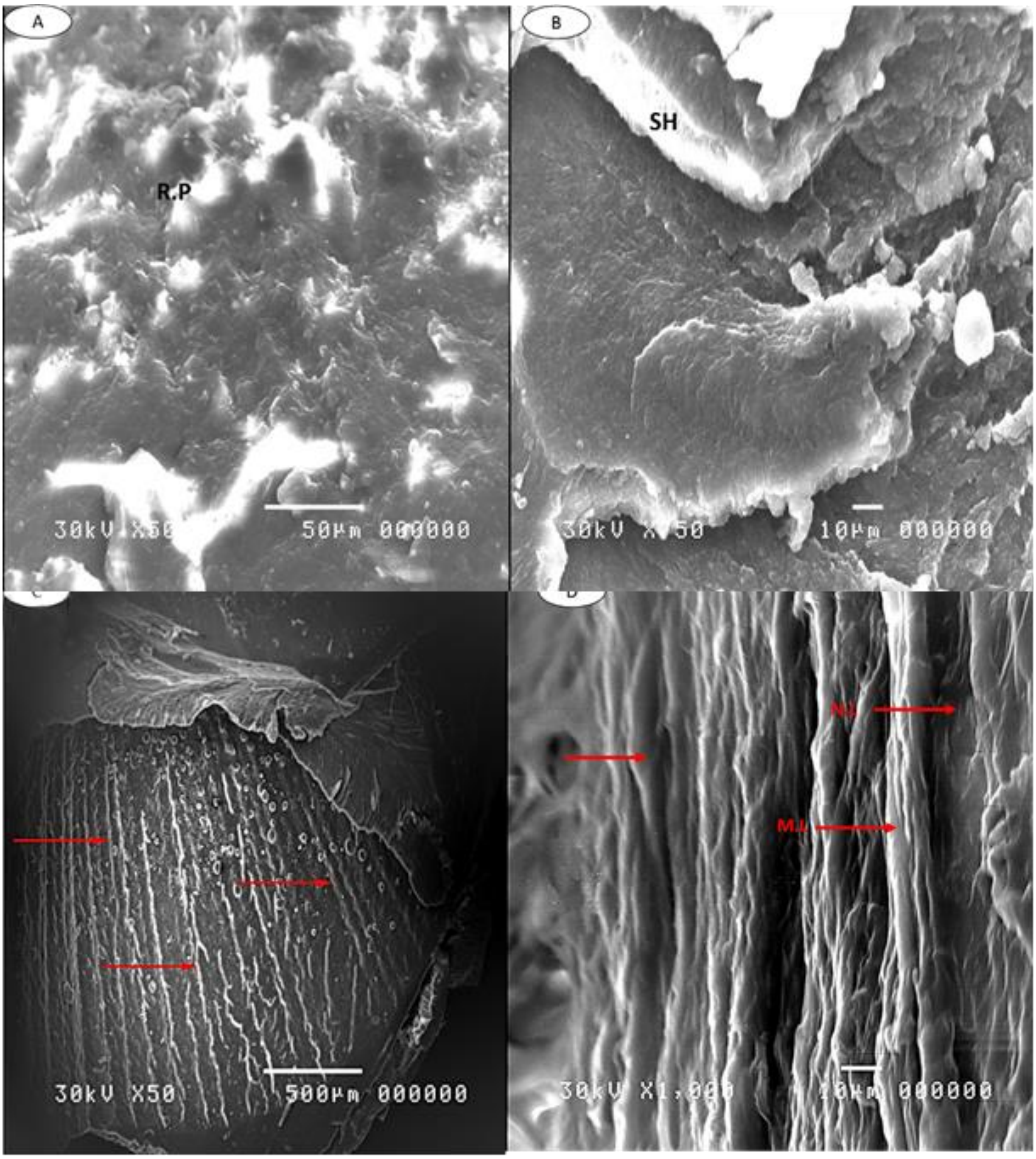

Fig. 3. Scanning electron micrographs for the upper beaks of *Octopus vulgaris* showing: **(A)** R.P (Rough points), **(B)** SH (shine micros microstructures of mucopolysaccharides) **(C)** growth lines (Red arrows), and **(D)** M.L (micro-layers)

Fig. 4. The lower beak of *Octopus vulgaris* showing: **(A)** L.W (Lateral wall); **(B)** W.F (wing fold), H (Hood region), and J.A (Jaw angle)

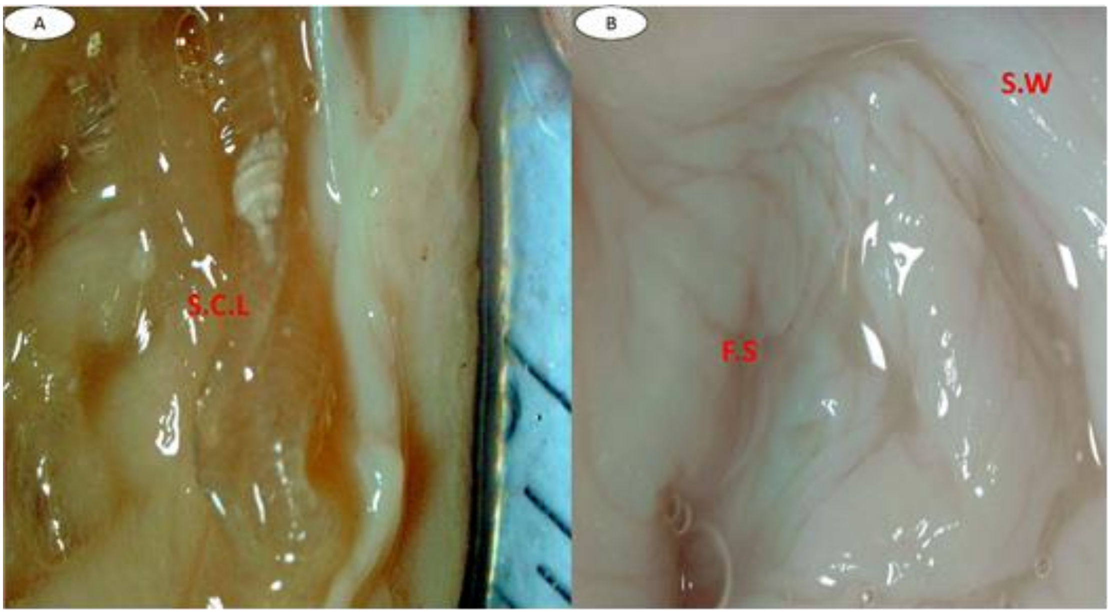

Fig. 5. Vertical view by binocular microscope for the whole stomach of *Octopus vulgaris* showing: **(A, B**) S.C.L (stomach cuticle layer) inside the lumen of the stomach and **(B)** F.S (free stomach) and S.W (stomach wall)

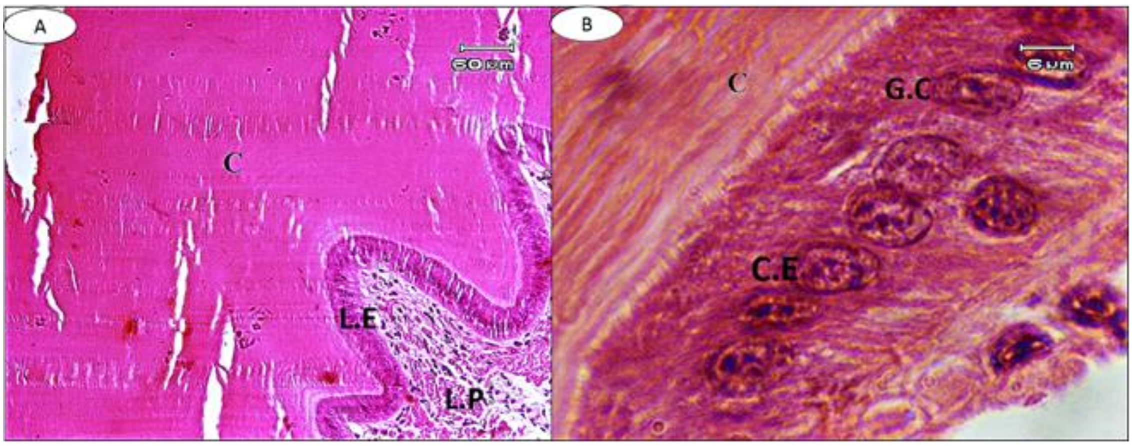

Fig. 6. The transverse section of the stomach showing: **(A)** L.P (lamina propria), L.E (lamina epithelia's), C (cuticle), and **(B)** C.E (columnar epithelial cell), G.C (Goblet cells)

DISCUSSION

The buccal mass in cephalopods is a very important structure containing some important organs which are varied in shape and functions to serve in feeding and survival animal. The buccal mass is a rounded structure and is easily removed or separated from an animal's body by observation during the anatomy of specimen. The beaks are important organs in feeding, classification, and estimation of age in cephalopods **(Clarke, 1962; Hernández-López *et al*., 2001; Lefkaditou & Bekas, 2004; Abd Elrheem, 2023; Duarte *et al*., 2023; Faiki, 2023; Wang & Fang, 2023)**.

The beaks are rounded organs consisting of upper and lower jaws, which play a crucial role in the initial step of digestion by cutting prey. Growth lines, which are microstructures within the beaks, are added periodically as the beaks grow **(Hernández-López *et al*., 2001; Faiki *et al*., 2023; Guerra-Marrero *et al*., 2023)**. The salivary papilla inside the buccal mass is used to paralyze the prey. Once the prey is paralyzed, the upper and lower jaws begin cutting it into pieces to facilitate the digestion process **(Fingerhut *et al*., 2018; Ramachandiran *et al*., 2020; Dill-Okubo *et al*., 2021; Abd Elrheem, 2022, 2023)**.

The stomach is the first site of true digestion and has a massive wall encompassed by two thick layers of cuticle, both internal and external to the lumen **(Lefkaditou & Bekas, 2004; Bastos, 2020; Andrews, 2022; Liu *et al*., 2022; Xuemei *et al.*, 2023; Perales-García *et al.*, 2024)**. The stomach contains goblet cells in the lamina epithelialis, which secrete mucus to facilitate the passage of food during the feeding process **(Lefkaditou & Bekas, 2004; Fernández-Álvarez *et al.*, 2020; Dill-Okubo *et al.*, 2021; Andrews *et al.*, 2022; Faiki *et al.*, 2023; Liu *et al.*, 2023; Murtia *et al.*, 2023; Nurutdinova *et al.*, 2024)**. The cephalopod digestive system is generally described simply during the feeding process, with specialization in humans involving the separation of all mixing organs into a digestive gland, which becomes the liver and pancreas and a true stomach through the evolutionary process. The beaks are significant organs, though they lose their hardness and stable color and are easily removed through decomposition. Therefore, the analysis of stomach contents in the same predatory animals often lacks beaks, indicating they are easily removed by simple contact and play a role in feeding rather than protection.

CONCLUSION

The study of the digestive system of the cephalopods by accurate methods depends on the true reading of each organ's function during the life cycle of animals. The study of the digestive system by morphology, histology, and function gives a large and easy understanding of the animal, feeding manner, and lifestyle.

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