

The Morphological Features of Sucker Ring Teeth of Cuttlefish *Sepia Pharaonis* from the Arabian Gulf

Marwa Elnagar*

Suez Canal University, Egypt

***Corresponding Author**

Marwa Elnagar, Suez Canal University, Egypt.

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Abstract

Amongst decapodiform cephalopods, Cuttlefishes have 'teeth' in the sucker rings of their arms and tentacles. They use their multiple arms for complex behaviors, including prey capture, grasping and transferring a batch of spermatophores to females. Understanding the unique morphologies of the sucker rings of these arms is critical for understanding the complex behaviors and reproductive strategies of cephalopods. Owing to the lack of previous reports a study was designed to explore the shape of the sucker teeth on the tentacle club, the regular arm and hectocotylus suckers of *Sepia pharaonis* Ehrenberg, 1831 from the Arabian Gulf area using scanning electron microscopy (SEM). The present study reports the morphological features of the sucker ring teeth; Investigating, counting and describing briefly the teeth of the sucker ring using scanning electron microscopy (SEM). The present study reported the piston-like muscle bed in the cavity of the tentacle sucker accompanied by the toothless chitinous ring for the first time assuming their mechanism of action and supporting the previous studies about cuttlefish hunting strategies. Our results not only linking the morphology and function of the sucker ring but also can be used in identification and discrimination between Decapodiforms species.

1. Introduction

Cephalopods have acquired numerous innovations and expanded their habitats to various marine environments as highly agile predators [1]. Multiple arms with suckers are regarded as one such innovative characteristics in cephalopods. For these arm functions, the presence of "suckers" plays key roles, so that suckers are thought to have contributed to the adaptive radiation of cephalopods as highly agile predators [2,3]. The sucker ring teeth are hard structures, and their shape is related to their function; for example, the sucker ring teeth on the arm are long and sharp, aiding in prey capture [4]. Most male cephalopods have a modified arm called the hectocotylus, which is used for transferring a batch of spermatophores to females [5,6]. The hectocotylized part has been described as having 'smaller' or 'reduced' suckers compared to the other arms [7]. Tentacles are morphologically adapted to capturing preys [8,9]. Tentacle suckers are completely concentrated in the distal part of the tentacle (tentacular club) and the number of longitudinal sucker rows is more than doubled [10]. The morphology of an organism reflects its own function for specific actions [11]. The shape of the sucker ring teeth is important for understanding its function. The existing morphological studies on suckers are limited to taxonomic descriptions and focused on the number and

alignment of the suckers [12]. This article is the first to explore the shape of the sucker teeth on the tentacle club, the regular arm and hectocotylus suckers of *Sepia pharaonis* Ehrenberg, 1831 from the Arabian Gulf area using scanning electron microscopy (SEM), linking the morphology to the function of the sucker ring and giving an identification method for *Sepia pharaonis* in Arabian Gulf area. It focused on the differences of suckers between the arms and tentacles. Actually, the mechanism of attachment as explained by the morphology of the sucker because it reflects their behavior, reproductive and hunting strategies.

2. Materials and Methods**2.1 Area of Investigation**

Kuwait's marine area occupies the western edge of the Mesopotamian shallow shelf of the northern part of the Arabian Gulf. The area of investigation lies between 28° 30' - 30° North and 47° 45' - 48° 45' east (Fig.1). The main target for this study was the reef areas where cuttlefish and squids live. Samples were also collected from the by-catch with fish and shrimp trawlers working in Kuwait bay. In addition, samples were also collected from Kuwait fish markets where cephalopods come as by-catch with fish and shrimp.

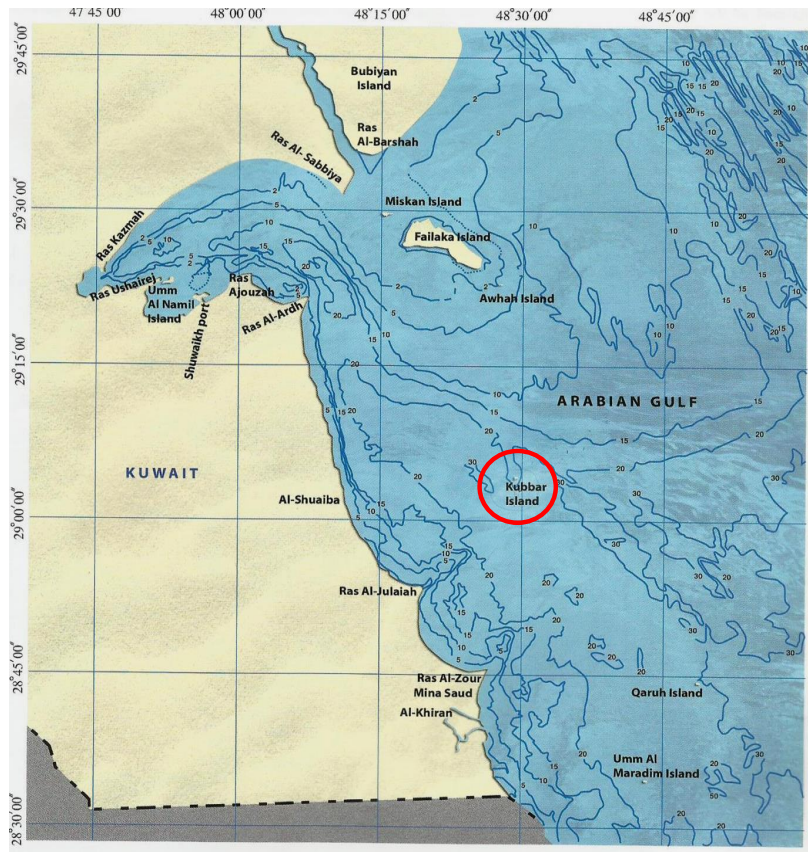


Figure 1: Map of the Kuwaiti Waters of the Arabian Gulf, Showing the Major Sampling Area around 261 Kubbar Island. (From Oceanographic atlas of Kuwait waters, KISR 2009)

2.2 Morphological Studies

Collected specimens were morphologically examined and photographed by using a camera Lucida. Sucker rings were removed from their cups then examined under a dissecting microscope and photographed. The chitinous rings of suckers from the arms and tentacular clubs were photographed using Scanning Electron Microscope. All morphological characters were examined in Kuwait Institution of Scientific Research labs, while Electron Microscope preparations were carried out in Kuwait university Electron Microscope unit.

2.3 Preparation of Specimens for the Electron Microscopy

Sample preparation involves processing, embedding and polymerization following the method of Harris (1996). Samples were fixed in 3% Glutaldehyde, washed with Cacodylate

Buffer 0.1 mol/L, treated with osmium tetra oxide, to increase contrast and stability of fine structures, after that they were washed, dehydrated in an ascending series of alcohol, infiltrated with Epoxy resin, embedded and coated with gold then viewed under the Transmitting Electron Microscope and photographed.

2.4 Ethical Statement

No experiments were carried out during the present research.

3. Results

Arm suckers of *S. pharaonis* are tetra serial (Fig. 2). They are sessile while tentacle club suckers are stalked (Fig. 3). The sucker rings of the regular arm are arranged along the arm oral surface. The chitinous rings of the arm suckers are examined using the Scanning Electron.

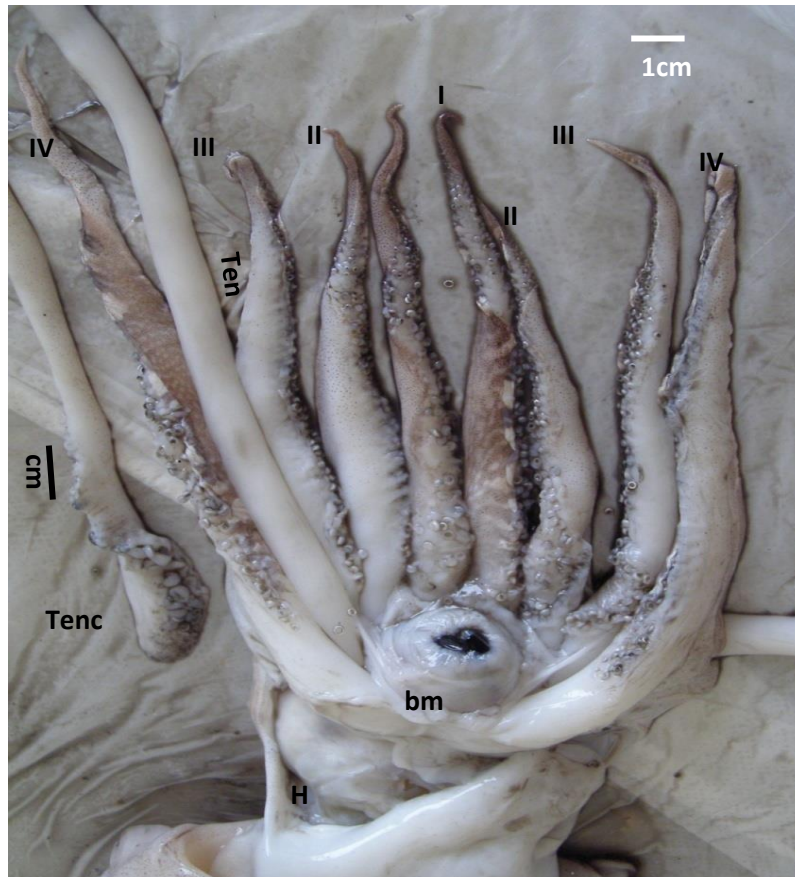


Figure 2: The Head of *Sepia Pharaonis* from the Ventral View x 1
 I (Arm No 1), II (Arm No 2), III (Arm No 3), IV (Arm No 4), bm (Buccal mass), H (head); Ten (Tentacle), Tenc (tentacle club)

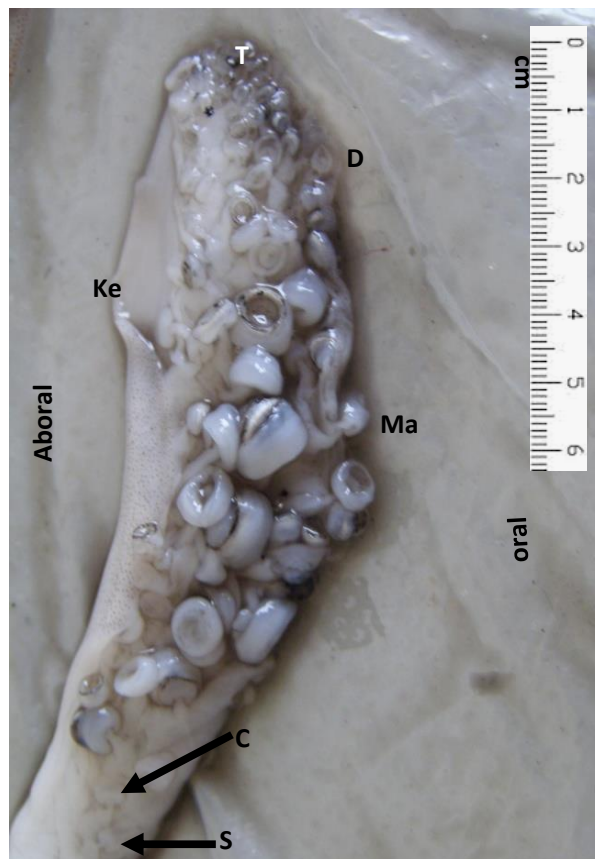


Figure 3: The Tentacle Club of *S. Pharaonis* x 5
 (Carpal locking apparatus), D (Dactylus), Ke (Dorsal Ma (Manus), S (stalk), T (Terminal pad)

Microscope (Fig. 4). The suckers are sessile (Fig. 4a), with strong muscular cups. The sucker rings are embedded into the muscular cup and covered externally with fibrous sheath (Fig. 4b) the covering sheath is lined with soft papillae. (Figs. 4 c- d) Each sucker consists of two parts, the upper rigid part having numerous adjacent, comb like semi-rectangular teeth, that are arranged in multiple rows (Fig. 4 e, f), these rows slightly curved inwards (Fig. 4f); The lower part is covered with papillated tissue

with, large cone shaped papillae (Figs. 4 g- h). The inner surface of the sucker facing the ring is lined with same type of papillae. The length and sharpness of the sucker teeth on the sucker ring did not evidently change over the entire circumference; furthermore no significant changes were detected in the length and sharpness of the sucker teeth between the oral and aboral sides of the ring (Fig. 4i).

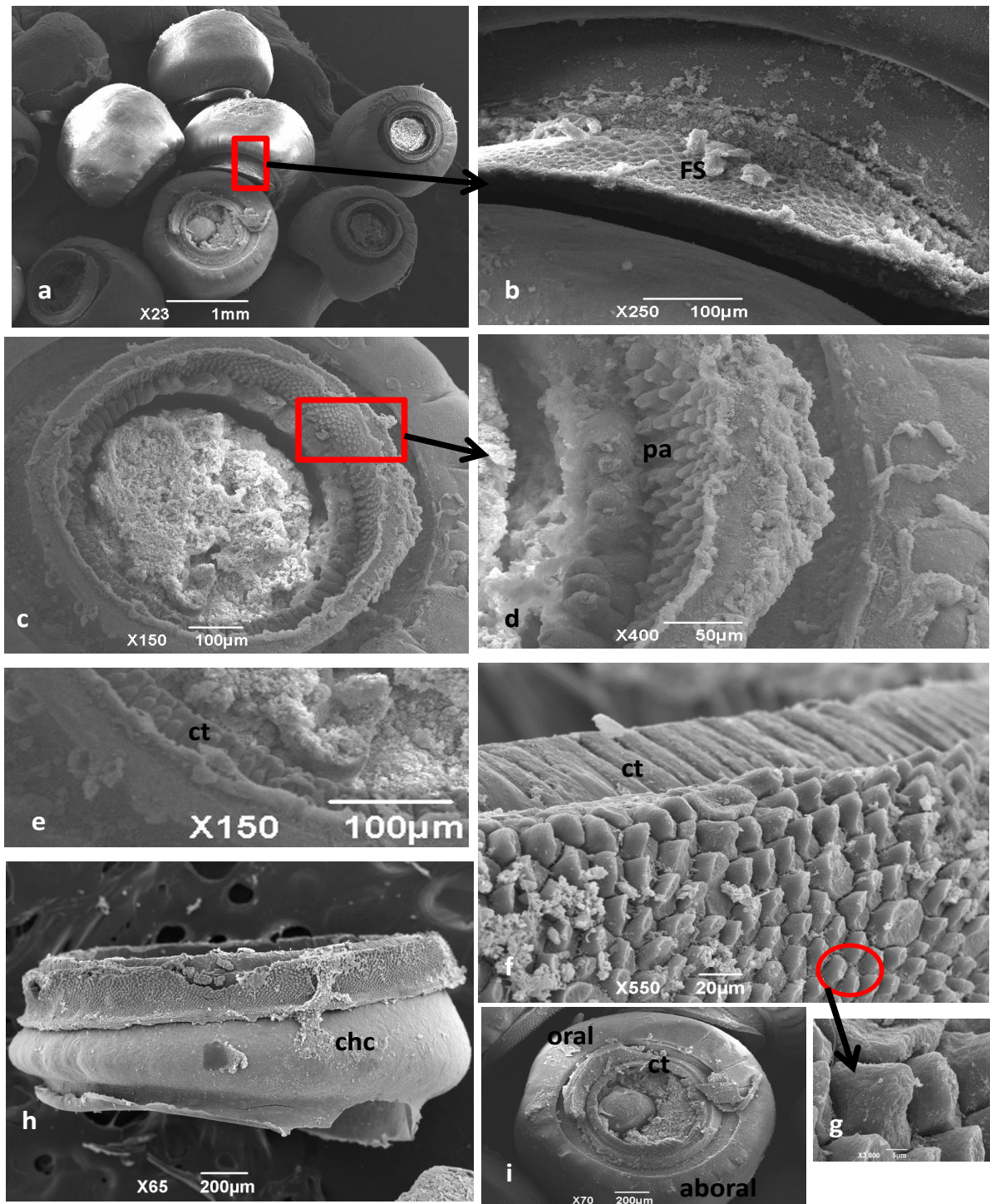


Figure 4: (a) SEM Micrograph of Normal Arm Suckers (from AII) of *S. Pharaonis* (b) Enlarged Part from the Lateral View of the Arm Sucker of *S. Pharaonis* (c) Frontal View of the Arm Sucker of *S. Pharaonis* (d) Enlarged Part of the Papillated Lining of the Sucker Facing the Sucker Ring of *S. Pharaonis* (e) The Comb Like Teeth of Normal Arm Suckers (from AII) of *S. Pharaonis* (f) Enlarged Part of the Papillated Coat of the Arm Sucker of *S. Pharaonis* (g) Enlarged Papillae of *S. Pharaonis* (h) Lateral View of the Sucker Ring of *S. pharaonis* (i) Frontal View of the Sucker Ring. chc (chitinous cup), ct (chitinous teeth), FS (fibrous sheath), pa (papillae)

Club sucker-bearing surface is flattened (Fig. 5), with eight suckers in transverse rows; suckers differ markedly in size: five or six median suckers enlarged (three or four of these are greatly enlarged). Each sucker consists of a deep muscular cup with a toothless chitinous ring for reinforcement (Fig. 5), each ring is covered with a coating of fibrous tissue may be for protection. The sucker ring is divided into three parts (Fig. 6), the chitinous

cup which is in direct contact with the muscular cup of the sucker and the toothless chitinous ridge which carries the sharp chitinous plate that emerges out of the sucker and is used for reinforcement (Fig. 7). A muscle bed in the middle of the sucker acts as a piston to strengthen the attachment of the sucker to the prey.



Figure 5: The tentacle club suckers of Sepia Pharaonis x 10 x 10. st (stalk), sr (sucker ring)

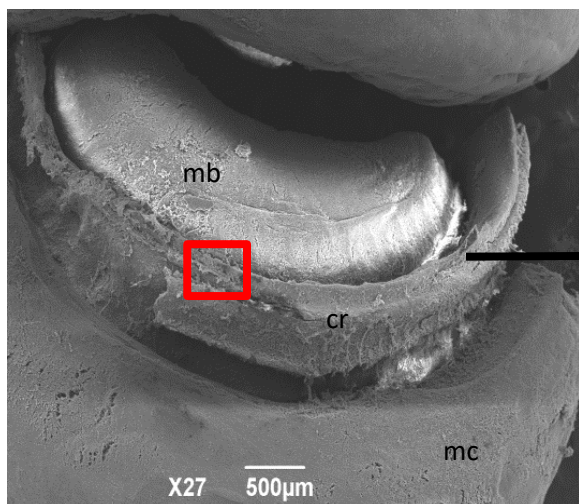


Figure 6: SEM Micrograph of the Tentacle Club Suckers of S. Pharaonis. chc (chitinous cup), cr (chitinous ring), mb (muscular bed), mc (muscular cup).

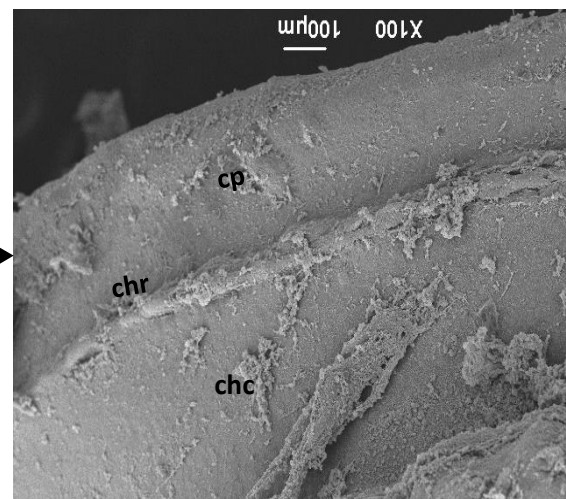


Figure 7: SEM Micrograph of Enlarged Part of the Tentacle Club Suckers Ring of S. Pharaonis chc (chitinous cup) , chr (chitinous ridge), cp (chitinous)

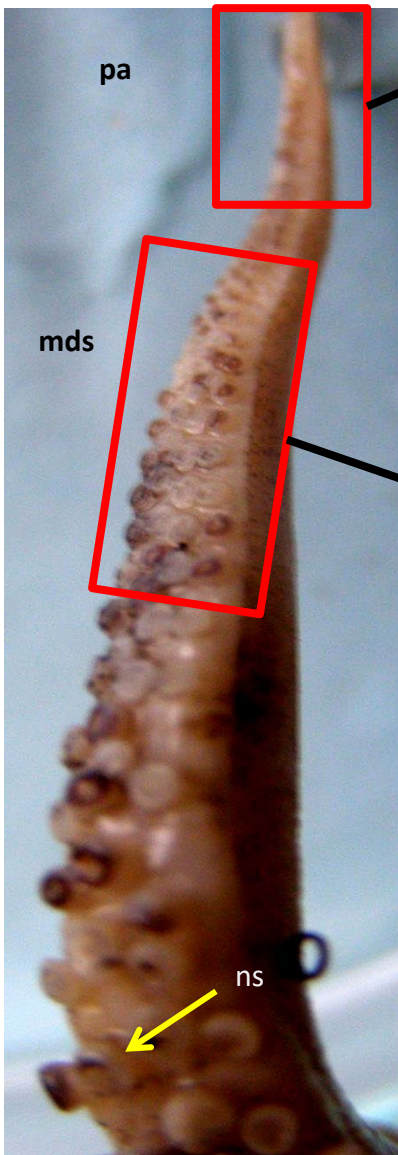


Figure 8: (a) The Hectocotylized arm of S. Pharaonis x 10

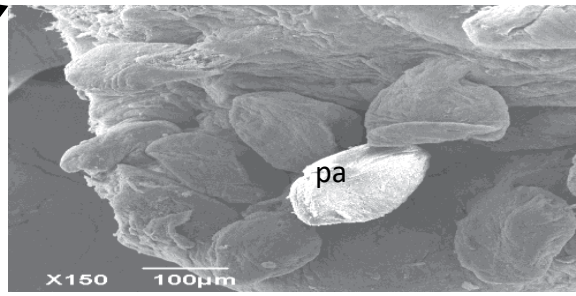


Figure 8: (b) SEM Micrograph of the Tip of Hectocotylized Arm of S. Pharaonis, Showing Papillae (pa)

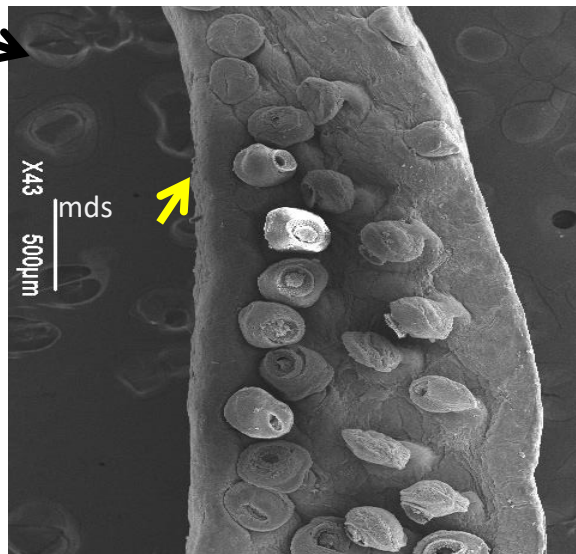


Figure 8: (c) SEM Micrograph of the Upper Part of Hectocotylized Arm of S. Pharaonis. Showing the Modified Suckers (mds).

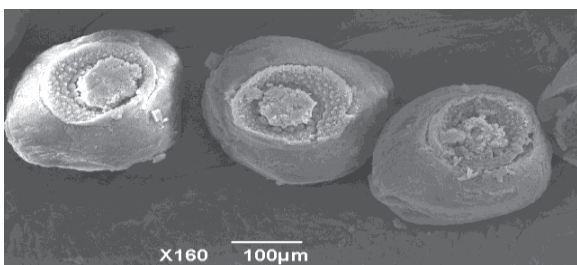


Figure 8: (d) SEM Micrograph of the Modified Suckers Found on Hectocotylized Arm of S. Pharaonis

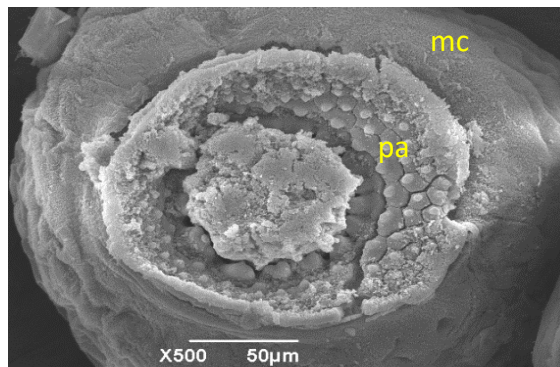


Figure 8: (e) SEM Micrograph of Modified Sucker of S. Pharaonis

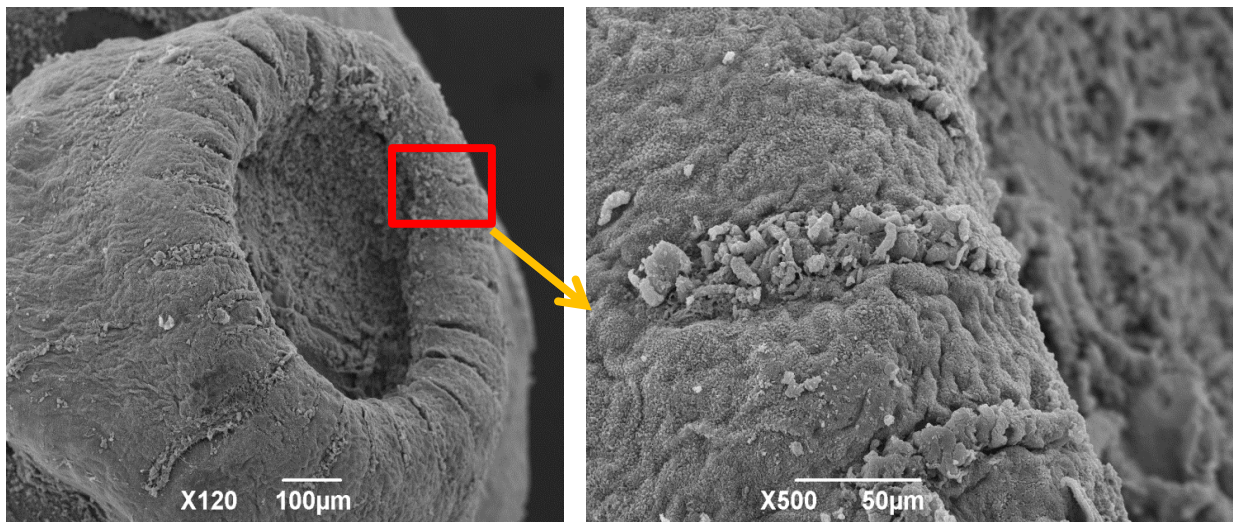


Figure 9: (a) SEM Micrograph of Buccal Membrane sucker of *S. Pharaonis* Figure 9: (b) Enlarged Part of the Sucker

Hectocotylus is found on left ventral arm of male *S. pharaonis*. The shape of the sucker varied between the hectocotylus and the arm, actually the Hectocotylus consists of 10 to 12 rows of normal size suckers proximally, six rows of modified suckers medially then papillated suckers without chitinous rings distally to arm tip (Figs. 8a -c). For the papillated sucker small papillae are located on the top of the cup surrounding a muscular ribbon and lined with soft papillated tissue ; at the bottom of the cup another type of larger papillae exists. Modified suckers (Figs. 8d -e), have no chitinous rings and lined with papillated tissue. The buccal membrane had a few, minute suckers, each lappet bearing one or two small suckers (Fig. 9). Each sucker consists of a flattened muscular cup with a few ridges on its outer surface (28- 29 ridges). There is no chitinous sucker ring in this type of suckers.

4. Discussion

Decapodiforms (cuttlefish and squid) are opportunistic predators, and their prey range is from invertebrates to vertebrates, that is, shrimp, crabs, and fishes.

The existing morphological studies on suckers are limited to taxonomic descriptions and focused on the number and alignment of the suckers [13].

The suckers of all decapods have horny rings. These rings often carry sharp claw-like teeth and in some species, these have been modified into hooks [14].

The present study is the first to report the morphological features of the sucker rings of *S. pharaonis* from the Arabian Gulf, based on structural observations obtained from Scanning Electron Microscopy.

Stated that “In cuttlefishes, the lateral epithelium of the arm expands to cover the immature sucker buds at the distal region, probably for the protection of immature suckers” [15]. Moreover, our observation contributes to the still unsolved discussion about that lateral epithelium, revealing that in *S. pharaonis* arms, there are cone shaped papillae covering the inner and outer surfaces

of the sucker cup, they are of unknown function yet; perhaps for sensory function or only for protection, further studies are needed to reveal their exact functions and mechanism of action.

The regular arm sucker rings have numerous blunt curved teeth ,this is surely an adaptation connected to the food and life style of *S. pharaonis*. Revealed that fish formed the major constituent of *S. pharaonis* food followed by prawns [16]. Described the unique feeding behavior of cuttlefishes as they extend two tentacles toward prey, catch the prey by tentacular club, retract the tentacles, and then hold and transfer the prey to their mouths by the remaining eight arms [17]. Suckers of coleoids have highly sophisticated structures which are flexible and capable of producing strong suction force [18]. The piston- like muscle bed in the cavity of the tentacle sucker ring accompanied by the toothless sharp chitinous plate that emerges out of the sucker are used for reinforcement. This unique structure allows the cuttle fish to attack the fast-moving fish efficiently and grasp them tightly; this would support the hypothesis of that cuttlefish mainly use an ambush strategy during hunting [19].

Furthermore, each ring is covered with a coating of fibrous tissue that is attached to chitinous ridge may be for support and protection, the long part of the inner sucker ring buries in this elastic fibrous tissue and attaches to it by the chitinous ridge as it joins the muscular sucker cup. The results of the present study are in harmony with that of which hypothesized that the acquisition of tentacles would be crucial for decapodiforms to efficiently catch agile prey while swimming around. Described a behavior of cuttlefishes as they are mainly benthic and they often walk on the ocean floor by using their arms, causing injuries of arm tips; this hypothesis may explain the presence of the protective papillated covering on their sucker rings.

The present study supports the results of describing the hectocotylyzation pattern of *S. pharaonis* as 10-12 basal transverse series of suckers normal followed by 7 series of modified suckers in the hectocotylyzed portion [20].

Decapodiforms sucker structures and the number of sucker rows

are varied among species [21]. The present study is the first to report the absence of horny rings in the hectocotylyzed part of *S. pharaonis* ventral arm in the Arabian Gulf. As the shape of sucker ring teeth is known to have a relation to its function, the morphological features of the suckers of the hectocotylus would be useful for mating behavior.

The hectocotylyzed part of the arm holds papillated suckers without chitinous rings to be suitable for grasping spermatophores without hooking and scratching and for smoothly transferring them to a female. small-diameter suckers without sharp rings would enhance the hectocotylus' ability to grasp spermatophores and transfers them to females. The small size suckers are concave lined with soft papillated tissue that can act together with a muscle rode in this cavity as an anti-slip device, and a site of spermatophore adherence.

The present study agrees with those described by that the buccal membrane of *S. pharaonis* has a few, minute suckers (each lappet bearing 1 or 2 small suckers) giving the detailed ultra-structure of this sucker using Scanning Electron Microscopy [22]. The muscular buccal suckers are of a unique structure having no chitinous rings attached to them, further studies are needed to reveal their functions.

Generally, the sucker teeth of Decapodiforms (including cuttlefish) are used to catch prey by hooking. Understanding the functional morphology of cephalopods' arms and tentacles requires comparing sucker shapes of many kinds of cephalopods to understand the relationship between sucker shape and feeding ecology.

5. Conclusion

- The present study is the first to report the shape and features of the sucker rings of *S. pharaonis* from the Arabian Gulf, based on morphological observations obtained from Scanning Electron Microscopy.
- Our findings revealed that in *S. pharaonis* arms, there are cone shaped papillae covering the inner and outer surfaces of the sucker cup, they are of unknown function yet. More studies are needed to understand their role in the sucker action mechanisms.
- The unique structure of tentacle club suckers represented by the piston like muscle bed in the cavity of the tentacle sucker ring accompanied by the toothless sharp chitinous plate allows the cuttle fish to attack the fast-moving fish efficiently.
- The hectocotylyzed part of the arm holds papillated suckers without chitinous rings to be suitable for grasping spermatophores without hooking and scratching and for smoothly transferring them to a female [23-27].
- Based on the morphological observation results in this study, it is suggested that making comparative studies among Cephalopod lineages will be necessary to reveal the intimate relation between the behavior and reproductive strategies of cephalopods.

Highlights:

- The present study is the first to report the shape and features of the sucker rings of *S. pharaonis* from the Arabian Gulf, based on morphological observations obtained from Scanning Electron Microscopy.
- For regular arm Each sucker consists of two parts, the upper

rigid part having numerous adjacent, comb like semi-rectangular teeth, and the lower part which is covered with papillated tissue with, large cone shaped papillae.

- For tentacular club suckers; there is a piston like muscle bed in the cavity of the tentacle sucker ring accompanied by toothless sharp chitinous ring, providing additional gripping power during prey capture and handling.
- The hectocotylyzed part of the arm holds papillated suckers without chitinous rings to be suitable for grasping spermatophores without hooking and scratching and for smoothly transferring them to a female.

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