ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

# Statolith Based Species Identification Method for Two Sepiidae Species from the Gulf of Kuwait

**El-Naggar[1], Marwa Abobakr[1]*, Yassien[2], Mohammed Hamed[2] and Manaf Behbehani[3]**

*[1,2]Marine Science Department Faculty of Science, Suez Canal University Egypt*

*[3]Department of Zoology, University of Kuwait, Kuwait*

***Corresponding Author**
Marwa Abobakr, Marine Science Department Faculty of Science, Suez Canal University Egypt.

**Submitted:** 2024, Oct 07; **Accepted:** 2024, Nov 01; **Published:** 2024, Nov 29

**Citation:** El-Naggar, Abobakar, M., Yassien, Hamed, M., Behbehani, M. (2024). Statolith Based Species Identification Method for Two Sepiidae Species from the Gulf of Kuwait. *J Gene Engg Bio Res, 6*(3), 01-08.

## Abstract

*The morphology of cephalopod statolith is closely related to its function, it is determined by the functional role of each of its parts together with complexly structured inner space of statocysts. Owing to a lack of previous reports concerning the ultra-structure and morphology of the equilibrium organs and statoliths in cephalopods of the Arabian gulf , a comprehensive study was therefore designed to explore the functional morphology of statocysts and statoliths of the broad club Sepia latimanus, and the pharaoh cuttlefish Sepia pharaonis using SEM in Kuwaiti waters. Significant differences are found in the external morphology of statoliths of the two species. The statolith of S. latimanus is typically demersal type statoliths characteristic of nearbottom decapods, while that of S. pharaonis is a pelagic type statoliths, these results demonstrate the strong impact of statolith morphology on the abundance of cephalopods and their distribution. The present study revealed the differentiation of the crystals forming the external surface of the statoliths of the two species under investigation, this surface is the site of interaction between the statolith and the statocyst. The results of this study will provide an essential basis for future investigations in the field, this may provide us with better understanding of yet unknown migration patterns of various cephalopod species.*

**Keywords:** Cephalopoda, Mollusca, Broad Club Cuttlefish Sepia Latimanus, Sepia Pharaonic, Statolith, Statocyst, Scanning Electron Microscopy

## 1. Introduction

Cephalopoda, the octopuses, squids and cuttlefishes comprise one of the most significant components of marine life. All are large, fast-growing, and active predators with highly evolved and specialized qualities of great inherent interest. Cephalopods constitute economically significant fisheries species in many parts of the world [1].

*Sepia* is a genus of cuttlefish in the family Sepiidae encompassing some of the best known and most common species. *Sepia latimanus*, also known as the Broad club Cuttlefish, is widely distributed from the Andaman Sea, east to Fiji, and south to northern Australia. It is the most common cuttlefish species on coral reefs, living at a depth of up to 30 m.

The pharaoh cuttlefish *Sepia pharaonis* is a broadly distributed species of substantial fisheries [2,3]. importance found from east Africa to southern Japan. *Sepia pharaonis* is a commercially harvested species.

Statoliths, are first described by, subsequently considered as true archives of cephalopod life cycles They can be used to investigate several aspects of cephalopod physiology, ecology and life style [4-7]. The Statoliths, are specialized gravity and angular acceleration receptor systems, they are designed to give the animal appropriate information about its position and movement in the water and enable it to compensate its eye movements [8]. The role of statoliths in the detection of angular accelerations was first hypothesized by [9]. Assumed that the statocysts can detect vibration stimuli, as well as detecting low-frequency sounds [10]. Statolith shape is species-specific, Statoliths are more precise for species identification of both recent and fossil cephalopods, compared to other hard structures, such as beaks and gladii, allowing cephalopod to be identified from the stomach contents of predators and even from fossil deposits [11-13].

The hypothesis that that statolith shape depends more on the evolutionary relationships of cephalopods rather than on the statoliths' function was confirmed by for squids and sepioids, they distinguished two main statolith morphologies: the "demersal", typical of near-bottom decapods, and the "pelagic", typical of all pelagic squids. The functional morphology of different parts of the decapod statolith has never been discussed in literature.

## 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 28o 30 – 30o North and 47o 45 - 48o 45 east (Fig.1).

The main target for this study is the reef areas where cuttlefish and squids live mainly. 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 bycatch with fish and shrimp.

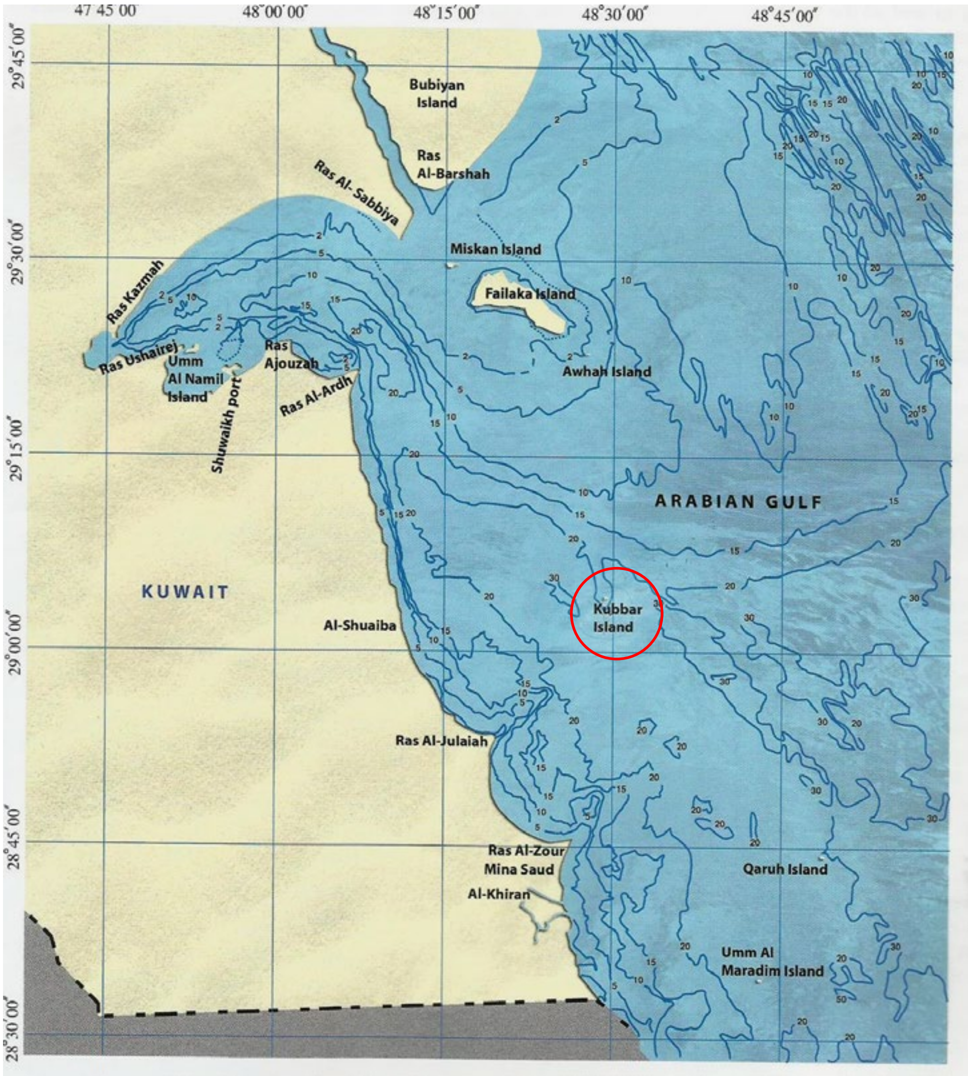


**Figure 1:** Map of the Kuwaiti waters of the Arabian Gulf, Showing the Major Sampling Area Around Kubbar Island. (From Oceanographic Atlas of Kuwait waters, KISR 2009

Samples were collected from using different known fishing methods working in Kuwait waters; these methods included steel traps (Fig.2) made by the fishermen from steel wires (gargoor) and trawl nets. In addition to purchasing samples from Kuwait fish markets.

**Figure 2:** Local Steel Trap Used for Cephalopod Collection. (Fahaheel fish market)

specimens were examined in Kuwait Institution of Scientific Research labs, while Electron Microscope preparations were carried out in Kuwait university Electron Microscope unit.

Using Techniques for surgical statolith extraction described by, statoliths of specimens of *S. latimanus*, and *S. pharaonis* were dissected under the zoom microscope with a fine needle and first placed into a fresh water to wash off organic impurities, after that statoliths were prepared for analysis or stored in a vial [14].

### 2.2 Preparation of specimens for the Electron Microscopy

Sample preparation following the method of, which involves processing, embedding and polymerization. Samples were fixed in 3% Glutaraldehyde, 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 [15].

## 3. Results

Statoliths of S. latimanus, and S. pharaonis are paired calcareous structures associated with the sensory epithelia lie in fluid filled cavities of the anterior chambers of the two adjacent sac-like equilibrium organs called statocysts, which are cavities of irregular shape, located in the posterior/ventral part of the cranial cartilage (Fig.3) and consist of two chambers (anterior and posterior), partially separated by finger-like projections and filled with liquid (Fig.3). Each statolith lies near the anterior end of the statocyst (Fig. 3), and is oriented with its long axis approximately in the dorsoventral plane of the cephalopod.

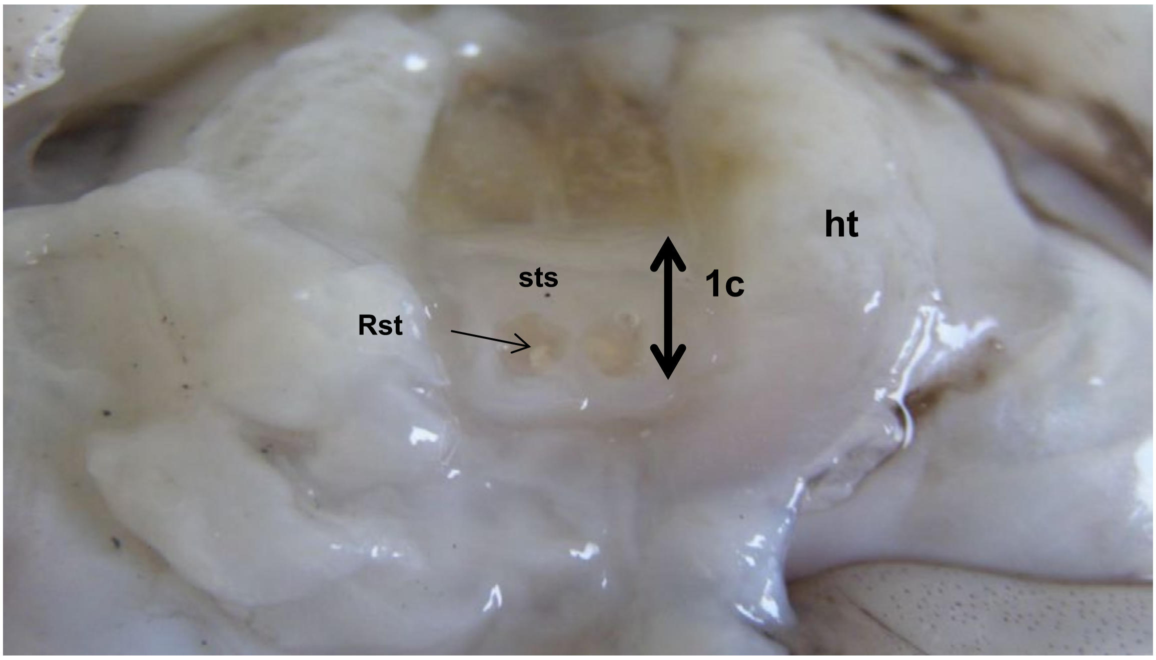


**Figure 3:** The Statocyst of S.Pharaonis

Statocysts are cavities of irregular shape placed within the cartilaginous cranium of the cephalopod situated ventro-posteriorly to the brain, they are a pair of fluid filled cavities. Many cartilaginous lobes protrude into the Statocyst cavity, anticristae (Fig. 4), that increase in size with the age of the animal as indicated by Clarke, 2003. The hair cells of the statocyst of *S.pharaonis* studied by the SEM are missing. Within each statocyst an irregularly shaped calcareous stone, the statolith (Fig.4), usually less than 2mm in length.

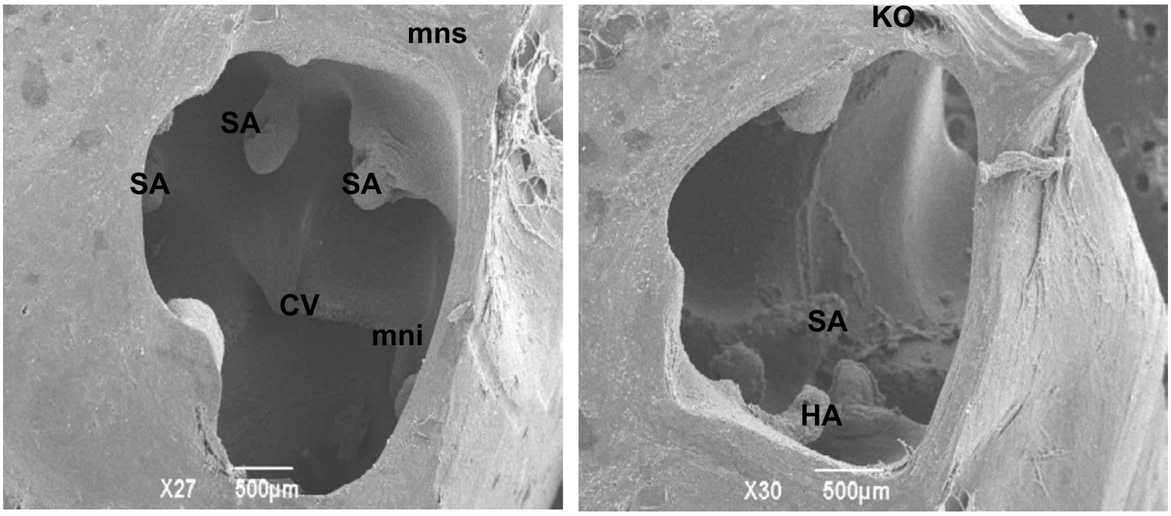


**Figure 4:** SEM Micrograph of the Statocyst of S.Pharaonis , After Transverse Vertical Cut. CV (Vertical Crista), HA (Hamuli), Ko (Opening of Kolliker's Canal), mni (Macula Neglecta Inferior), mns (Macula Neglecta Superior), SA (Straight Anticrista)

Statoliths are characterized by four main regions: the dorsal dome, the lateral dome, the rostrum and the wing, (Fig.5).

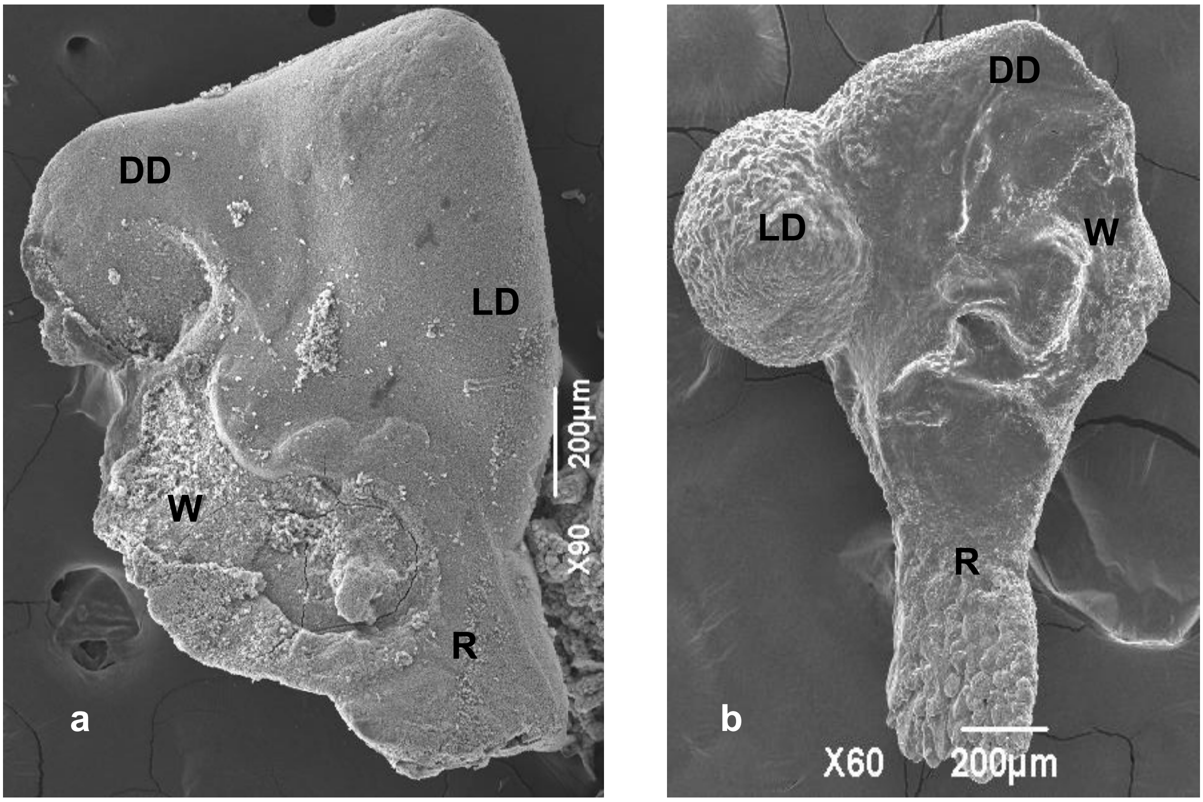


**Figure 5:** (a) SEM Micrograph of the Dorsal Side of Right Statolith of S. Pharaonis(b) SEM Micrograph of the Dorsal Side of Right Statolith of S. Latimanus.. DD (Dorsal Dome), LD (Lateral Dome), R (Rostrum),W (Wing).

The statolith of *S. pharaonis* has thickened inner wall (Fig. 6a), the lateral dome is broadest at its dorsal end , the lateral dome represents a large area in anterior and posterior aspects, it is very much boarder and longer than the rostrum, the lateral and dorsal domes are not well-separated. (Fig. 6a). The wing of the statolith, which is the area of attachment to the statocyst wall have a dorsal and a ventral indentation. The wing is wide and long, it does not reach the end of the rostrum leaving less than one-fourth of its distal part free (Fig. 6a). The wing and rostrum are separated by a crescent shape groove.

The rostrum is seen to be short, broad and rectangular in outline and is at an angle to the main axis of the lateral dome, and wide at its base . The lateral margins of the dome are thick, smoothly curved, without inferior lobe, The ventral surface of the dorsal dome is covered with randomly arranged crystals (Fig. 6b). The wing is long, extending almost to the rostrum distal end and overlapping most of the dorsal dome, margins of the wing have several randomly arranged rectangular indentations (Fig. 6c), these indentations, give the impression of arranged balls of sharp thorny spiks stacked together in a repeated form on the surface (Figs. 6d) The spur is small and sharply divided from the wing by a groove.

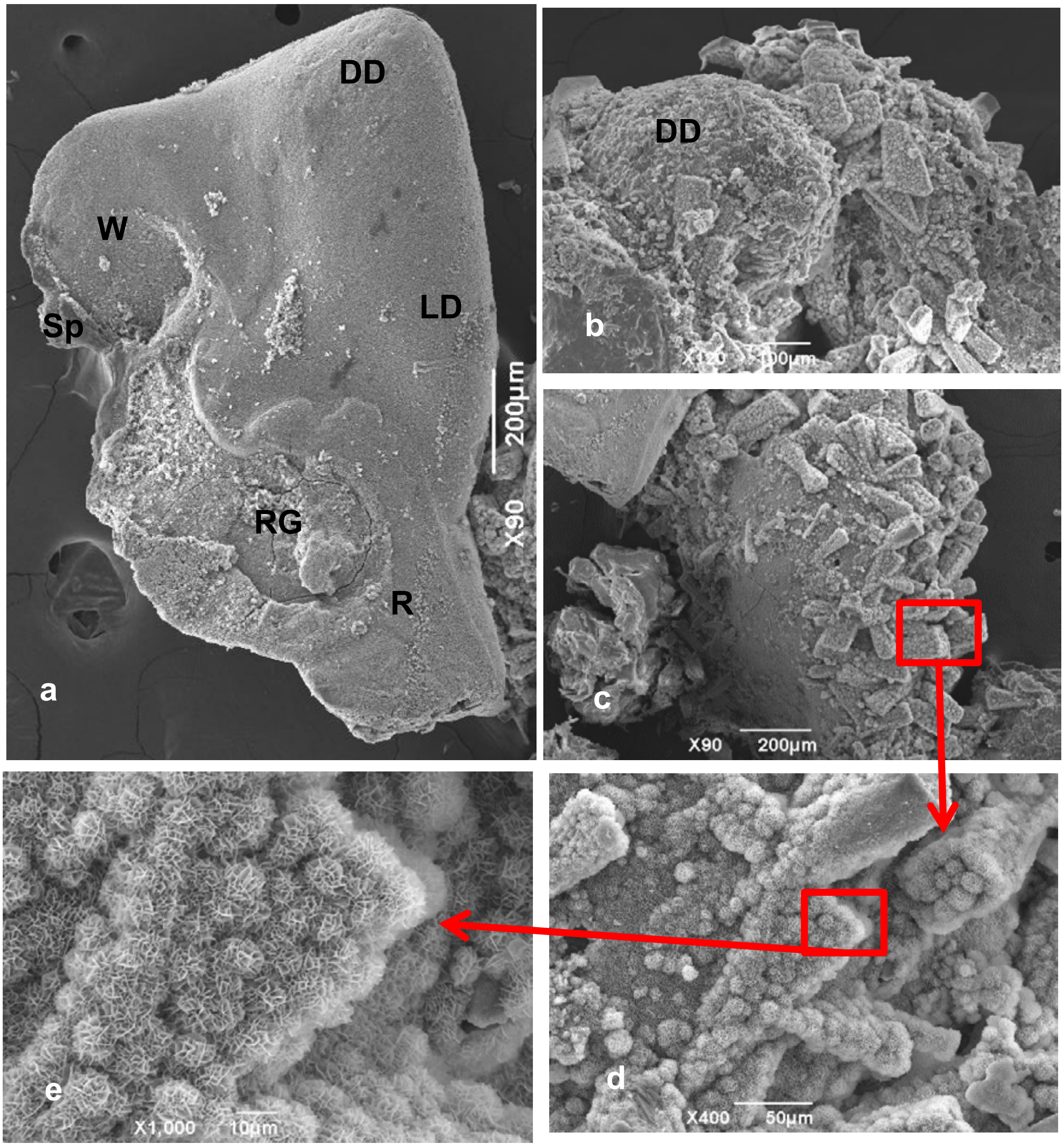


**Figure 6:** (a) SEM Micrograph of the Dorsal Side of the Right Statolith of S. pharaonis. DD (dorsal dome), LD(lateral dome), R (rostrum), RG (rostral groove),W (wing), Sp (spur). (b) SEM micrograph of the arterio-ventral side of the dorsal dome of right statolith of S. pharaonisshowing ultra-structureand the arrangement of the crystals on its surface . (c) SEM micrograph of the lateral view of right statolith of S. pharaonis, showing the structure of wing indentations and their arrangements . (d) SEM micrograph of enlarged part of the wing indentations of the right statolith of S. pharaonis showing the arrangement the crystals forming each indentation. (e) SEM micrograph of the wing indentations of right statolith of S. pharaonis showing the spikey like structure of each indentation.

The statolith of S .latimanus has large paddle-shaped rostrum, which is long, flattened, serrated at its end. The lateral and dorsal domes are well-developed and separated by a distinct groove, the lateral dome is large and semi-spherical. The wing of the statolith of S. latimanus is thinner and shorter than that of S. pharaonis , with curvy broad edges (Fig.7). The rostrum of S.

latimanus statolith is much longer than that of S. pharaonis. Unlike S. pharaonis the rostrum of S. latimanus has a rough surface at its ventral side and roughness at its tip (Fig.7). The crystals forming this surface looks coarse lump like arranged in adjacent rows in the anterior part of the rostrum (Fig.7a), while they look much smoother on the posterior side (Fig.7b). The dorsal dome of S. latimanus statolith is small and translucent, while the lateral dome is larger and elongated, it is coarse and solid covered with sharp small crystals on its arterio-ventral side and a larger softer crystal posteriorly, the structure and function of these crystals are unknown need more extensive studies to be revealed. The shapes of the right and left statoliths from the same specimen are mirror-symmetrical.

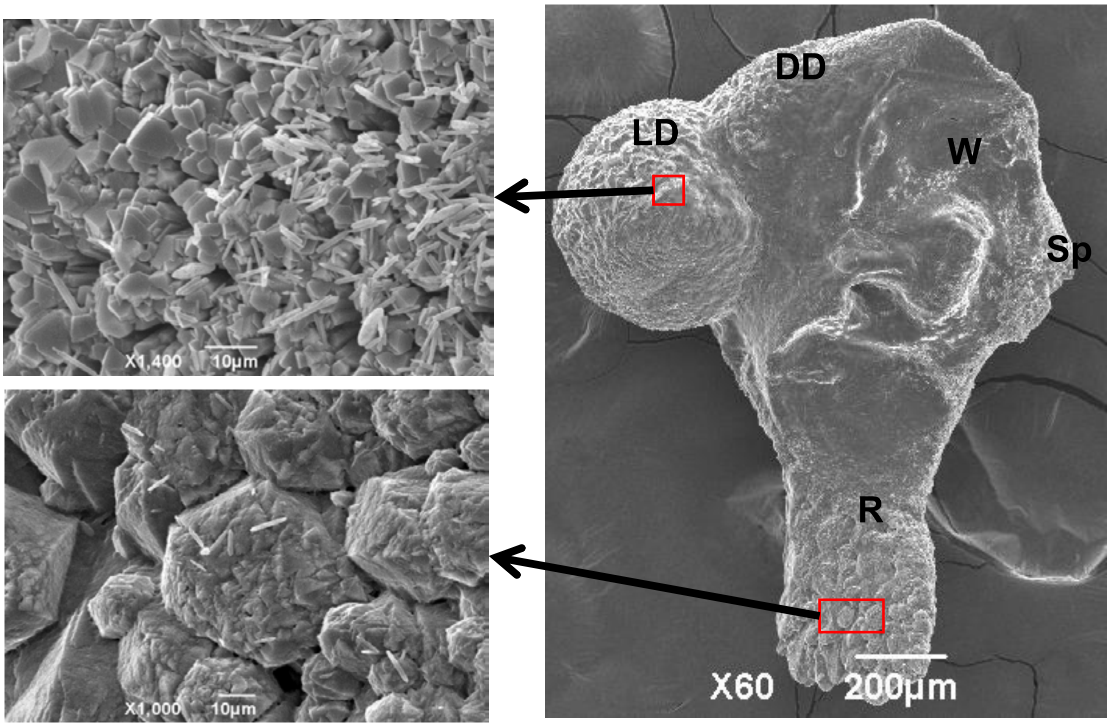


**Figure 7a:** SEM Micrograph of the Anterior view of Right Statolith of S. Latimanus , Showing Its Ultra Structure and the Arrangement of the Crystals Forming the Statolith Surface. DD (Dorsal Dome), LD(Lateral Dome), R (Rostrum),W (Wing), Sp (Spur)

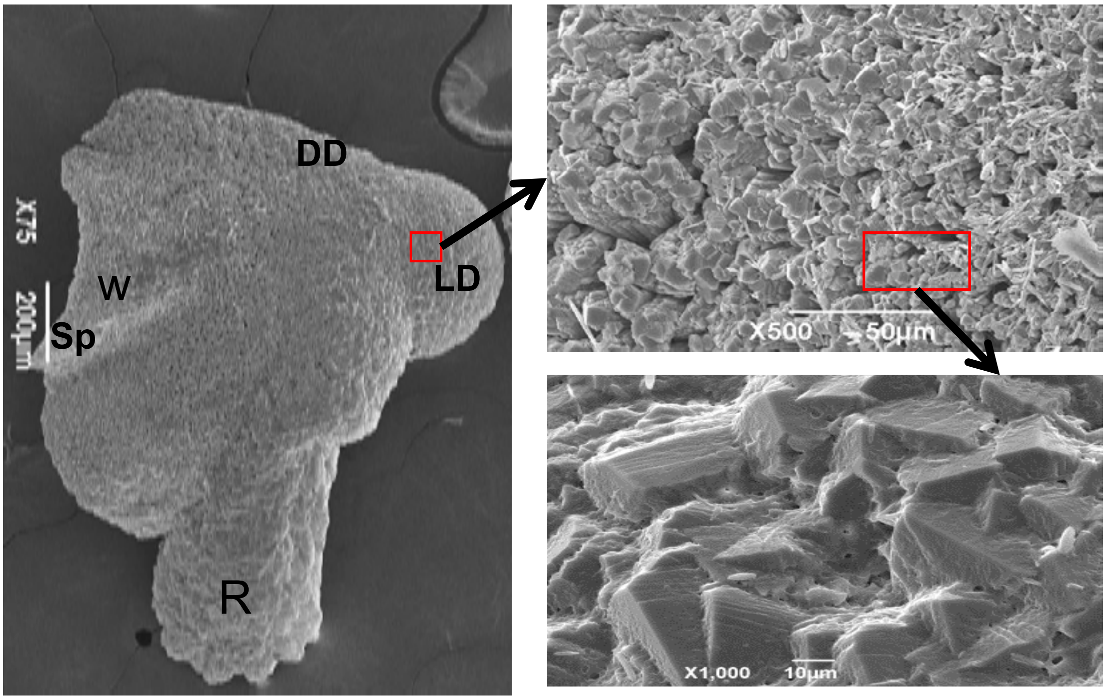


**Figure 7b:** SEM Micrograph of the Posterior View of Right Statolith of S. Latimanus , Showing Its Ultra-Structure And The Arrangement of the Crystals Forming the Statolith Surface. DD (Dorsal Dome), LD (Lateral Dome), R (Rostrum), W (Wing), Sp (Spur), W (Wing)

## 4. Discussion

Kuwait bay is considered as one of the characterized features of the Kuwaiti marine environment, which is an elliptically shaped bay that protrudes from the Arabian Gulf in Westward direction at its Northwestern corner. Kuwait Bay is of a moderate size (850 $km^2$) with an average water depth of 5 m and a maximum depth of 20 m at the entrance [16].

According to the Kuwait Diving Team which announced on September 2010, (based on a comprehensive surveys of the territorial waters) 90 percent of the coral reefs surrounding the Kuwaiti shores died due to pollution problems, their study covered Um AlMaradim, Kheiran, Ras Al-Zor, Garouh, Um Diera, Teyler, Kubbar and Oraifjan resulted in 90 percent of "bleaching" of the coral reef [17-19]. In a study done by squids, octopuses and cuttlefishes exposed to sound had damaged statocysts. Hair cells were ruptured and sometimes missing altogether. The nerve fibers that carry signals from the hair cells were swollen. In some cases, there were lesions and holes in the sensory surfaces of the statocysts. The statocyst damage raised the possibility that noise played a role in the squid deaths, but no one had tested whether low-intensity sound can cause that kind of damage. The results of the present work shows that the hair cells of the statocyst of S.pharaonis studied by the SEM are missing this are may be due to the Noise caused by oil industry in this area besides the noise caused by oil tankers and shipping ports.

These results of the present study indicate that the harmful effects of marine noise pollution cause marine animals like cephalopods in Kuwait to drift away. These results are supported by the results of that the epidermal hair cells and statocysts of cephalopods are remarkably similar to the lateral lines and inner ears of fish [20]. Also, performed electrophysiological experiments on Sepia officinalis and Lolliguncula brevis that showed that the epidermal hair cells of these cephalopods were sensitive to local water movements produced by a vibrating sphere located 6–13 mm away [21]. In cephalopods, the statocyst contains the macular and crista systems. The macular system acts as an analog to the otolith organ and contains a dense calcareous statolith attached to sensory hair cells. Showed that, the cephalopod statocyst appears to be sensitive to kinetic sound components because it detects vibrations; they also demonstrated that cephalopods are sensitive to particle motion, but not to sound pressure [22]. Furthermore, they suggested that statocysts would detect particle motion in the same manner as fish otolith organs.

The present study shows that the statolith of S .latimanus is characteristically demersal , with large Oar-shaped rostrum, which is long, flattened, and well-developed lateral and dorsal domes. According to the oar-like rostrum of the demersal decapod statolith provides a greater sensitivity during rolling at low accelerations [23]. The more massive and structurally differentiated statolith of demersal type ensures better sensitivity to angular accelerations and provides accurate movements such as roll and swerving, in highly structured space of near-bottom environment [24]. Thus, features of the statolith shape in S .latimanus gives it greater sensitivity to low angular accelerations in all possible planes compared to those of S. pharaonis. Peculiarities of the statolith shape in pelagic species (especially long and wide wing achieving the rostrum tip and short sharpened rostrum) make these statoliths hardly movable around both longitudinal and transversal axes that considerably diminish the sensitivity of these animals to angular accelerations in pitching and rolling planes.

The statolith of S. pharaonis, is typically pelagic. Pelagic type statoliths are more adopted to monitor accelerations in the water column where there are no obstacles for the jetting swimming. As compared with that of S. latimanus it has shorter rostrum and larger dorsal dome. On the other hand, statolith of S. latimanus has a longer rostrum and a smaller dorsal dome, and its morphological structure is obviously different. Statoliths of demersal squids are two or more times larger than those of pelagic squids among animals of the same size class. Dorsal and lateral domes of the pelagic statolith are poorly separated from each other and function as a unit; rostrum is short and wing is long, extending almost to the rostrum distal end and overlapping most of the dorsal dome. In response to acceleration, the statolith moves within the cavity and stimulates hair cells projecting inwards from the walls of the statocyst [25]. The hair cells are orientated in several planes, so that the number and position of those excited by the movement convey precise information to the brain about the movement of the animal in three dimensions. These hair cells were missing in the present study [26-29].

The statolith of S.pharaonis has a shorter rostrum than that of S.latimanus this indicates that S.latimanus statolith shape gives it greater sensitivity to low angular accelerations in all possible planes compared to those of S.pharaonis. According to this result S. pharaonis lives in deeper water most likely on the sea bed, while S. latimanus exhibit shallow waters near the coral reefs. This also indicates the two fishing methods required to catch both species, as bottom trawling is the most efficient method to capture observed that S. pharaonis migrates to spawning ground from fishing ground.

## Acknowledgement

I would like to express my deep gratitude to the Mariculture and Fisheries Department of the Kuwait Institute for Scientific Research for their kind cooperation, advice and encouragement. Dr. Sulaiman Al-Mattar of the same department has been helpful in facilitating sample collections and deserves our thanks. Professor Abdul-Majeed Safar, Director of the Nanoscopy Center at the Faculty of Science, Kuwait University is hereby acknowledged for his kind support. I also thank, Mr. Mohammad Behbehani, Technician at the Nanoscopy Center for his efforts and cooperation in preparing the electron microscopy images. for their valuable cooperation and patience. Sincere thanks to all staff of KISR for supporting; Cooperation and guidance during the course of the present study.

## References

1. Roper, C. F., Sweeney, M. J., & Nauen, C. E. (1984). FAO species catalogue: an annotated and illustrated catalogue of species of interest to fisheries. Cephalopods of the World. *Food and Agriculture Organization of the United Nations*.
2. Ehrenberg, C. G. (1831). Cephalopoda living in the Red

Sea. Invertebrate animals excluding insects. Physical Symbols, or Icons and Descriptions of New or Less Known Natural Bodies, Taken from Travels through Libya, Egypt, Nubia, Dongal, Syria, Arabia, and Abyssinia, Zoological Part, Hemprich, PC, and Ehrenberg, CG, Ed., Berlin, 4, 6.
3. Nesis, K.N. (1987). Cephalopods of the world. T. F. H. Publications, Neptune City, NJ.
4. Ovsjannikov, P.H. and Kowalewsky, A. (1867). On the central nervous system and the hearing organ of cephalopods. *Acad. Imp. Sci. St Petersbourg 11*: 1-36.
5. Lipinski, M. R. (2001). Statoliths as archives of cephalopod life cycle: a search for universal rules. *Folia Malacologica, 9*(3).
6. Arkhipkin, A. I. (2005). Statoliths as 'black boxes' (life recorders) in squid. *Marine and freshwater research, 56*(5), 573-583.
7. Villanueva, R., Moltschaniwskyj, N. A., & Bozzano, A. (2007). Abiotic influences on embryo growth: statoliths as experimental tools in the squid early life history. *Reviews in fish Biology and Fisheries, 17,* 101-110.
8. Budelmann, B. (1977). Structure and function of the angular acceleration receptor systems in the statocysts of cephalopods. In *Symp Zool Soc Lond* (Vol. 38, pp. 309-324).
9. Arkhipkin, A. I., & Bizikov, V. A. (1998). Statoliths in accelerometers of squids and cuttlefish. *Ruthenica, 8*(1), 81-84.
10. Arkhipkin, A. I., & Bizikov, V. A. (2000). Role of the statolith in functioning of the acceleration receptor system in squids and sepioids. *Journal of Zoology, 250*(1), 31-55.
11. ARKHIPKIN, A. I. (2003). Towards identification of the ecological lifestyle in nektonic squid using statolith morphometry. *Journal of Molluscan Studies, 69*(3), 171-178.
12. Clarke, M. R. (1978). The cephalopod statolithan-introduction to its form. *Journal of the Marine Biological Association of the United Kingdom, 58*(3), 701-712.
13. Clarke, M. R., Fitch, J. E., Kristensen, T., Kubodera, T., & Maddock, L. (1980). Statoliths of one fossil and four living squids (Gonatidae: Cephalopoda). *Journal of the Marine Biological Association of the United Kingdom, 60*(2), 329-347.
14. Dawe, E. G. and Natsukari, Y. (1991). Light microscopy . In Jereb. P, Ragonese, S., Boletzky, S. von (eds.) Squid age determination using statoliths. *Journal of Fisheries and Aquatic Sciences,* pp.53–66.
15. Harris, J.R. (1997). Negative staining and cryoelectronic microscopy: The thin film techniques. BIOS scientific publishers, Ltd., Oxford, United Kingdom.
16. Al-Ghadban, A. N. (2004). Assessment of suspended sediment in Kuwait Bay using Landsat and SPOT images. *Kuwait Journal of Science and Engineering, 31*(2), 155.
17. Sheppard, C., Al-Husiani, M., Al-Jamali, F., Al-Yamani, F., Baldwin, R., Bishop, J., & Zainal, K. (2010). The Gulf: a young sea in decline. *Marine pollution bulletin, 60*(1), 13-38.
18. Sale, P. F., Feary, D. A., Burt, J. A., Bauman, A. G., Cavalcante, G. H., Drouillard, K. G., & Van Lavieren, H. (2011). The growing need for sustainable ecological management of marine communities of the Persian Gulf. *Ambio, 40,* 4-17.
19. Al-Cibahy, A. S., Al-Khalifa, K., Böer, B., & Samimi-Namin, K. (2012). Conservation of marine ecosystems with a special view to coral reefs in the Gulf. In *Coral Reefs of the Gulf: Adaptation to Climatic Extremes* (pp. 337-348). Dordrecht: Springer Netherlands.
20. André, M., Solé, M., Lenoir, M., Durfort, M., Quero, C., Mas, A., ... & Houégnigan, L. (2011). Low-frequency sounds induce acoustic trauma in cephalopods. *Frontiers in Ecology and the Environment, 9*(9), 489-493.
21. Kaifu, K., Akamatsu, T., & Segawa, S. (2008). Underwater sound detection by cephalopod statocyst. *Fisheries science, 74,* 781-786.
22. Budelmann, B. U., & Bleckmann, H. (1988). A lateral line analogue in cephalopods: water waves generate microphonic potentials in the epidermal head lines of Sepia and Lolliguncula. *Journal of Comparative Physiology A, 164*(1), 1-5.
23. Packard, A., Karlsen, H. E., & Sand, O. (1990). Low frequency hearing in cephalopods. *Journal of Comparative Physiology A, 166,* 501-505.
24. Ceriola, L. and Milone, N. (2007). Cephalopods Age Determination by Statolith Reading: a Technical Manual. Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea. GCP/RER/010/ITA/TD-22. *AdriaMed Technical Documents, 22*:78pp.
25. Arkhipkin, A. I., Bizikov, V. A., Doubleday, Z. A., Laptikhovsky, V. V., Lishchenko, F. V., Perales-Raya, C., & Hollyman, P. R. (2018). Techniques for estimating the age and growth of molluscs: Cephalopoda. *Journal of Shellfish Research, 37*(4), 783-792.
26. Boyle, P., & Rodhouse, P. (2008). Cephalopods: ecology and fisheries. *John Wiley & Sons.*
27. Boletzky, S. V. (1983). Sepia officinalis. *Cephalopod life cycles,* 31-52.
28. Carpenter, K. E. (1997). Living marine resources of Kuwait, Eastern Saudi Arabia, Bahrain, Qatar, and the United Arab Emirates. Food & Agriculture Org.
29. Gabr, H. R., Hanlon, R. T., Hanafy, M. H., & El-Etreby, S. G. (1998). Maturation, fecundity and seasonality of reproduction of two commercially valuable cuttlefish, Sepia pharaonis and S. dollfusi, in the Suez Canal. *Fisheries research, 36*(2-3), 99-115.