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Note

Occurrence of morphological aberrations in Pharaoh cuttlefish *Acanthosepion pharaonis* (Ehrenberg, 1831) from the Arabian Sea

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Abstract

The pharaoh cuttlefish *Acanthosepion pharaonis* (Ehrenberg, 1831) (=*Sepia pharaonis*) is a widely distributed commercial cephalopod species in Indo-Pacific waters. This study documents two separate mature female specimens of *A. pharaonis* from the south-eastern Arabian Sea, one exhibiting an unusual double bifurcation of the right second arm and the other possessing an abnormal cuttlebone. The specimens had dorsal mantle lengths of 252 and 163 mm, and corresponding body weights of 1181 and 318 g, respectively. This represents the first documented case of double arm bifurcation in a cuttlefish from the region, contributing to the limited knowledge of morphological abnormalities in cuttlefish.

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Cephalopod molluscs, which include cuttlefish, squid, and octopus, offer a viable alternative to other regeneration models (Murata *et al*., 1981). The arms of these organisms are as physically and structurally complex as many vertebrate appendages (Kier and Stella 2007; Kier and Schachat, 2008). The quantitative information on arm regeneration in cuttlefish proves that they are capable of fully regenerating lost arms, and the regeneration process is predictable and consistent (Tressler *et al*., 2014). All cephalopods investigated to date are capable of regenerating many of their tissues, including arms, tentacles, and fins (Imperadore and Fiorito, 2018).

Parona (1900) described and figured bifurcation in the arm of the musky octopus *Eledone moschata* (Lamarck, 1798), *Octopus vulgaris* Cuvier, 1797 and an additional arm in the lesser octopus *E. cirrhosa*, from the Mediterranean Sea. Subsequently, other cases were reported for the common octopus from the Japanese and adjacent waters (Okada, 1937, 1965), and the anatomy of octopuses with seven arms was studied in detail (Gleadal, 1989), with special reference to the arrangement of brachial nerves. Kumpf (1960) described a male specimen of *Octopus briareus* G. C. Robson, 1929, in which the third left arm was divided into two branches; with a well-developed web between the divisions, resembling the web present at the bases of normal arms in this species.

Toll and Binger (1991) documented different cases of arm anomalies in Octopoda. The most common anomalous condition of the arms of octopuses is the bifurcation or polyfurcation of the arms, particularly at the tips (Toll and Binger, 1991). Gonzalez and Guerra (2008) reported cases of bilateral hectocotylisation in squids and cuttlefish. In contrast, dichotomy of the arms in Teuthoidea and Sepiida seems to be rarer than in Octopoda. To date only one case has been reported in a decapod cephalopod (*Sepia esculenta* = *Acanthosepion esculentum* (Hoyle, 1885) as documented by Okada (1937), while Jereb *et al.* (1997) reported four cases of anomalies in the copulatory arms in the Sepiolinae. The arms of squid and cuttlefish play crucial roles in prey capture, object manipulation, locomotion and reproduction (Hanlon and Messenger, 1996).

The cuttlebone serves as a permanent record of injuries and developmental abnormalities because, unlike the soft tissues of cephalopods, it is retained throughout the animal's life and

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remains intact after death (Feral, 1978). Consequently, abnormal or scarred cuttlebones have been documented in several species of the genus *Sepia* (Battiato, 1983; Boletzky and Overath, 1991; Sherrill *et al*., 2000; Bello and Paparella, 2003), providing valuable evidence of past trauma and the healing response in wild cuttlefish. More recently, Venkatesan *et al*. (2020) reported a scar-bearing cuttlebone in *Acanthosepion pharaonis* (Ehrenberg, 1831), from the Arabian Sea, further demonstrating the utility of cuttlebone morphology for recognising and interpreting abnormalities in natural populations. The Pharaoh cuttlefish, *A. pharaonis* is one of the most important species exploited along the Arabian Sea (Geetha Sasikumar, 2013). It is a neritic demersal species endemic to the tropical waters of the Indo-Pacific region, spanning from the Red Sea, Arabian Sea, Andaman Sea to the South China and East China Seas, Japan, eastern Indonesia, and southern Australia, including the Gulf of Carpentaria (Ried *et al*., 2005). Previously, Venkatesan *et al.* (2020) reported an abnormal cuttlebone of the species from the Arabian Sea. This study documents occurrence of arm bifurcation and abnormal cuttlebone in the Pharaoh cuttlefish *A. pharaonis* from the Arabian Sea.

A female *A. pharaonis* with a bifurcated arm was found among a sample of 34 specimens collected from the Sakthikulangara Fishing Harbour, Kollam, on 31 October 2023, while another female with an abnormal cuttlebone was collected from the Cochin Fisheries Harbour (CFH), in May 2024 in Kerala, India. Both were caught in bottom trawl, at a depth of 40–80 m, from the south-eastern Arabian Sea (08°56'N; 76°11'E and 10°11'N; 76°21'E respectively). The specimens were iced immediately after collection and transported to the laboratory. Taxonomic identification was conducted using the key provided by Ried *et al*. (2005). Morphometric measurements and indices of specimens were measured according to Roper and Voss (1983) before fixation in a solution of 10% buffered formalin. Indices of length were expressed as a percentage of dorsal mantle length. The sexual maturity of the specimen was assessed according to Silas *et al*. (1985). The gonadosomatic index (GSI) and nidamental gland index (NGI) were calculated as:

GSI = (GW/ BW) x 100

NGI = (NGW/BW) x 100

where GW = Gonad weight (total reproductive system)
NGW = Nidamental gland weight and BW = Body weight.

Among the two mature females of *A. pharaonis,* one individual exhibited an unusual bifurcate arm morphology, while the other showed deformation of the cuttlebone. Their dorsal mantle lengths measured 252 and 163 mm, respectively. The dissected animal with split arm was deposited in the Designated National Repository (DNR), of ICAR-Central Marine Fisheries Research Institue (ICAR-CMFRI), Kochi, India with accession number CMFRI DNR No.: DE.1.1.1.2.1.

The dorsal mantle length (DML) of the cuttlefish with abnormal arm was 252 mm, and the body weight (BW) was 1181g (Fig. 1). The main morphometric details of the specimen are given in Table 1. The right second arm (dorso-lateral) was split into two branches; the shorter branch measuring 113 mm and the longer branch measuring 145 mm (Fig. 1, 2). The suckers were arranged in four rows (tetraserial arrangement) in the normal fashion from the base of the arm (Fig. 2b). The longest branch had 138 suckers, whereas the shortest had 98 suckers. The web depth between the two branches was 4 mm. The arm formula, which indicates the relative order of arm length on each side was IV>II>I>III for the left arms and IV>I>IIa>III>IIb for the

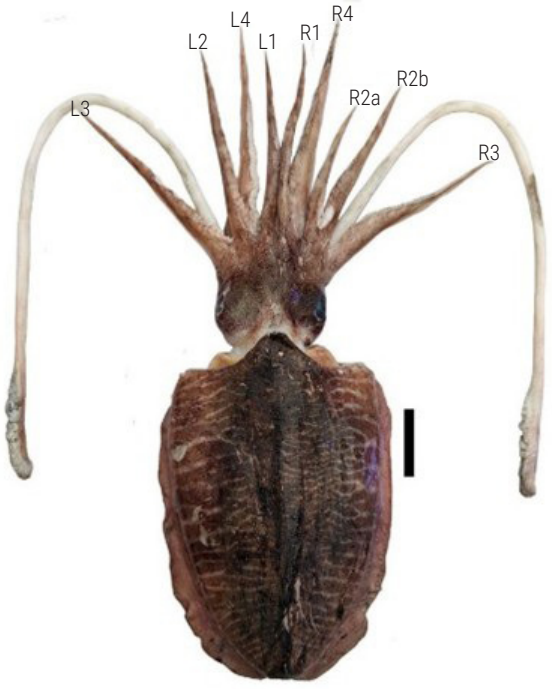

Fig. 1. Dorsal view of *A. pharaonis* with split arm (R2a and R2b), from the Arabian Sea (Scale bar = 5 cm)

(a) (b)

Fig. 2. (a) Dorsal and (b) ventral view of split arm of *A. pharaonis* (scale bar = 2 cm)

right arms (Table 1). All other arms were morphologically normal. The individual was a mature female with an empty stomach. The GSI of the individual was 5.5%, and the NGI was 2.3%.

The mature female specimen with the abnormal cuttlebone had 163 mm DML and 318 g BW (Fig. 3). The cuttlebone was bent (~60°C) upwards in the posterior region (Fig. 4). Details of the observations and morphometric measurements of the abnormal cuttlebone are shown in Table 2. Fracture on the dorsal side was completely healed, while the fracture was still visible on the ventral side (Fig.4d).

Table.1. Morphometric measurements and indices (as percentage of DML) of *A. pharaonis* with abnormal right arm

Variables	Measurements (mm)	Indices
Dorsal mantle length	252	-
Mantle width	154	61
Head length	72	29
Head width	76	30
Fin length	236	94
Fin width	30	12
Right arm 1	160	63
Right arm 2a	113	45
Right arm 2b	145	61
Right arm 3	150	60
Right arm 4	198	79
Left arm 1	149	59
Left arm 2	155	62
Left arm 3	148	59
Left arm 4	180	71
Right tentacle length	560	222
Left tentacle length	525	208
Total weight (g)	1181	-
Sex	Female	-
Maturity stage	Spawning	-
Nidamental gland weight (NGW) (g)	27*	2.3
Accessory nidamental gland weight (ANGW)(g)	08*	0.7
Ovary weight (O) (g)	30*	2.5
Gonad weight (NGW+ANGW+ O) (g)	65*	5.5

*****Weight

Cephalopods exhibit bilateral symmetry along the mid-sagittal plane, which is also reflected in the arrangement of their internal organs, including the the paired gills, branchial hearts and salivary glands and in females, the paired oviducts (Pelseneer, 1906). Post-traumatic regeneration of arms and tentacles is a well documented a process

Table. 2. Morphometric measurements and indices (as percentage of DML) of *A. pharaonis* with abnormal cuttlebone

Variables	Measurements (mm)	Indices
Dorsal mantle length	163	-
Mantle width	100	61
Head length	52	32
Head width	55	34
Fin length	155	95
Fin width	15	9
Right arm 1	90	55
Right arm 2	D	-
Right arm 3	92	56
Right arm 4	105	64
Cuttlebone length	150	92
Cuttlebone width	60	37
Body weight (g)	318	-
Sex	Female	-
Maturity stage	Mature	-
Nidamental gland weight (NGW) (g)	24*	8
Accessory nidamental gland weight (ANGW) (g)	3*	0.9
Ovary weight (O) (g)	14*	4.4
Gonad weight (NGW+ANGW+ O) (g)	41*	12.9

D: Damaged, *Weight

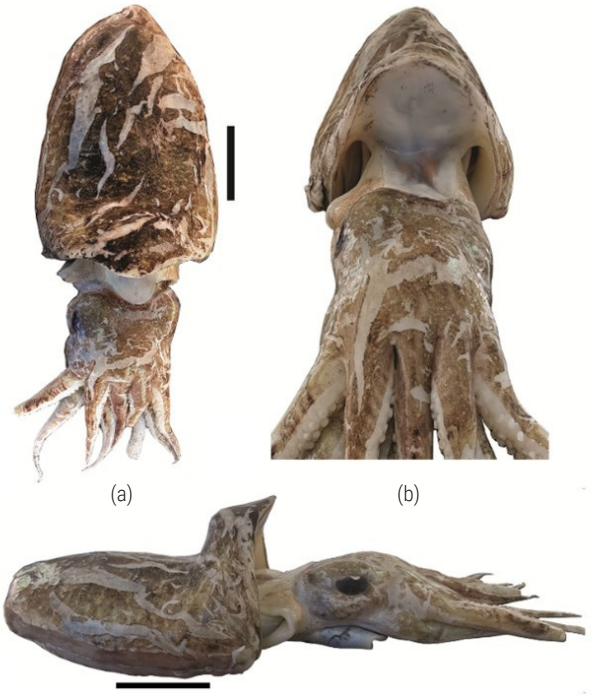

Fig. 3. (a) Dorsal view; (b) front view and (c) side view *A. pharaonis* with of abnormal cuttlebone from the Arabian Sea (Scale bar = 5 cm)

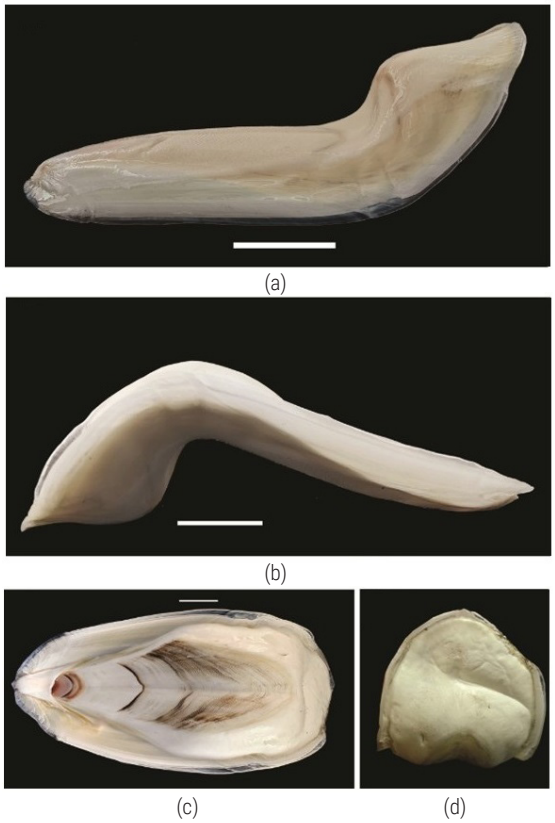

Fig. 4. Abnormal cuttlebone of *A. pharaonis* (a) Side view dorsal side; (b) Side view dorsal side down; (c) Ventral view and (d) Posterior front view (Scale bar = 5 cm)

in cephalopods (Féral, 1978, 1979; Bello, 1995). In *Sepia officinalis*, regeneration of a sectioned arm is a complex process comprising six distinct phases (Feral, 1978, 1979). Complete regeneration occurs within approximately three months, restoring the structure and function of the arms, to a state comparable to those of the other arms. Similar processes were observed in sepiolids and octopods (Feral, 1978, 1979). Previously, Kumpf (1960) reported arm abnormality (split arm) in the Caribbean reef octopus *Octopus briareus* Robson, 1929, from Big Pine Key, Florida, USA, and suggested this type of growth does not appear to be the result of an arm injury. The abnormalities, such as bifurcation and regeneration of the tentacular clubs, observed in a specimen of the deep-sea squid *Moroteuthopsis ingens* (Smith, 1881) from the South Atlantic, have been attributed to Hox gene mutations. Hox genes encode evolutionarily conserved transcription factors involved in skeletal patterning during embryonic development (Gonzalez and Guerra, 2008). Earlier, scar-bearing cuttlebone was reported from wild-caught *A. pharaonis* from the Arabian Sea (Venkatesan *et al*., 2020), indicating that the species is prone to predator induced injuries.

Congenital deformities are rarely reported in cephalopods, including cuttlefish, and are generally associated with disturbances during embryonic development resulting from genetic or environmental factors. Such abnormalities should be distinguished from traumatic injuries and subsequent regeneration of appendages, which are relatively common in cephalopods (Bloor *et al*., 2013). Given the nature of the observed abnormality, it could be attributed to a predator attack or accidental injury. Previous studies have reported GSI values ranging from 3.6 to 6.2 in mature *A. pharaonis* from the Arabian Sea (Sasikumar *et al*., 2015), suggesting that the observed arm abnormality did not adversely affect the maturity of the animal. This specimen did not exhibit any anomalies other than the arm bifurcation (Fig. 1). However, the individual with the abnormal cuttlebone had lost its tentacles and right second arm, which may have occurred during trawl operations. Nevertheless, this study represents the first report of an arm abnormality in *A. pharaonis* and provides preliminary information on the condition. Further studies involving larger sample sizes are required to better understand the occurrence and possible causes of arm abnormalities in cephalopods.

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