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# Occurrence of large mature Diamond-back squid *Thysanoteuthis major* (Cephalopoda: Thysanoteuthidae) from the southeastern Arabian Sea with remarks on its biology

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## Abstract

This article provides evidence of mature male and female *Thysanoteuthis major* caught from the southeastern Arabian Sea. The mature individuals had a dorsal mantle length (DML) of 570 to 670 mm. The detailed measurements of the individuals' morphometric features and indices are presented. Microstructural analysis of statoliths showed that the female individual was 194 days old with a 3.63 mm DML/day growth rate and the male had an age of 158 and 168 days with a growth rate of 3.38 and 3.62 mm DML/day. Two parasite species were found in the female stomach identified as genera cestode *Phyllobothrium* sp. and nematode *Anisakis simplex*. The presence of mature individuals provided further evidence that the southeastern Arabian Sea may be a spawning ground for the species.

**Keywords:** *Arabian Sea, Diamondback squid, mature, parasite, spawning, statolith*

## Introduction

The Arabian Sea is recognized as a highly diverse region for oceanic cephalopods (Aravindakshan and Sakthivel, 1973). Besides that, the fauna of oceanic cephalopods in the Arabian Sea are poorly explored when compared to other marine organisms (Paitkowski and Welsch, 1991). The diamond-back squid *Thysanoteuthis rhombus* Troschel, 1857 is a large squid that is believed to be the sole member of the family Thysanoteuthidae (Nesis, 1992) and distributed in tropical and subtropical oceans of the world (Nigmatullin and Arkhipkin, 1998). A recent study identified three distinct putative species with distributions aligned with the major ocean basins (Deville *et al.*, 2023). Accordingly, *Thysanoteuthis*

*major* (Gray, 1828), the most prevalent species, is widely found in the North Pacific Ocean, North Indian Ocean, and the southern limits of the Atlantic Ocean, while, *T. rhombus* Troschel, 1857, occurs in both the North and South Atlantic Oceans as well as the Mediterranean Sea, and *Thysanoteuthis filiferum* (Hoyle, 1904), in the southwestern Pacific Ocean. Therefore, it is assumed that all the previous records of the *T. rhombus* from the Indian waters are considered *T. major*.

Nigmatullin and Arkhipkin (1998), estimated the biomass of *Thysanoteuthis* spp. based on observations from night-light surveys and trawl catches, at 1.5-2.5 million tons from the world oceans. Commercial fishery for this species is reported from Japanese waters (Miyahara *et al.*, 2006a; 2008), the eastern Atlantic Canary Island and Jamaica (Perez *et al.*, 2012). The reproductive biology, morphology and anatomy of the reproductive system were illustrated by Nigmatullin *et al.* (1991).

Parasitic helminth fauna of large pelagic jumbo squid *Disidicus gigas* (Shukhgalter and Nigmatullin, 2001) from the central east Pacific and *T. rhombus* (Nigmatullin *et al.*, 1998) from the Atlantic and Pacific oceans were reported. Recently, parasitic helminths were found in *Sthenoteuthis oualaniensis* (giant form) from the Arabian Sea (Sajikumar *et al.*, 2021).

However, nothing is known about the morphology of the reproductive system and helminth fauna of mature male and female *T. major*. Therefore, the present article presents a report on the reproductive aspects of mature male and female *T. major* from the southeastern Arabian Sea. Also, estimate age and growth rate based on statolith increments analysis.

## Material and methods

### Sampling

Single individuals of mature males and females of *T. major* caught from the southeastern Arabian Sea by using drift gillnet during 17<sup>th</sup> October 2016, 15<sup>th</sup> November 2017, and 3<sup>rd</sup> January 2022. The sample was immediately iced and transported to the laboratory, where measurements were taken in millimeters for DML (dorsal mantle length), MW (mantle width), A1 (first arm), A2 (second arm), A3 (third arm) and A4 (fourth arm), HL (head length), HW (head width), FL (fin length) and FW (fin width) following the methods of Roper and Voss (1983). The length indices were calculated as a percentage of the dorsal mantle length. The total weight of the squid was recorded to the nearest gram, and the gonads were dissected out and weighed after ascertaining the maturity stage macroscopically.

### Maturity

The maturity stage for each specimen was based on Lipinski (1979). The sexual system of both males (spermatophoric gland and Needham sac) and females (Ovary, Oviducts, Oviductal gland and nidamental gland) were dissected and weighted to the nearest 1 g. One male (575 mm DML) specimen's reproductive system weight could not be taken because of the damaged condition of the reproductive system.

The gonadosomatic index (GSI) and maturity coefficient (MI) were determined (Nigmatullin *et al.*, 1995) as

Where GW is gonad weight and BW is the body weight.

$SSW = \text{Total weight of the sexual system}$

### Age estimation

The squid was aged using statolith increment analysis (Arkhipkin and Shcherbich, 2012).

Growth increments were assumed to be daily, based on the validation of growth increments of the same species from Pacific waters (Miyahara *et al.*, 2006b). Recent and past research supports the one-day-one increment hypothesis (Jackson, 2004). From the dates of capture, the hatching date was estimated. The daily growth rate (DGR) was determined using the equation from Jackson *et al.* (1997):

$DGR = (\text{Dorsal mantle length} - \text{hatchling size}) / (\text{age in days})$

Where, hatchling size was assumed to be 1.6 mm DML (Miyahara *et al.*, 2006b).

### Parasite estimation

The helminthological examination of individuals followed Zuev *et al.* (1985). The larvae of cestodes and anisakid nematodes were identified following Yamaguti (1959) and Berland (1961).

## Results

The mature female individual was caught from 09°17' N; 73°21' E and male 09°44' N; 75°38' E region, which is one of the major fishing grounds in the southeastern Arabian Sea (Fig. 1). The female individuals measured 670 mm in dorsal mantle length and weighed about 11.05 kg and the male, with severed arms, measured 570 mm DML with BW of 5.5 kg and 575 mm DML with BW of 7.2 kg. The arm formula of the present individuals was 3>2>4>1. In Comparison with DML, the highest indices were observed for fin width (94.8-97%) and fin length (87.8-92.5%) while the lowest was for arm I (25.2-28.7%). Among the arms, the third pair of arms was the largest (right: 52.2-61.2%; left 45.5-57%). The detailed morphometric measurements and morphometric indices of the specimen are provided in Table 1.

### Age and growth

The statolith measured from 1197 to 1358  $\mu\text{m}$  in length for males and females respectively, forming about 0.21 and 0.20-0.21 % of the dorsal mantle length. About 158 to 194 growth increments were recorded for males and female respectively (Table. 2, Fig. 3). Considering one-day-one increment, the squids had a growth rate of 3.63 mm DML/day for females whereas, in male, it was 3.38 and 3.62 mm DML/day.

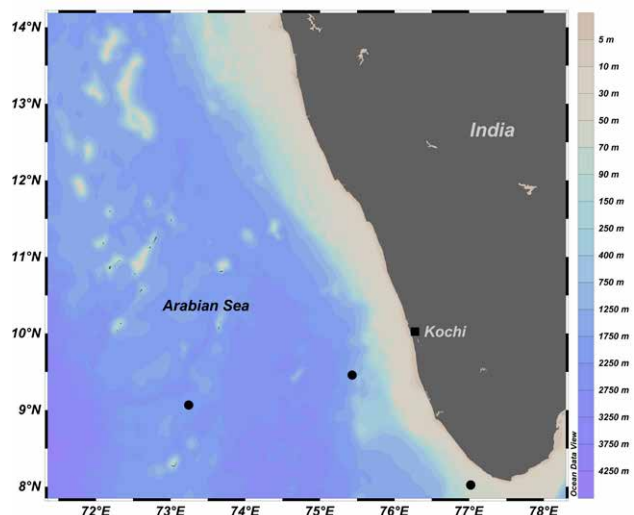


Fig. 1. Map showing the location of mature individuals of *Thysanoteuthis major* caught from the southeastern Arabian Sea



Table 1. Morphometric measurements and indices of mature *Thysanoteuthis major* caught from the southeastern Arabian Sea

Measurements (mm)	Specimen 1 ♀	Indices	Specimen 2 ♂	Indices	Specimen 3 ♂	Indices
Dorsal mantle length	670	100.0	570	100	575	100.0
Mantle width	210	31.3	192	33.6	185	32.2
Fin length	620	92.5	490	85.9	505	87.8
Fin width	650	97.0	525	92.1	545	94.8
Head length	115	17.2	104	18.2	108	18.8
Head width	170	25.4	158	27.7	120	20.9
Right arm 1	179	26.7	-	-	165	28.7
Right arm 2	230	34.3	-	-	195	33.9
Right arm 3	350	52.2	-	-	355	61.7
Right arm 4	230	34.3	-	-	200	34.8
Left arm 1	190	28.4	-	-	145	25.2
Left arm 2	235	35.1	-	-	190	33.0
Left arm 3	305	45.5	-	-	328	57.0
Left arm 4	220	32.8	-	-	195	33.9
Maturity stage	Mature	-	Mature	-	Mature	-
Total weight (g)	11050	-	5940	-	7280	-

### Female reproductive system

The oviducal gland was greatly enlarged, white and firm. The oviducts were comparatively small (12.3 % of DML), were swollen and the oviducts and ovary were pinkish (Fig. 4a, b). The oviducts with a straight distal portion were arranged with zigzag tubes of mature oocytes. In the ovary, ova were embedded in a thin branched blood vessel in the mantle cavity. The ovary fills about 30 % of the coelom volume. Oblate spherical ova filled in the oviducts ranged in size from 680 to 820  $\mu\text{m}$ , the average size being 790  $\mu\text{m}$  ( $n=30$ ) (Fig. 3c, d).

The oviducts of the spawning female contained 204 g of ova, representing 1.84% of the total weight of the specimen. The gonad weight (ovary, oviducts) was 524 g. The ratio of gonad weight (GSI) to body weight was 4.7%. The maturity coefficient (MI) was 10.3%.

### Male reproductive structure

The reproductive system of the mature male consists of a single testis and spermatophore complex with a total weight of 46 g and 48 g (Fig. 5) respectively. A spiral sperm duct was observed (Fig. 5a). The male left fourth arm was characterised

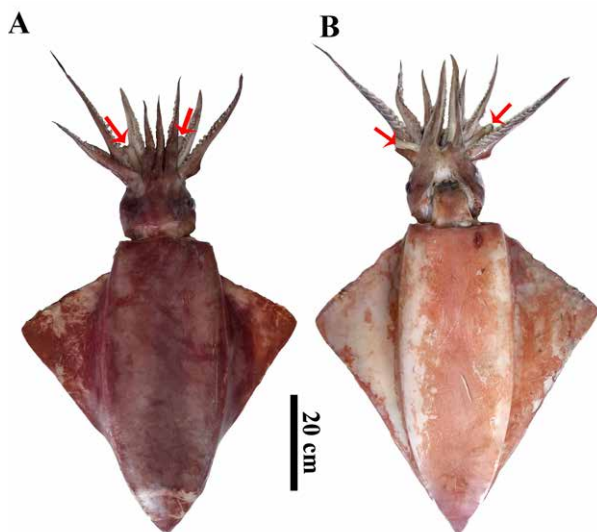


Fig. 2. Dorsal (A) and ventral (B) view of mature female *Thysanoteuthis major* caught from southeastern Arabian Sea (arrow mark indicates the remains of tentacles)

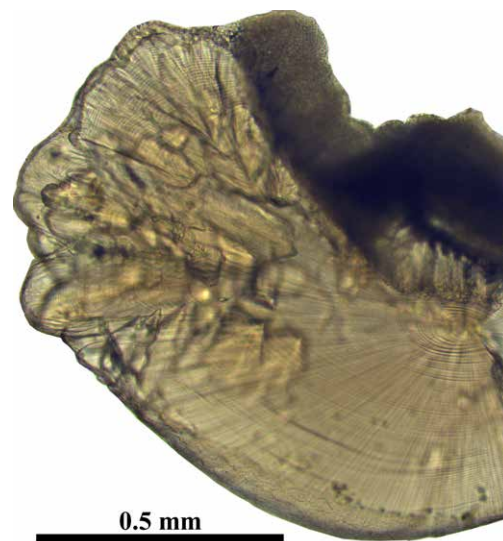


Fig. 3. Statolith microstructure of *Thysanoteuthis major* (male) from the Arabian Sea

Table 2. Statolith based age and growth details of *Thysanoteuthis major* from the Arabian Sea

SL. No	DML	Statolith length (μm)	Age (days)	Growth rate (mm DML/day)
1	570	1197	168	3.38
2	575	1245	158	3.62
3	670	1358	194	3.63

as hectocotylied with 56 and 54 suckers (Fig. 5b) and the right arm had 40 and 42 suckers. The length of the testis of the mature male is about 20.2% DML, and weight is 0.42% BW (Fig. 5c). The gonadosomatic index of the mature male was 0.77% BW. The Needham's sac is an elongated tube with a 390 mm length with 68.4% of the DML. The Needham's sac had 13 spermatophores with an average size of 58 mm (range=54-63 mm, sd± 2.73) and an average weight of 0.15 g (0.13 to 0.16 g, sd± 0.01) (Fig. 5d).

### Parasites

The stomach content of *T. rhombus* was examined to identify prey, however, the stomach was empty. We have found 13 alive parasites in the stomachs of female individuals. Among 13 individuals, nine individuals identified as the cestode (tapeworm) genera *Phyllobothrium* sp., and four individuals of nematode *Anisakis simplex* (Fig. 6a, b).

The total length of range of *Phyllobothrium* sp. ranges from 4.32 to 13.17 mm and maximum widths from 0.3 to 0.8 mm. The scolex width ranges from 0.78 to 1.84 mm (Table 3). The individuals have four folded bothridia with wavy edges, and each bothridia has an additional sucker (Fig. 6a). The

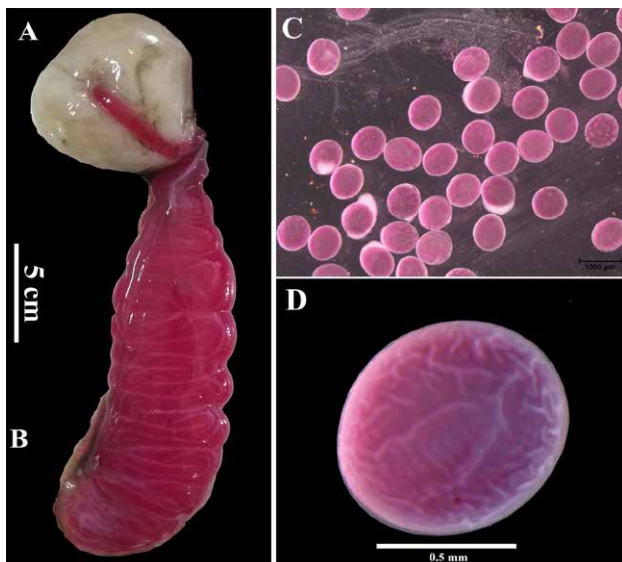


Fig. 4. Reproductive system of the mature female of *Thysanoteuthis major*: A) first section of oviducal gland; B) oviduct with eggs; C) eggs separated from oviduct; D) single mature egg

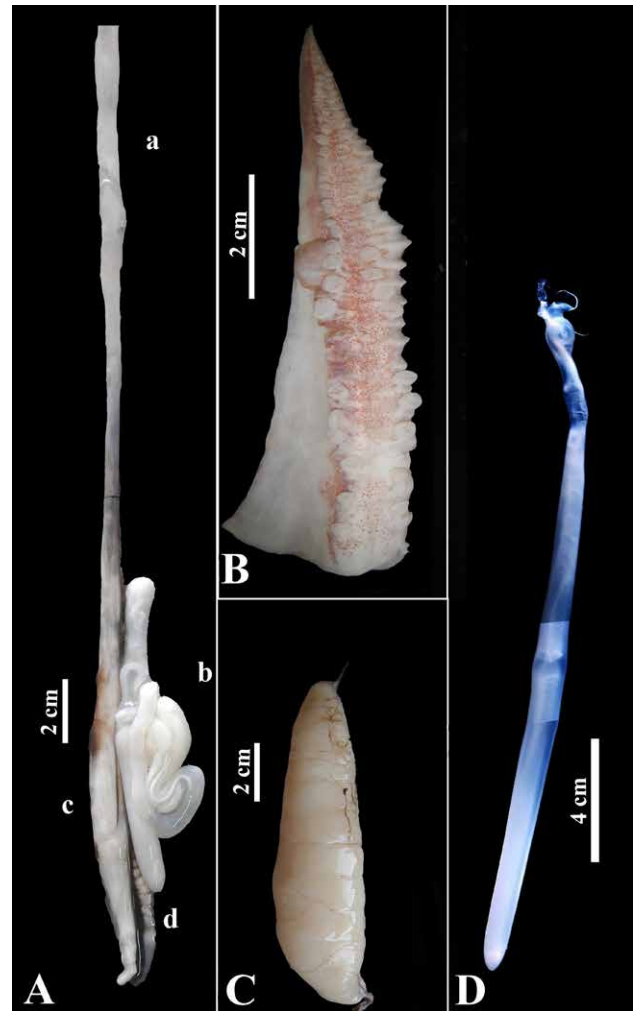


Fig. 5. Reproductive system of mature male of *Thysanoteuthis major* with 570 mm mantle length; A: a) Penis; b) Spermatophoric complex; c) Needham's sac; d) spermatid; B: hectacotylied arm; C: Spermatophoric gland; D: general view of spermatophore

nematode *Anisakis simplex* size ranged from 14.07 to 20.35 mm and maximum body width ranged from 0.31 to 0.47 (Table 4). The body's cephalic end is rounded, and its large ventral cuticular tooth is strongly pointed and bent ventrally. The tail is rounded, and long, ending in a sharp cuticular spike (Fig. 6b).

### Discussion

A recent study found that a sister relationship exists between *T. rhombus* and *T. major*, while a third species, *T. filiferum* was identified as the most divergent species (Deville *et al.*, 2023). *Thysanoteuthis* spp. is well known to fishermen from various parts of the world by several names such as box squid, sleeved squid, barrel squid, umbrella squid, and red squid (Roeleveld and Pfeiffer, 1987). Sajikumar *et al.* (2020) recorded fast growth in *Thysanoteuthis* spp. (*T. major*) distributed in tropical waters of the Southeastern Arabian Sea, while Nigmatullin *et al.*

Table 3. Details of morphometric measurements of cestode *Phyllobothrium* sp. found in the stomach content of *T. major* from the southeastern Arabian Sea

Measurements (mm)	1	2	3	4	5	6	7	8	9
Total length	3.56	4.32	4.90	5.81	9.38	9.71	11.3	11.5	13.17
Filament width	0.30	0.42	0.51	0.39	0.68	0.73	0.68	0.82	0.66
Maximum scolex width	0.87	0.78	1.24	1.4	0.97	0.98	1.45	1.84	1.41

Table 4. Detailed morphometric measurements of nematode *Anisakis simplex* observed in the stomach of *T. major* from the southeastern Arabian Sea

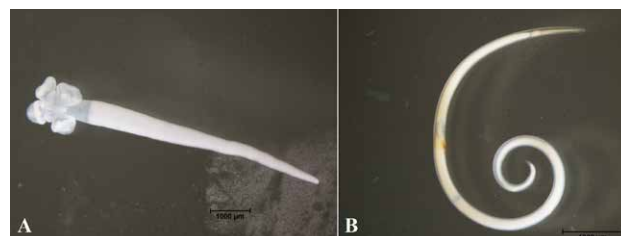
Measurements (mm)	1	2	3	4
Total length	20.35	14.26	16.72	18.23
Maximum width	0.47	0.31	0.38	0.41
Tail mucron length	0.13	0.09	0.10	0.11

(1995) reported slow growth in subtropical *Thysanoteuthis* spp. All three species delimited (Deville *et al.*, 2023) from the Mediterranean, Southern Japan, and the Southeast Pacific which spawns mainly in the warm seasons.

The capture of mature female *T. major* squid on 15 November 2017 represents the first record of a female in such an advanced stage of maturity from the southeastern Arabian Sea. The present mature female individual is larger than all previously known individuals from the Arabian Sea (Mohamed *et al.*, 2001; Sundaram *et al.*, 2007; Baby, 2011). Earlier, a mature male with a dorsal mantle length of 620 mm was reported from 09°26' N to 75° 31' E off Alleppey (Mohamed *et al.*, 2001). One mature female individual with a dorsal mantle length of 700 mm was reported from the Gulf of Mannar on the east coast of India (Kasim *et al.*, 1998).

The indices of the statolith were 0.20%, also reported in mature individuals from the tropical Atlantic (Nigmatullin *et al.*, 1995). The periodicity of increment formation as "one day, one increment" was validated in *T. rhombus* (*T. major*) from the southern Sea of Japan (Miyahara, 2006b). The age-DML relationship indicated that the present individual grew faster than those in subtropical and tropical waters of the world (Miyahara *et al.*, 2006a; Nigmatullin *et al.*, 1995) possibly because the southeastern Arabian Sea experienced a higher average temperature (~28 °C) than in subtropical waters (Jeswal *et al.*, 2012). It is well known that rising seawater temperatures will accelerate the growth of cephalopods (Forsythe, 2004). The results from a previous study from the southeastern Arabian Sea also support faster daily growth rates (Sajikumar *et al.*, 2020).

According to Nigmatullin *et al.* (1995), the spawning ground for *T. rhombus* was identified based on the areas in which egg masses, paralarvae, and fully mature squid were observed. Individual spawning for *T. rhombus* is reported to last for about 3–4 months when a female releases at least 8–12 egg masses during this period

Fig. 6. Parasites A) *Phyllobothrium* sp.; B) *Anisakis simplex* identified from the stomach of *T. major* from the southeastern Arabian Sea

(Nigmatullin and Arkhipkin, 1998). The intermittent (pulse, batch) spawning with several egg masses spawned during a more or less extended time (Harman *et al.*, 1989) is typical for most studied nektonic and micronektonic squids including Thysanoteuthidae *T. rhombus* (Nesis, 2002). The ratio of the relative weight of the reproductive systems (10.27%) is a bit lower than in Ommastrephidae (Laptikhovskiy, 1995). Previous studies of the relative weight of mature female reproductive systems ranged from 10–17% of BW and 1–1.2% of BW for males (Nigmatullin *et al.*, 1991). The comparatively lower relative weight of the reproductive system and reduced total number of eggs in the ovary compared to 2.7 to 4.8 million oocytes reported by Nigmatullin *et al.*, 1995, indicates that the current female individual may be in the last stage of intermittent spawning. Therefore, the fecundity of the individual in the oviduct could be the number of eggs released during each batch or the batch fecundity.

Earlier parasites from the stomach content of *T. rhombus* were reported (Nigmatullin and Arkhipkin, 1998). *Phyllobothrium* sp. and *Anisakis simplex* were observed in the stomachs of Ommastrephidae squids *Dosidicus gigas* from eastern Pacific waters (Riser, 1956; Dollfus, 1964; Shukhgalter and Nigmatullin, 2001). Snyder (1998) also found parasites in the stomach, mantle cavity and ovary of purpleback flying squid, *S. oualaniensis* (giant form) from the Arabian Sea. The main route of parasite infection is via ingestion of infected prey (Naidenova *et al.*, 1985).

The present findings of mature individuals (Nigmatullin *et al.*, 1995) indicate the southeastern Arabian Sea is a spawning ground of this large pelagic squid.

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## Author contributions

Conceptualization: KKS; Methodology: TBR; Data Collection: RJN, ARA; Data Analysis: KKS; Writing Original Draft: KKS; Writing Review and Editing: GS; Supervision: KSM

## Data availability

The data are available and can be requested from the corresponding author.

## Conflict of interests

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

## Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of sensitive live samples/ protected environments.

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