

# Reproductive system and the spermatophoric reaction of the mesopelagic squid *Octopoteuthis sicula* (Rüppell 1844) (Cephalopoda: Octopoteuthidae) from southern African waters

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Manuscript received April 2008; accepted October 2008

Reproductive features of the poorly known oceanic squid *Octopoteuthis sicula* are described and quantified to gain insight into the reproductive biology of the species. The data are based on 39 complete and partial specimens from southern African waters, collected between 1975 and 2005. The specimens ranged in mantle length from 38 mm to 290 mm and included juveniles and mature females and males. The species shows female-biased sexual size dimorphism. Ovulation is asynchronous, indicating a repeated spawning strategy. Males transfer spermatophores presumably by using their

long terminal organ. Spermatangia (discharged spermatophores) were found implanted in several parts of the body in both females and males, including in the anterior dorsal and ventral rugose, semi-gelatinous mantle tissue of maturing and mature females. This modified mantle tissue was only well developed in females. The morphologies of the spermatophore and the spermatangium of *O. sicula* are described. The spermatophoric reaction is reconstructed, using various stages of discharge, to provide insight into the process of intradermal implantation of spermatangia of this species.

**Keywords:** Cephalopoda, implantation, mating, *Octopoteuthis sicula*, reproductive system, spawning, spermatangium, spermatophore, spermatophoric reaction, squid

## Introduction

Squid are carnivorous nectonic marine molluscs with well-developed senses and inhabit all the world oceans from the continental shelf to the abyss. The sexes are separate and, although they have different spawning strategies (Rocha *et al.* 2001), reproduction is semelparous, i.e. there is not more than one reproductive cycle and no regeneration of the gonads (Nesis 1987).

Male gametes are packed into spermatophores — complex structures that are produced in a series of glands in the spermatophoric organ — and spermatophores are stored in the male reproductive system (Drew 1919). During copulation, spermatophores are transferred to the female and discharge into spermatangia during a process known as the spermatophoric reaction. Male squid do not fertilise the eggs internally but deposit spermatangia on the female. This happens either in or close to seminal receptacles consisting of modified tissue, or spermatangia are implanted into unmodified female tissue. The latter sometimes happens in such a way that spermatangia are completely covered by female tissue. The position of the seminal receptacles and implanted spermatangia varies among species (Hoving *et al.* 2004, Jackson and

Jackson 2004, Nesis 1995, Norman and Lu 1997, O'Shea *et al.* 2007). Males of some species use a modified arm, the hectocotylus, for the transfer of spermatophores. In species that lack a hectocotylus, a long terminal organ (an extension of the Needham's sac sometimes termed penis) is apparently used instead (Nesis 1995).

The mechanism responsible for deep spermatangium implantation is poorly understood but to be found in the spermatophoric reaction (Hoving and Laptikhovskiy 2007). The spermatophoric reaction has only been studied for a few cephalopod species: *Enteroctopus dofleini* (Wulker 1910), *Todarodes pacificus* (Steenstrup 1880) and *Loligo pealei* (LeSueur 1821) (Marchand 1913, Drew 1919, Austin *et al.* 1964, Hanson *et al.* 1973, Takahama *et al.* 1991). None of these species implant spermatangia deeply.

After initiation of the spermatophoric reaction, the process of spermatangium implantation may be observed directly on spermatophores that are removed from fresh male squid (Hoving and Laptikhovskiy 2007). In preserved male squid, partly discharged spermatophores are sometimes found inside the terminal organ. In such cases, the spermatophores probably started inverting during capture or handling

of the specimen, and the spermatophoric reaction stopped after the spermatophores came into contact with the preservative (formalin or alcohol). A wide variety of spermatangia was described by Marchand (1913). By studying the different stages of discharged spermatophores, the functional components of the spermatophore can be tracked and the spermatophoric reaction can be reconstructed. Therefore, detailed morphological studies of spermatophores, partly discharged spermatophores and implanted spermatangia can provide insight in the mechanism of spermatangium implantation in the absence of fresh or frozen specimens.

The Octopoteuthidae is a poorly known squid family that implants spermatangia into unmodified tissue, probably with aid of its long terminal organ (Nesis 1995). Members of this family inhabit the meso- and bathypelagic waters of the world oceans and are an important component in the diet of oceanic predators like cetaceans (Clarke 1996, Roper and Vecchione 1993). Currently, the family comprises seven poorly defined species. The specimens studied here seemed to belong to a single species and all had two light organs in the posterior ventral mantle, light organs on the base and close to the core of the ventral arms III and IV, and accessory cusps on the arm hooks. We tentatively gave it the available name *Octopoteuthis sicula*, but the systematics of the genus *Octopoteuthis* is unstable.

In the tropical Atlantic, *Octopoteuthis* spp. seem to spawn seasonally, with a peak between March and June (Stephen 1986). Except for some reproductive features described for *Octopoteuthis rugosa* (Clarke 1980) from the stomachs of sperm whales *Physeter catodon* (Clarke 1980), there is little information on the general reproductive biology (reproductive morphology, fecundity, mating and ovulation) of any species in the genus.

This study presents for the first time data on sexual dimorphism, male and female fecundity and ovulation of *O. sicula*. The presence, structure and transfer of spermatophores and spermatangia of *O. sicula* were also investigated. Finally, the spermatophoric reaction and the implantation process of spermatangia were reconstructed using different stages of discharged spermatophores. This allowed a discussion about intradermal implantation of squid spermatangia, a mechanism that is widely used among oceanic squid, but poorly understood.

## Material and Methods

A total of 39 complete and partial *O. sicula* were studied. Animals were collected between 1975 and 2005, either by trawls or removed from the stomachs of predators (sperm whales or tuna), and were fixed and stored in 10% formalin. Fishing was done in an area between 27° S and 36° S and between 14° E and 31° E by the research vessels FRS *Africana*, RV *Dr Fridtjof Nansen* and *Boulonnais*. Depth of trawling was between 100 m and 1 478 m. Details of the localities and collections are given in Table 1. In every specimen, the dorsal mantle length (ML), total spermatophore length (TSL), terminal organ length (TOL) and the length and maximum width (maxW) of various structures were measured to the nearest 0.1 mm using a caliper. The body mass (BM) and the mass of specific organs

were measured to the nearest 0.1 g. A simplified maturity scale was used, where J = juvenile (sex undetermined); Stage I = maturing (sex can be determined but specimens are not mature) and Stage II = mature (ova in oviducts or spermatophores in terminal organs).

To estimate the number of oocytes inside the ovary, the oocytes and resorptive oocytes in a 0.5 g subsample of the ovary were counted and measured along their longest axis. The total number of (resorptive) oocytes in the ovary was estimated by extrapolating the number of oocytes in the subsample with known mass to the total mass of the ovary.

Different stages of partly inverted spermatophores were found in the terminal organ of some mature males and these were studied and measured using a stereomicroscope with a calibrated graticule. The spermatophoric reaction of *O. sicula* could be reconstructed by studying different stages of spermatophore discharge and by comparing those with published information on the spermatophoric reaction of *L. pealeii* (Drew 1919) and of *T. pacificus* (Takahama *et al.* 1991). The terminology used is based on Drew (1919) using the Tree of Life webpage illustration by Young *et al.* (2000) of the reconstruction of the spermatophoric reaction of *L. pealeii*.

## Results

### Sexual dimorphism

Mature females had a ML between 195 mm and 290 mm and a BM ranging from 328 g to 700 g ( $n = 7$ ) (Figure 1). Mature males had a ML range of 117–200 mm and a BM of 96–476 g ( $n = 7$ ). Although the sample size is small and overlap exists, there appears to be sexual size dimorphism with mature females attaining generally a larger body size than mature males.

There seems to be sexual dimorphism in the morphology of the anterior exterior mantle tissue. Maturing and mature females have rugose anterior part of the mantle (Figure 2) in the form of very regular, or branching, deep longitudinal fleshy strips covered with gelatinous tissue. Mature males also have some rugose structure, but no strips, and rather irregular, shallow and delicate wrinkles that are on the lateral surfaces only.

### Female reproductive system

The reproductive system of females consists of an ovary, paired oviducts with oviducal glands and nidamental glands.

The ovary is situated in the posterior half of the mantle cavity and its mass ranged between 25 g and 39 g (3.6–6.2% BM) in three females. Oviducts were situated laterally under the visceral sac and opened near the base of the gills. The length of the oviducts in the mature females ranged between 41 mm and 63 mm (20–23.8% ML) with a maxW of 7–23 mm. The oviducts had 12–14 convolutions. The number of ova held in the oviducts was counted in three females, and the number increased with mantle length. The ova measured approximately 2 mm in length. The oviductal eggs numbered 262 and 293 in one female (219 mm ML), 425 and 450 in another (230 mm ML), and 3 126 and 1 421 in a female of 265 mm ML. The potential

**Table 1:** Locality and collection details of the examined juvenile, male and female specimens of *O. sicula*

Catalogue no./specimen no.	Sex/stage	ML (mm)	Locality	Gear	Date	Depth (m)	Ship
SAM-S1992	M II	–	34°48.8' S–18°8.1' E	MT	3/10/1988	700	Africana
SAM-S3456	M II	200	34°0.5' S–27°10.4' E	MT	18/10/1991	786	Africana
Nan-1	M II	195	32°43' S–16°34' E	BT	2/07/2003	752	Dr Fridtjof Nansen
SAM-S3864	M II	155	–	–	–	–	–
SAM-S3457	M II	152	27°22.3' S–14°3.7' E	BT	15/08/1988	880	Africana
SAM-S3460	M II	150	30°47.8' S–15°9.9' E	MT	3/09/1991	1 478	Africana
SAM-S2036	M II	117	33°38.1' S–17°24.1' E	MT	3/06/1988	910–990	Africana
SAM-S3457	M I	150	27°22.3' S–14°3.7' E	BT	15/08/1988	880	Africana
Nan-3	M I	108	–	BT	2/04/2002	–	Dr Fridtjof Nansen
Nan-4	M I	99	–	BT	3/02/2004	650	Dr Fridtjof Nansen
Nan-5	M I	93	–	BT	21/02/2001	960	Dr Fridtjof Nansen
Nan-6	M I	85	32°43' S–16°36' E	BT	26/02/2005	682	Dr Fridtjof Nansen
SAM-S3454	M I	63	36°45.4' S–21°50.1' E	MT	24/10/1992	750	Africana
SAM-S3454	M I	53	36°45.4' S–21°50.1' E	MT	24/10/1992	750	Africana
SAM-S3574	M	195	30°10.0' S–14°33.1' E	BT	17/08/1988	855	Africana
SAM-S3402	M	180	35°31.0' S–18°49.0' E	T	10/05/1996	853	Boulonnais
Nan-2	M	118	–	BT	2/04/2002	754	Dr Fridtjof Nansen
SAM-S3954	F II	290	–	–	–	–	–
Nan-7	F II	265	31°55' S–16°02' E	BT	22/02/2005	668	Dr Fridtjof Nansen
SAM-S2707	F II	235	35°9.8' S–23°37' E	MT	19/10/1992	990–1 110	Africana
Nan-8	F II	230	32°43' S–16°34' E	BT	2/07/2003	752	Dr Fridtjof Nansen
SAM-S1049	F II	230	33°35' S–17°10' E	RMT 8	26/07/1982	165–100	Africana
Nan-9	F II	205	32°43' S–16°35' E	BT	26/02/2005	753	Dr Fridtjof Nansen
SAM-A29638	F II	195	33°41.0' S–17°29.0' E	Ex. TT	13/05/1961	–	–
SAM-S643	F I	192	30°43.0' S–31°10.0' E	Ex. SW	4/03/1975	–	–
SAM-S987	F I	102	31°27.0' S–16°37.8' E	–	22/08/1982	–	–
Nan-10	F I	101	32°43' S–16°36' E	BT	26/02/2005	306	Dr Fridtjof Nansen
Nan-11	F I	50	–	BT	24/01/2000	924	Dr Fridtjof Nansen
Nan-12	J	47	–	BT	24/01/2000	924	Dr Fridtjof Nansen
Nan-13	J	44	–	BT	22/02/2001	950	Dr Fridtjof Nansen
Nan-14	J	43	–	BT	2/11/2001	880	Dr Fridtjof Nansen
Nan-15	J	38	–	BT	2/11/2001	880	Dr Fridtjof Nansen
Nan-16	J	39	–	BT	24/01/2000	924	Dr Fridtjof Nansen
SAM-S2066	J	35	34°53' S–18°5.1' E	MT	3/10/1988	913–918	Africana
SAM-S3455	–	Head	36°23.9' S–19°21.8' E	MT	23/01/1992	1 000	–
SAM-S2066	–	Head	34°53.0' S–18°5.1' E	MT	3/10/1988	913–918	Africana
SAM-S3459	–	Remains	–	MT	23/05/1982	600	Africana
SAM-S643	–	180	30°43.0' S–31°10.0' E	Ex. SW	4/03/1975	–	–
–	–	175	30°43.0' S–31°10.0' E	Ex. SW	4/03/1975	–	–
SAM-S2392	–	145	30°10.2' S–14°33.5' E	BT	20/02/1988	814	Africana

SAM-xxx = catalogue number of the South African Museum in Cape Town, South Africa

Nan-x = specimen collected by RV *Dr Fridtjof Nansen*

M = male; F = female; J = juvenile

MT = midwater trawl; BT = bottom trawl

ex. TT = from the stomachs of tuna; ex. SW = from the stomachs of sperm whales

fecundity for the latter two females was 132 000, and 216 000 (+ 72 000 resorptive oocytes) respectively. In the ovaries of both females, unripe oocyte size ranged from 0.08 mm to 1.75 mm.

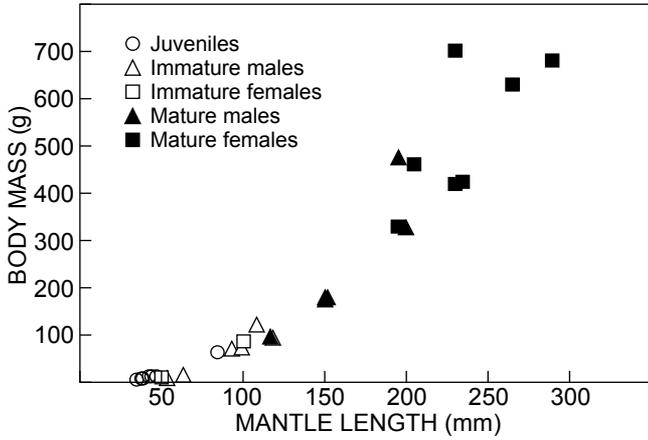


Figure 1: Relationship between the mantle length and body mass of juvenile, male and female *O. sicula*

Oviducal glands were 20–35 mm in length (9.4–13.9% ML) and had a maxW of 10–13 mm (3.4–5.7% ML) for mature females. Nidamental glands of mature females (205–290 mm ML;  $n = 5$ ) ranged between 54 mm and 100 mm (26.3–35.7% ML). MaxW of the nidamental glands was 13–28 mm (6.3–10.9% ML).

The relative mass of the reproductive system ranged between 7% and 13.1% BM for three mature females 230–290 mm ML.

**Male reproductive system**

Not all seven mature males had completely intact viscera or complete bodies. The total mass of the male reproductive system including the testis was determined for four individuals and ranged between 4.1% and 6.8% BM (150–200 mm ML). The mass of the testis in six mature males ranged between 3.1 g and 12 g (2–3.7% BM; 117–200 mm ML). In six mature males (117–200 mm ML), the terminal organ was 104–180 mm (82.2–92.3% ML) long, 11–20 mm (5.5–10.3% ML) wide, was muscular and extended between 37.4–54.7% ML beyond the mantle cavity (Figure 2). In three immature males (93–108 mm ML), the total reproductive system mass

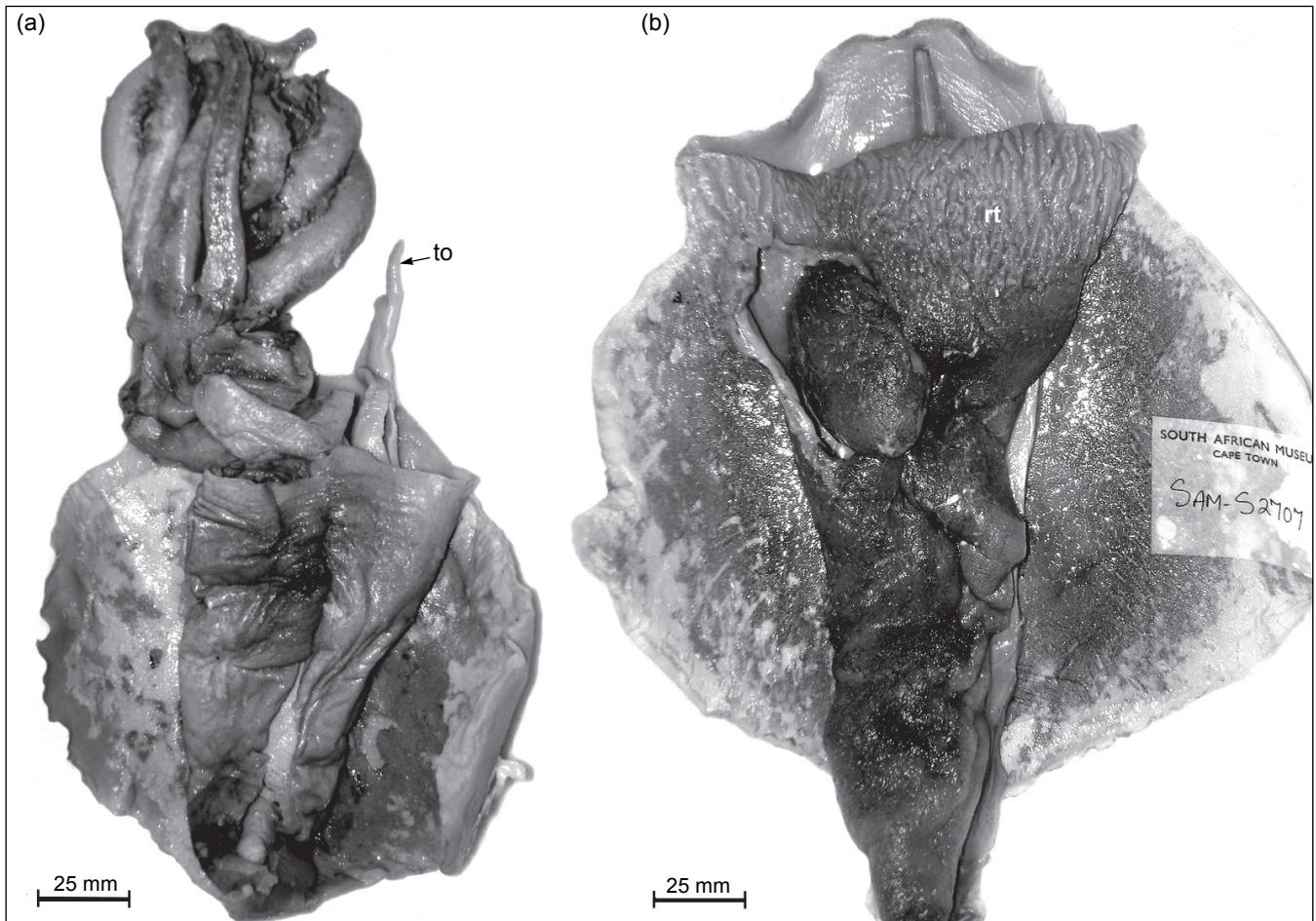


Figure 2: Ventral view of the mantle of *O. sicula*: (a) a mature male with the terminal organ [to] indicated, (b) a mature female with the rugose mantle tissue [rt] indicated

was 0.8–1.0% BM with the testis weighing between 0.2 g and 0.4 g. In four immature males (85–108 mm ML), the terminal organ was 27–43 mm (31.8–49.1% ML) and MaxW 3–4 mm (3–4.7% ML).

### Spermatophores

Spermatophore length seems to increase with mantle length. Measurements in five mature males (ML 117–200 mm) showed the smallest spermatophores (10.9–11.4 mm TSL; mean =  $11.2 \pm 0.2$  mm,  $n = 11$ ) for the smallest male and the largest spermatophores (16.5–17.5 mm TSL; mean =  $17.1 \pm 0.36$  mm;  $n = 10$ ) for the largest male. Total number of spermatophores in five males ranged between 100 and 1 050, where the greatest number was for a male 195 mm ML and the smallest number for a male 200 mm ML.

As in loliginid squid (Drew 1919), the spermatophore consists of an ejaculatory apparatus, a cement body and a sperm mass (Figure 3a). The sperm mass is relatively long (approximately 75% TSL) and is connected to the

cement body by a small tube (Figure 3b). The cement body (approximately 13% TSL) consists of two morphological components (Figure 3b). The first component, closest to the sperm mass, seemed to be filled with transparent cement and the second component extends orally from the first as a tube. The oral part of the tube broadens into the ‘anchor’ (Hess 1987). The ejaculatory apparatus (approximately 12% TSL) extends orally from the anchor. The coiling of the ejaculatory apparatus increases orally. The inner tunic and middle membrane are indicated in Figure 3b.

### Spermatangia implants

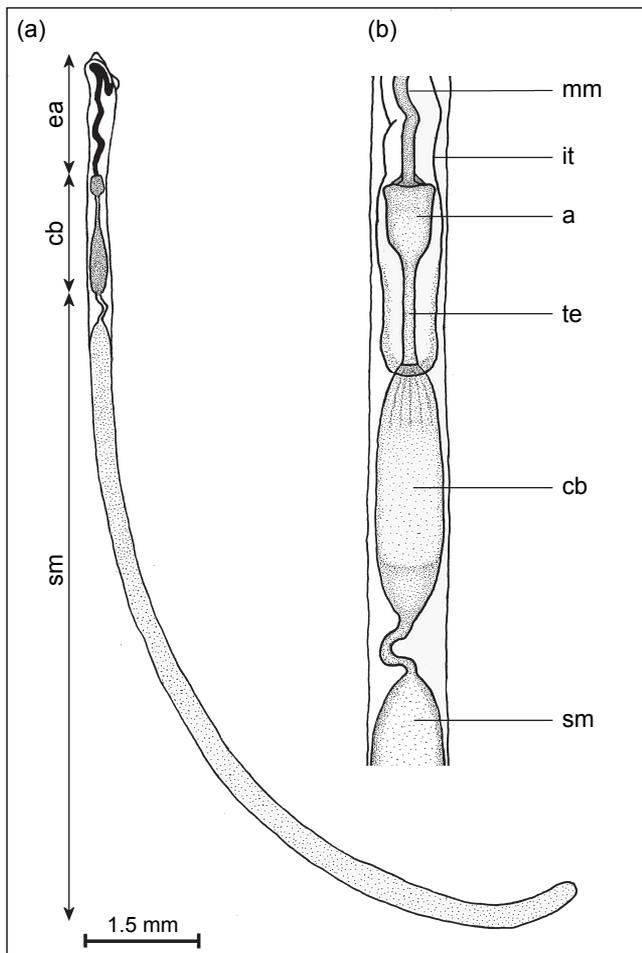
Spermatangia were found implanted in nine females, four males and two animals of undetermined sex (Table 2, Figure 4). The position and sometimes the number of spermatangia were recorded (Table 2).

The smallest female that had mated measured 102 mm ML and was immature. Also, an immature male without spermatophores in its terminal organ (SAM-S3457: 150 mm ML) had spermatangia embedded ventrally under its left eye. Three mature males had spermatangia on the outside of their arms, one of which had another single spermatangium in the ventral anterior mantle.

In nine mated females, spermatangia were found implanted on the outside of some arms, in the dorsal part of the head, around the eyes, in the mantle, in the rugose anterior mantle tissue and in the fin. When only the presence of spermatangia on these positions was taken into account for the nine females, six of these females were mated in their mantle, of which four also had spermatangia in the semi-gelatinous rugose mantle tissue. Four females were mated in their head, three females were mated in their arms and eyes and two females had spermatangia in their fin. In two of these females, spermatangia were found around the two light organs in the posterior ventral mantle. One of the two specimens that could not be sexed had spermatangia in its fin, and the other had spermatangia in the mantle and semi-gelatinous rugose mantle tissue.

The number of spermatangia at one place varied from one up to 60 (Table 2), but the number of spermatangia was not recorded for all specimens. The spermatangia were often superficially implanted, mainly in the gelatinous tissue on the above mentioned locations. However, in one female (SAM-S643: 192 mm ML), spermatangia actually penetrated into the mantle cavity through the mantle. Another exception was the finding of superficially implanted spermatangia in the outer collar on both sides of the nuchal cartilage.

Spermatangia were approx. 2.5 mm long with a maxW of 1 mm (Figure 5a). Each consisted of a central bulbous region containing the sperm mass and a thin aboral open tube. This open aboral end was sticking out of the tissue of the female whereas the rest of the spermatangium was covered by tissue. Orally, the spermatangium had another tube that often folded back and adhered to the central bulb (Figure 5a). This latter tube ended in a club-shaped structure, which most likely is the anchor in the intact spermatophore. The loose spermatangia from the terminal organ (discharged but not implanted) had their oral tube still pointing forward, showing the anchor (Figure 5b). A close-up



**Figure 3:** The spermatophore of *O. sicula*: (a) overview of the whole spermatophore, (b) detail of the cement body. Abbreviations: ejaculatory apparatus (ea); cement body (cb); sperm mass (sm); middle membrane (mm); inner tunic (it); anchor (a) and tubular extension (te)

**Table 2.** Position and number (in parenthesis) of implanted spermatangia in male and female *O. sicula*. 'Present' is used when the exact position was not recorded

Catalogue no./ specimen no.	Sex/ stage	ML (mm)	Arms	Eyes	Mantle	Head	Gelatinous folds anterior mantle	Fin
SAM-S1992	M II	–	Base LI Base RII, RIII					
SAM-S3457	M I	150		Ventral under left				
Nan-1	M II	195	Base RIII, RIV		Ventral anterior			
SAM-S3456	M II	200			(1)			
SAM-S987	F I	102	Base LI					Dorsal anterior margin
SAM-S643	–	175						Right ventral anterior margin (5)
	–	180			Anterior ventral right (5)		Present	Dorsal anterior margin (25)
SAM-A29638	F I F II	192 195		Around and in right (40)	Ventral anterior right		Present (60)	Dorsal anterior margin
Nan-9	F II	205			Around posterior light organs (6)		Present (40)	
SAM-S1049	F II	230			Ventral anterior left			
Nan-8	F II	230	Base LIII, LIV	Dorsal above right	Ventral anterior left Light organs			
SAM-S2707	F II	235			Complete ventral		Present	
Nan-7	F II	265	Base RIV	Above left (60) Under left (10)	Dorsal anterior gelatinous Dorsal anterior gelatinous (10)		Present (20)	
SAM-S3954	F II	290			Ventral anterior right			Dorsal Ventral base LRIV

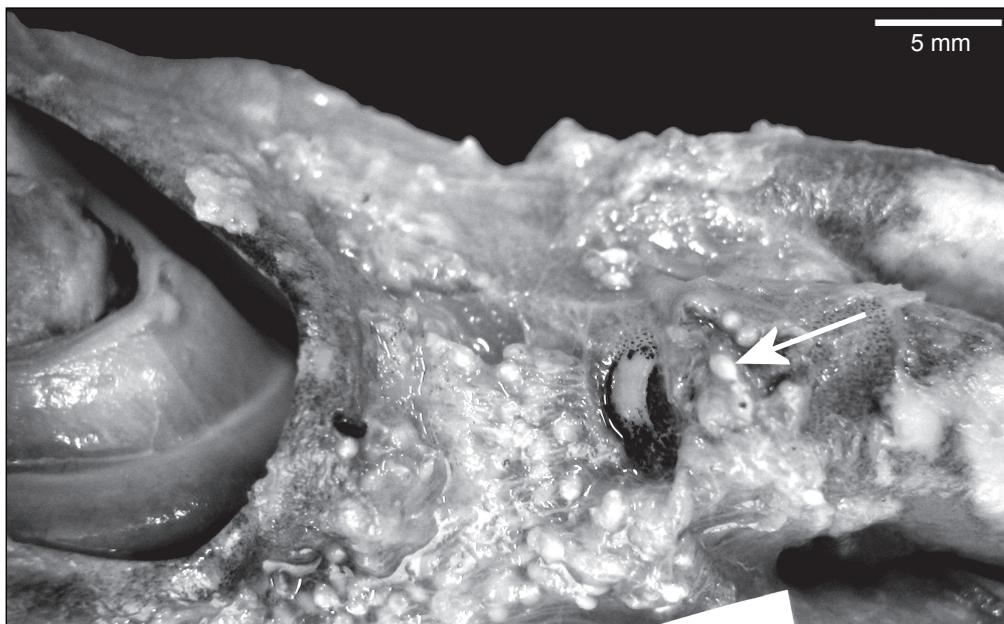
SAM-xxx = catalogue number of the South African Museum in Cape Town, South Africa

Nan-x = specimen collected by RV *Dr Fridtjof Nansen*

M = male; F = female; J = juvenile

R and L refer to right and left arms respectively

I–IV refer to arm pair number



**Figure 4:** Implanted spermatangia (a spermatangium is indicated by the arrow) on the head of a mature female *O. sicula* (SAM-S1049: 230 mm ML)

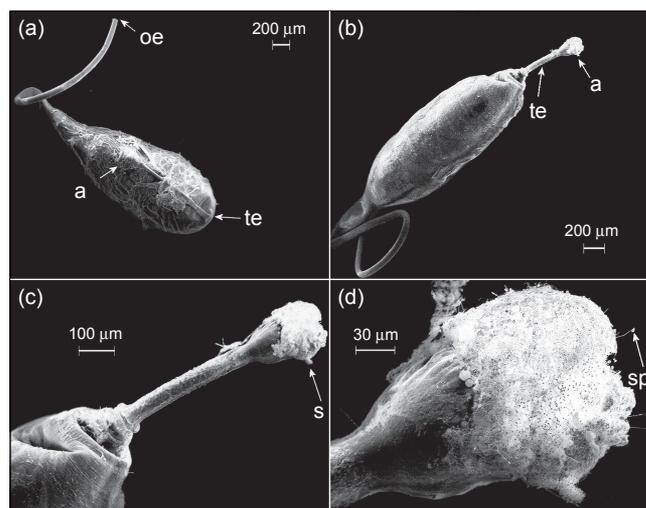
of the anchor of one of these spermatangia showed the presence of spermatozoa (Figure 5c, d).

**Discussion**

Male *O. sicula* do not have hectocotylied arms and presumably transfer spermatophores with their large terminal organ. The morphology of the spermatophore of *O. sicula* resembles that of *Octopoteuthis rugosa* (Clarke 1980). However, what that author defines as the ejaculatory apparatus is here regarded as the cement body. Hess (1987) proposed that the variations in the ejaculatory apparatus described by Clarke (1980) are most likely the result of rupture of the spermatophore. This is confirmed by the morphological similarity between the spermatophores of Clarke (1980) and the partly discharged spermatophores in this study.

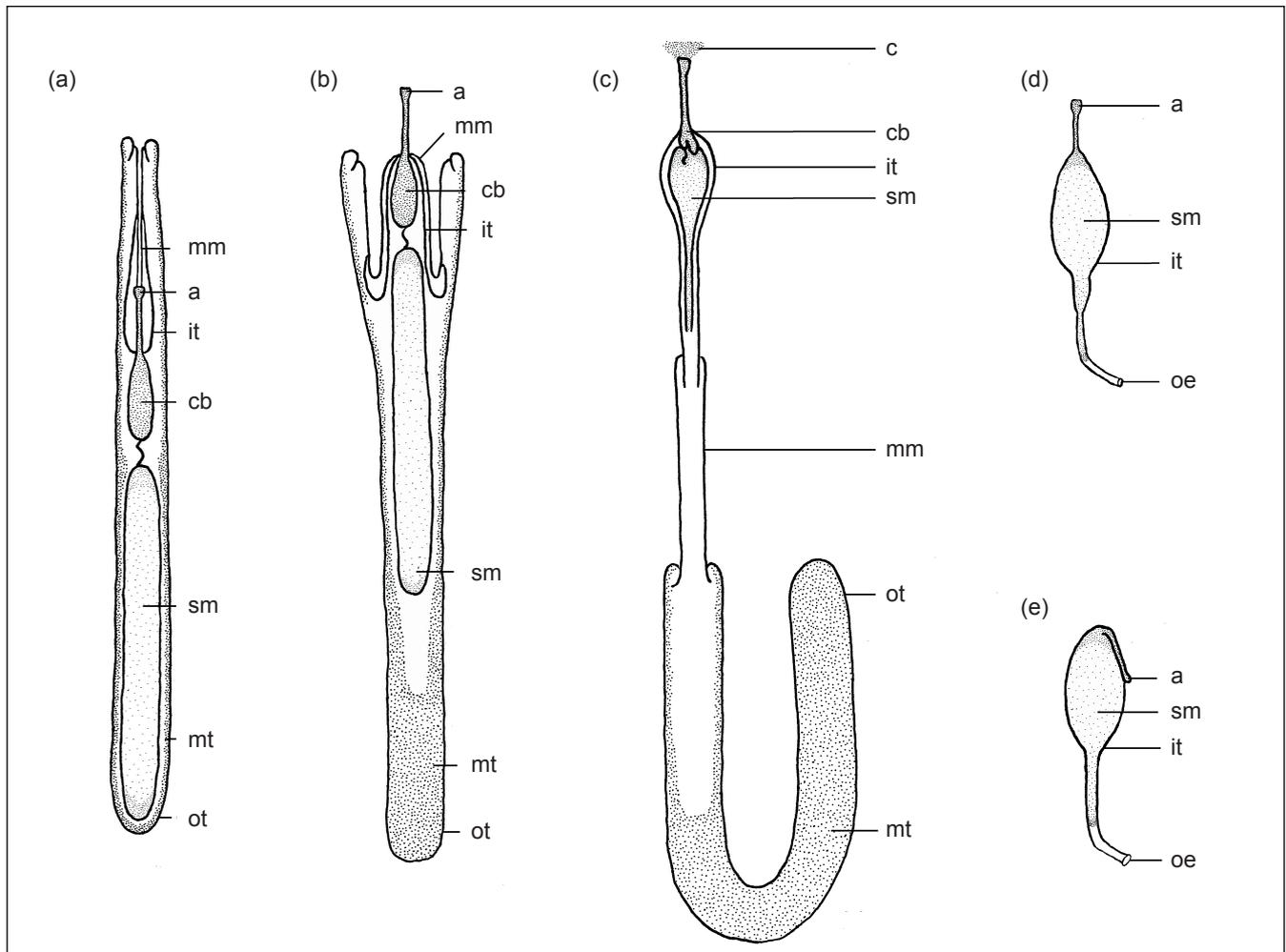
Male *O. sicula* implant spermatangia on various places on the female bodies, even before the females reach full maturity. Spermatangia were also found implanted in four males, possibly from their own spermatophores. This could have been caused by trauma during capture or by accidentally implanting them during mating with a female. The same has been proposed for mated males of *Architeuthis* (Hoving *et al.* 2004, Kjennerud 1958, Norman and Lu 1997). On the other hand, the finding of spermatangia in an immature male *O. sicula* suggests that male-to-male mating can occur.

Nesis (1995) suggests that octopoteuthids implant spermatangia at different locations of the body, but that only those that are properly located will be used by the female during spawning. So what is the proper place for spermatangium deposition in octopoteuthids? Clarke (1980) found spermatangia implanted in the anterior ‘grooved mantle jelly and in the head jelly’ in *O. rugosa*, as was observed in the present study. When only the presence of implanted spermatangia is taken into account (and not the number), the mantle, especially the semi-gelatinous rugose mantle



**Figure 5:** (a) An implanted spermatangium removed from female tissue showing the tubular extension [te] and anchor [a] adhered to the spermatangium, and the open aboral end [oe]; (b) a spermatangium removed from the terminal organ showing the tubular extension [te] with anchor [a] pointing orally; (c) details of (b) showing a bundle of sperm [s] coming out of the anchor of the spermatangium; (d) details of the anchor and bundle of sperm showing individual spermatozoa [sp]

tissue, and the head seem to be the preferred places for spermatangium deposition in *O. sicula*. Although some spermatangia were found implanted in the muscular mantle wall, the semi-gelatinous tissue that is present on the head and mantle may be a better medium for implantation, and positioning the spermatophores there during mating may perhaps reduce sperm loss. Clarke (1980) did not find the mantle jelly to be grooved in a male specimen



**Figure 6:** Schematic reconstruction of the spermatophoric reaction in *O. sicula*: (a) the intact spermatophore; (b) the spermatophore at the beginning of the spermatophoric reaction with the anchor of the cement body leaving the spermatophore first; (c) the forward-moving sperm mass pushes the cement body through the tubular extension and anchor; (d) the released spermatangium with the anchor pointing forwards; and (e) the final implanted spermatangium with the anchor adhered to the spermatangium. Abbreviations: middle membrane (mm); anchor (a); inner tunic (it); cement body (cb); sperm mass (sm); middle tunic (mt); outer tunic (ot); cement (c) and open aboral end (oe)

of *O. rugosa* and he proposed the furrows to constitute a sexual difference. This proposition was confirmed in this study for *O. sicula* where the semi-gelatinous, rugose longitudinal strips on the mantle were only well developed in females. The same observation was found on a collection of *Octopoteuthis deletron* from the north Pacific. Only females of this species have the grooved semi-gelatinous anterior mantle tissue (HJTH, pers. obs.). The association of spermatangia with the grooved semi-gelatinous mantle tissue indicates that this modification is specifically for seminal reception. However, this would suggest that the male is not very accurate in situating the spermatangia because they were also found implanted in other female body parts. Modified tissue for the reception of spermatangia is found in oceanic squid families. In the Enoploteuthidae, Lycoteuthidae and Pyroteuthidae, the receptacle is located in the nuchal area of females (Burgess 1998, Hoving *et al.* 2007, Young and Harman 1998). In the Ommastrephidae, spermatangia are placed

in a ring around the mouth on the buccal membrane and spermatozoa are transported from the spermatangia into special seminal receptacles for storage (Nesis 1995).

It is unknown how female squid mobilise sperm from implanted spermatangia, but if *O. sicula* spawn eggs through the funnel, the ventrally located spermatangia may be best situated during fertilisation. For fertilisation of the eggs, the spermatozoa may exit through the aboral tubular trailing end of the implanted spermatangium, which opens to the exterior. Or the female may peel open the rugose gelatinous tissue with their hooked arms, which would expose spermatozoa from the spermatangia to the spawned eggs. If the latter is true, spent females would show considerable damage to their ventral mantles. However, spent female *O. sicula* remain undescribed.

The presence of oocytes of all sizes inside the ovary and ripe ova in the oviduct indicates that *O. sicula* has asynchronous vitellogenesis leading to a repeated spawning strategy, where females produce several egg batches over time. It is

not known whether or not females continue to grow between spawning events. In one ovary of *O. sicula*, 25% of all the oocytes in the ovary were resorptive. Oocyte resorption is a known phenomenon in cephalopods and may function to remove surplus oocytes for which there are inadequate energy reserves to proceed with oocyte maturation (Melo and Sauer 1998).

In some squid, both the removal of the spermatophore and the initiation of the spermatophoric reaction are done by the hectocotylus. For *Moroteuthis ingens*, a species lacking a hectocotylus, the spermatophores move forward in the penis, probably by peristaltic movement of this organ, and the spermatophoric reaction is initiated by the tip of the terminal organ, which either pulls the thread or presses the oral end of the spermatophore (Hoving and Laptikhovskiy 2007). Because *O. sicula* lacks a hectocotylus and has a similar reproductive morphology as *M. ingens*, the spermatophoric reaction in *O. sicula* is probably also initiated by the terminal organ. Hess (1987) described the distal tip of the penis of *Octopoteuthis* sp. A to form a spatulate disc with an irregular transverse aperture. No such shape was found in the squid under study. The terminal organ of *O. sicula* extends freely from the mantle margin and the distal tip may be easily damaged during capture in the net, which may explain why we did not find this structure. The spermatophoric reaction of *O. sicula* could well be initiated by the distal tip of the terminal organ if it had the shape described by Hess (1987).

Using knowledge on the spermatophoric reaction from the literature and the morphologies of spermatangia of *O. sicula* in different stages of discharge, the spermatophoric reaction is reconstructed and illustrated in Figure 6. It is likely that, after initiation of the spermatophoric reaction of spermatophores of *O. sicula*, the main driving process behind all other actions in the reaction that involve movement are caused by osmotic pressure as a result of the uptake of sea water by the semipermeable spermatophore (Drew 1919, Hanson *et al.* 1973). The uptake of water by the spermatophore results in the forward movement of the sperm mass (Hanson *et al.* 1973) (Figure 6b). This causes oral secretion of the cement body (Drew 1919, Takahama *et al.* 1991). For spermatophores of *O. sicula*, this would indicate that the cement body with tubular extension and the anchor are the first parts extruded and to contact female tissue before implantation starts (Figure 6b). During discharge, the bulbous component of the cement body probably receives pressure from the forward moving sperm mass and empties its contents (the cement) through the tube and anchor of the cement body (Figure 6c). The tube may serve to create some distance between the extruded cement gland contents and the cement body. This is possible because the chemicals of the cement body, which aid in adhesion to or dissolution of tissue, may be noxious (Nesis *et al.* 1998). The anchor may control the distribution of the cement chemicals. Once all the cement is secreted, the forward moving sperm mass makes the tubular extension (and anchor) of the cement body to bend aborally (Figures 4a, 6d). Perhaps some sperm gets pushed out of the anchor following the ejected contents of the cement body, explaining the finding of spermatozoa on the anchor (Figure 4d). Once the spermatangium is implanted into the tissue of the female, the remaining

empty parts of the spermatophore (the middle membrane attached to the outer and middle tunic) will separate from the spermatangium and fall off. The implanted spermatangium is completely covered by the female's tissue, except for the open end of the aboral tube, which will presumably be the exit for the spermatozoa when fertilisation takes place (Figure 6e).

This study provides the first information on the reproductive biology and spermatophoric reaction of *O. sicula*. The morphologies of the spermatophore and spermatangium, together with the reconstruction of the spermatophoric reaction, reveal a complex process of intradermal implantation of spermatangia. However, the mechanics that are responsible for the penetration of spermatangia into the tissue remain unknown.

**Acknowledgements** — The comments of Dick Young and Vladimir Laptikhovskiy on an earlier draft of the paper are greatly appreciated. Miranda Waldron of the Electron Microscopy Unit of the University of Cape Town is thanked for her assistance in making the SEM photographs. Deniz Haydar is thanked for her help with English and illustrations. This paper is dedicated to Martina AC Roeleveld who sadly passed away during the study.

## References

- Austin CR, Lutwak-Mann C, Mann T (1964) Spermatophores and spermatozoa of the squid *Loligo pealii*. *Proceedings of the Royal Society B: Biological Sciences* **161**: 143–152
- Burgess LA (1998) A survey of seminal receptacles in the Enoptoteuthidae In: Voss NA, Vecchione M, Toll RB, Sweeney MJ (eds) *Systematics and Biogeography of Cephalopods Vol. 1. Smithsonian Contributions to Zoology* **586**: 271–276
- Clarke MR (1980) Cephalopoda in the diet of sperm whales of the southern hemisphere and their bearing on sperm whale biology. *Discovery Reports* **37**: 1–324
- Clarke MR (1996) Cephalopods in the World's Oceans: Cephalopods as prey. III. Cetaceans. *Philosophical Transactions of the Royal Society of London Series B* **351**: 1053–1065
- Drew GA (1919) Sexual activities of the squid *Loligo pealii* (Les.). II. The spermatophore; its structure, ejaculation and formation. *Journal of Morphology* **32**: 379–435
- Hanson D, Mann T, Martin AW (1973) Mechanism of the spermatophoric reaction in the giant octopus of the North Pacific, *Octopus dofleini martini*. *Journal of Experimental Biology* **58**: 711–723
- Hess SC (1987) Comparative morphology, variability, and systematic applications of cephalopod spermatophores (Teuthoidea and Vampyromorpha). PhD thesis, University of Miami, USA
- Hoving HJT, Laptikhovskiy V (2007) Getting under the skin: autonomous implantation of squid spermatophores. *Biological Bulletin* **212**: 177–179
- Hoving HJT, Lipiński MR, Roeleveld MAC, Durholtz MD (2007) Growth and mating of *Lycoteuthis lorigera* (Steenstrup, 1875) (Cephalopoda; Lycoteuthidae). *Reviews in Fish Biology and Fisheries* **17**: 259–270
- Hoving HJT, Roeleveld MAC, Lipiński MR, Melo Y (2004) Reproductive system of the giant squid *Architeuthis* in South African waters. *Journal of Zoology, London* **264**: 153–169
- Jackson GD, Jackson CH (2004) Mating and spermatophore placement in the onychoteuthid squid *Moroteuthis ingens*. *Journal of the Marine Biology Association, UK* **84**: 783–784
- Kjennerud J (1958) Description of a giant squid, *Architeuthis*, stranded on the west coast of Norway. *Universitetet i Bergen. Arbob* 1958, *Naturvitenskapelig rekke* **9**: 1–14

- Marchand W (1913) Studien über die Cephalopoden. II. Über die Cephalopoden. *Zoologica* **26**: 171–200
- Melo Y, Sauer WHH (1998) Ovarian atresia in cephalopods. In: Payne AIL, Lipiński MR, Clarke MR, Roeleveld MAC (eds) *Cephalopod Biodiversity, Ecology and Evolution. South African Journal of Marine Science* **20**: 143–151
- Nesis KN (ed) (1987) *Cephalopods of the World*. TFH Publications, Neptune City, New Jersey
- Nesis KN (1995) Mating, spawning and death in oceanic cephalopods: a review. *Ruthenica* **6**: 23–64
- Nesis KN, Nigmatullin ChM, Nikitina IV (1998) Spent females of deepwater squid *Galiteuthis glacialis* under the ice at the surface of the Weddell Sea (Antarctic). *Journal of Zoology, London* **244**: 185–200
- Norman MD, Lu CC (1997) Sex in giant squid. *Nature* **389**: 683–684
- O'Shea S, Jackson GD, Bolstad KS (2007) The nomenclatural status, ontogeny and morphology of *Pholidoteuthis massyae* (Pfeffer, 1912) new comb (Cephalopoda: Pholidoteuthidae). *Reviews in Fish Biology and Fisheries* **17**: 425–435
- Rocha F, Guerra Á, González ÁF (2001) A review of reproductive strategies in cephalopods. *Biological Reviews* **76**: 291–304
- Roper CFE, Vecchione M (1993) A geographic and taxonomic review of *Taningia danae* Joubin, 1931 (Cephalopoda: Octopoteuthidae), with new records and observations on bioluminescence. In: Okutani T, O'Dor RK, Kubodera T (eds) *Recent Advances in Fisheries Biology*. Tokai University Press, Tokyo, pp 441–456
- Stephen SJ (1986) The distribution of larvae of the genus *Octopoteuthis* Ruppell 1844 (Cephalopoda, Teuthoidea). *Vie et Milieu* **35**: 175–179
- Takahama H, Kinoshita T, Sato M, Sasaki F (1991) Fine structure of the spermatophores and their ejaculated forms, sperm reservoirs, of the Japanese common squid, *Todarodes pacificus*. *Journal of Morphology* **207**: 241–252
- Young RE, Harman RF (1998) Phylogeny of the “Enoploteuthid” families. *Smithsonian Contributions to Zoology* **586**: 257–271
- Young RE, Vecchione M, Mangold KM (2000) Cephalopod spermatophore terminology. Tree of Life webpage, available at [http://www.tolweb.org/accessory/Cephalopod\\_Spermatophore\\_Terminology?acc\\_id=1972](http://www.tolweb.org/accessory/Cephalopod_Spermatophore_Terminology?acc_id=1972)