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Structure of the reproductive system and hectocotylus in males of lesser flying squid *Todaropsis eblanae* (Cephalopoda: Ommastrephidae)

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This paper introduces new data on *Todaropsis eblanae* morphology, morphometry and functional aspects of the male reproductive system and hectocotylus. Spermatophores differ in specimens from the Atlantic Ocean (average length, 18.28 ± 1.45 mm, $15.63 \pm 0.8\%$ of mantle length; weight, 2.0–12.0 mg) and the Indian Ocean (average length, 24.8 ± 2.85 mm, $16.9 \pm 2.1\%$ of mantle length; weight, 35.0–39.6 mg) ($t = 3.14$; $p < 0.01$ for absolute sizes and $t = 0.711$; $p > 0.01$ for relative sizes). An additional important distinctive trait is the form of connection of the cement body with the ejaculatory tube. In recent years, *T. eblanae* has been regularly caught in the Barents Sea, meaning its range has extended to subarctic waters. The morphology and morphometry of the spermatophoric complex of organs did not vary in investigated parts of its range. Hectocotylus patterns and some important spermatophore traits distinguish *Todaropsis* from other Ommastrephidae.

Keywords: Cephalopoda; *Todaropsis*; male reproductive system; hectocotylus; the Barents Sea

Introduction

The monotypic genus *Todaropsis* (Ball 1841) is a primitive taxon of the family Ommastrephidae, and is significantly important for understanding the early stages of the family's evolution (Nigmatullin 2000, 2007). The structure and functioning of the reproductive system in the lesser flying squid *Todaropsis eblanae* (Ball 1841) has been studied unevenly between the sexes. Female reproductive biology is known relatively well (Hastie et al. 1994; Nigmatullin and Laptikhovskiy 1999; Lordan 2001; Hernandez-Garcia 2002; Robin et al. 2002), in contrast to male reproductive biology. Data on morphology of the male reproductive system is sparse. A short description of the hectocotylus in Atlantic specimens was made originally by Hoyle (1892) and then supplemented with further material by Jatta (1896), Pfeffer (1912) and Naef (1923). These descriptions, including schematic figures, were based on immature or damaged specimens. Later, the hectocotylus was described by Adam (1952), including relatively good figures of ontogenetic development of both hectocotylus arms. Thereafter, the figure of mature hectocotylized arms produced by Adam (1952) was repeatedly reproduced with short descriptions but without new data (Lu and Dunning 1982;

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Roper et al. 1984; Nesis 1987; Okutani 1995; Dunning and Lu 1998; Roper et al. 2010). Lu and Dunning (1982) briefly described the hectocotylus in Australian specimens.

Ontogenetic variability of quantitative indices in the spermatophoric complex of organs (SCO; after Roper 1966), without morphological description, was described relatively recently (Hastie et al. 1994; Lordan 2001; Hernandez-Garcia 2002; Rosa et al. 2005). Fragmentary data on the length and number of spermatophores are numerous enough (Mangold-Wirz 1963; Roper et al. 1984; Hastie et al. 1994; Sabirov 1995; Hernandez-Garcia 2002; Nigmatullin et al. 2003). However, there are no figures or detailed morphological and morphometric descriptions for the SCO and spermatophores of *T. eblanae*, with the exception of a brief description of spermatophores by Hess (1982).

Todaropsis eblanae is a shelf–upper slope demersal species with a disjunctive vast range spreading from tropical to temperate waters (Figure 1) (Lu 1982; Nesis 1987; Wormuth 1998; Nigmatullin 2000; Roper et al. 2010). The size of adult squid depends on latitude of habitat and season of spawning. The largest males reach 22 cm mantle length (ML) and the females reach 29 cm (Robin et al. 2002; Roper et al. 2010). The limits of variation of male size at maturity are 8.5–22 cm, and they mostly mature at an ML of 10–16 cm (Mangold-Wirz 1963; Lu 1982; Gonzales et al. 1994; Wormuth 1998; Arkhipkin and Laptikhovskiy 2000; Lordan 2001; Hernandez-Garcia 2002; Zumholz and Piatkowski 2005). Males grow more slowly than females. Life cycle duration is nearly 1 year but about 6 months in the tropical parts of its range (Arkhipkin and Laptikhovskiy 2000; Nigmatullin 2007).

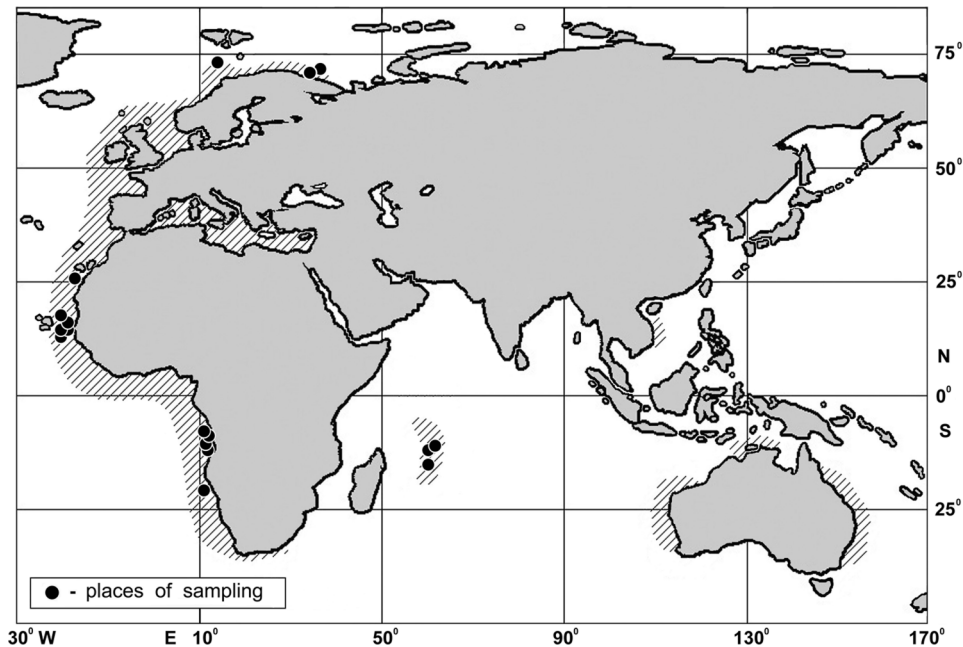


Figure 1. The range of *Todaropsis eblanae* and the sampling locations.

The aim of this paper is to describe the male reproductive system and hectocotylus in the lesser flying squid from different parts of its range, including a new location at which it has been found, the Barents Sea (Sabirov et al. 2009).

Materials and methods

Thirty-eight males of *T. eblanae* of ML 7.0–16.7 cm were obtained from trawl catches of fishery survey trawlers in 1969–1988 in the shelf and upper slope of the Eastern Atlantic and the western part of the Indian Ocean. One specimen was sampled on the coast of southern Morocco (catch depth 140 m; IV maturity stage, ML 11.4 cm); 29 specimens on the coast of Mauritania (catch depth 101–345 m; 10 specimens, III maturity stage, ML 7.0–11.0 cm; five specimens, IV maturity stage, ML 10.0–13.0 cm; 14 specimens, V₂ maturity stage, ML 11.6–16.7 cm); four specimens on the coast of Angola (catch depth 200–208 m; V₂ maturity stage, ML 12.5–13.7 cm); one specimen on the coast of Namibia (catch depth 200 m; V₁ maturity stage, ML 7.6 cm); and three specimens in the area of the Mascarene Ridge of the Indian Ocean (catch depth 214–385 m; V₂ maturity stage, ML 14.2–15.0 cm). In addition, since 2006 *T. eblanae* has been caught and noted in the Barents Sea on a few occasions: (a) from the research cruise R/V “*F. Nansen*” on 20 August 2006 using pelagic trawl A-8-623 at 71°13' N, 36°38' E (catch depth 60 m; V₁ maturity stage, ML 9.2 cm); (b) during diving sessions of Polar Research Institute (PINRO) divers on 26 August 2008 photographs were taken in Ura Bay (depth of location, 15 m) of the Barents Sea; and (c) from the research cruise R/V “*Jan-Mayen*” on 9 September 2010, 19 specimens were caught by demersal trawl “Campelen-1800” at 73°34' N, 15°35' E (depth of location 464–468 m), but biological analysis was not obtained (Figure 1).

The biological analysis for all specimens held onboard ship included measurement of ML, identification of sex and stage of maturity. Maturity was assigned using a scale for ommastrephids (Nigmatullin et al. 2003). This scale can be briefly described, as follows. 1. Juveniles: sex is not visually distinguishable; 2. Immature (stages I and II); 3. Physiological maturation: maturation of testis and tentative spermatophorogenesis (stages III–IV); 4. Functional maturation: initial accumulation of normal spermatophores in spermatophoric (Needham’s) sac (substage V₁); 5. Functional maturity: active spermatophorogenesis with proper spermatophore accumulation and release at mating (substages V₂ and V₃); 6. Functional maturity with gradual degeneration of testis and decrease of its functioning (stage VI); 7. Spent (stage VII). Substages from V₁ to V₃ differ mainly by quantity of spermatophores in the spermatophoric sac.

During squid ontogenesis, males form a sequential series of spermatophore-like productions. This has also been found in *T. eblanae* and is as follows. Tentative spermatophores look like curved tubes with shapeless contents (see: Results). They have no spermatozoa, and are formed during the immature stages. False spermatophores are formed during the maturing stage, and appear like normal (proper) spermatophores, but with low sperm content in a short translucent seminal reservoir. Neither tentative nor false spermatophores are usually stored for any length of time in the spermatophoric sac. Proper spermatophores are formed during the mature stage, and have a normal form and inner structures with dense sperm concentration in large seminal reservoirs (Nigmatullin et al. 2003).

The stomach, digestive gland, testis, SCO and sometimes the squid body were weighed. The testis, SCO and hectocotylized arms were preserved in 4–5% formalin solution.

The following indices were calculated: maturity index (*MI*), SCO index (*SCI*), testis index (*TI*) and index of ratio of SCO: testis (*ISC/T*).

$$MI = (W_{SCO} + W_t) / W_b;$$

$$SCI = W_{SCO} / W_b;$$

$$TI = W_t / W_b;$$

$$ISC/T = W_{SCO} / W_t;$$

where: W_{SCO} is the weight of SCO, W_t is the weight of testis and W_b is the weight of body.

Detailed biological analysis included measurements of structural parts of the hectocotylus, SCO and components of the spermatophores (Figures 2, 3). Special terminology and methods of studying the morphology of SCO and spermatophore in Ommastrephidae were developed (reviews: Nigmatullin et al. 2003; Sabirov 2009).

A total of 1605 spermatophores were studied using morphometric analysis. The volume of a single seminal reservoir was calculated as a cylinder with rounded ends:

$$V = 0.262 * (3l - d) * d^2,$$

where V is the volume in mm^3 ; l is the length of seminal reservoir in mm; and d is the average width of seminal reservoir in mm.

The total volume of spermatozoa packed in all spermatophores was calculated by multiplying the volume of a single seminal reservoir volume by the total number. To estimate the spermatozoa concentration in seminal reservoirs, the spermatozoa were dissolved in 2 ml water for 3–4 hours. The numbers of spermatozoa in 15 large squares of a Gorjaev's chamber were counted and the total number of spermatozoa in the spermatophore was calculated according to:

$$N = M * 225 * v' / 0.9,$$

where N is the number of spermatozoa in spermatophore; M is the average number of spermatozoa in one large square of the chamber; 225 is the number of large squares in the chamber; 0.9 is the chamber's volume in mm^3 ; and v' is the suspension volume in mm^3 .

The concentration of spermatozoa in 1 mm^3 was determined by dividing the spermatozoa number by the volume of the seminal reservoir. It was established for a maturing male from the Barents Sea (ML 9.2 cm, spermatophore length 13.5–15.5 mm).

To investigate evidence for copulations (spermatangia) and the number of seminal receptacles, we studied 21 females of ML of 4.7–11.5 cm from the shelf of Morocco. They were collected from trawl catches in 1980 and 1988.

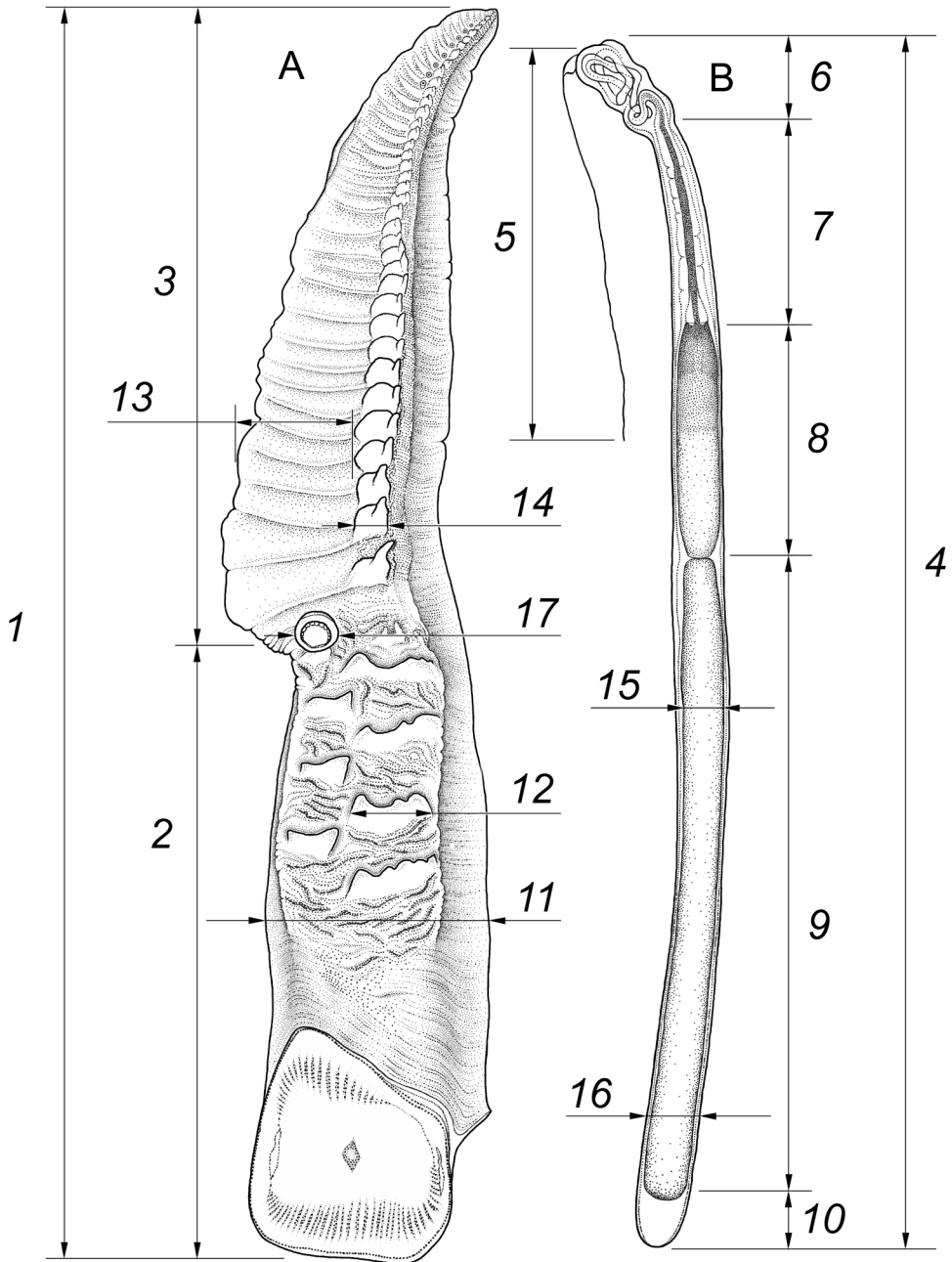


Figure 2. Scheme of measuring of (A) hectocotylus and (B) spermatophore. Length of: 1. hectocotylized arm; 2. modified proximal part; 3. hectocotylized distal part; 4. spermatophore; 5. tread; 6. head; 7. ejaculatory tube; 8. cement body; 9. seminal reservoir; 10. posterior empty part. Width of: 11. modified proximal part; 12. coarse crests; 13. ventral membrane of distal part; 14. leaf-like plates; 15. seminal reservoir; 16. spermatophore; 17. diameter of sucker.

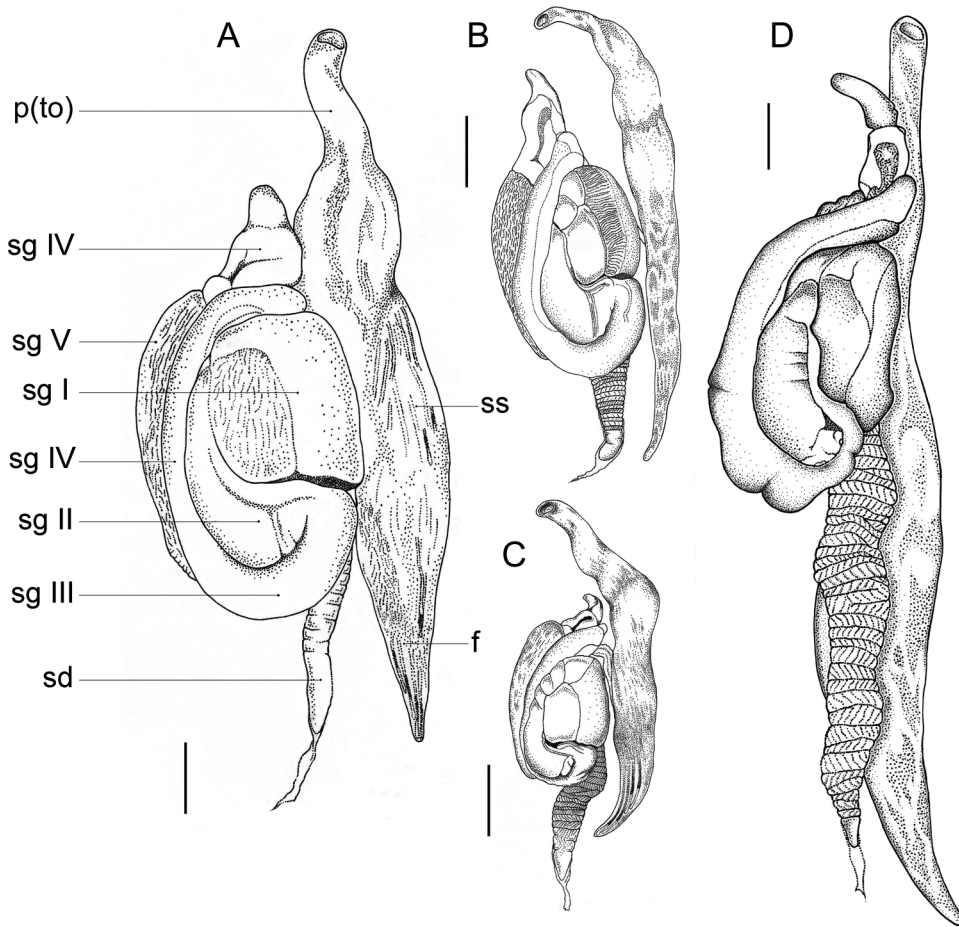


Figure 3. The spermatophoric complex of organs of *Todaropsis eblanae* from (A) the Barents Sea [mantle length (ML) 9.2 cm, V₁ maturity stage]; (B) the shelf of Morocco (ML 11.4 cm, IV maturity stage); (C) the shelf of Namibia (ML 7.6 cm, V₁ maturity stage); and (D) the Mascarene ridge (ML 14.2 cm, V₂ maturity stage). Parts of SCO: sd, sperm duct; sg I–VI, spermatophoric gland parts; ss, spermatophoric sac; p(to), penis (terminal organ); f, fundus. Scale bars: 5 mm.

Statistical analysis used the programs STATGRAPHICS (v. 5.0, StatPoint Technologies inc., Warrenton, VA, USA) and MS EXCEL. This included mean sizes, average sizes, standard error and deviation, correlation coefficient, variation coefficient (*VC*) and Student's *t*-test (*t*).

Results

Spermatophoric complex of organs

A single SCO lies in the left half of the mantle cavity and in mature males is 30–50% of ML. *MI* varied in studied males from 4.8 to 6.1%, *SCI* from 2.7 to 3.0%, *TI* from 2.1 to 5.0% and *ISC/T* from 60.0 to 80.0 %.

The sperm duct is massive (25–35% ML), and consists of about 50 coils laid spirally. There is an ampoule with muscular walls in the proximal part of the sperm duct. Its distal end opens subterminally into part I of the spermatophoric gland (SG). Six consecutive parts of the SG are morphologically distinct from each other (Figure 3) and have the form of an S-shaped figure. Parts I and II of the SG are sac-shaped and their lengths are 11.8–12.6% ML and 10.2–11.1% ML, respectively. Parts III (10.2–11.2% ML) and IV (20.7–22.4% ML) of the SG appear like a bent tube with a furrow on their border. Part IV of the SG is connected with part V by a narrow tube (an intermediate duct). A thin excretory tube with ciliated epithelium deviates from the intermediate duct and opens freely into a cavity of the genital bag. Part V of the SG looks like an extended sac with a length of 21.7–23.7% ML. It adjoins at the dorsal side compactly laid in one plane to the previous parts of the SG (Figure 3). The walls of part V of the SG are soft and friable.

Part VI of the SG is separated from part V by an easily visible coupler. Part VI of the SG is the smallest, with a length of 10.2–10.5% ML. The glandular tissue of this part is jelly-like and transparent, and the duct is bent in a loop. From part VI of the SG, completely formed spermatophores move into the fundus of the spermatophoric sac. It has an elongated form and, when filled by spermatophores, reaches 50% ML. The interior walls of the spermatophoric sac have spiral lamellae, so the accumulated spermatophores are set spirally. Additionally, its walls are muscular and strong, and capable of contraction. The sac is widest in the median part and narrows before the penis (the terminal organ).

Geographical variability in morphology and morphometry of the SCO is not revealed.

The SCO in the male from the Barents Sea was enlarged (61% ML) and swollen (Figure 3A). *ISC/T* of this specimen was 129.6%, whereas in males at the same stage of maturity from the Eastern Atlantic it was an average of 20–30%. Its *MI* value was 4.8%, and *SCI* was 2.7%. The length of the sperm duct reached 25.0% ML; however, it was thin and semi-translucent and was laid in only 12 slightly visible coils. All parts of the SG, especially II, III and IV, were obviously lengthened: their lengths were 13.4%, 15.8% and 27.2% ML, respectively.

Spermatophores

The spermatophores appear as slightly bent tubes (Figure 4) with a distinctive thick outer tunic of light brown colour. Spermatophoric length (SL) varies significantly from 11.6 to 31.5 mm (12.4–21.1% ML) ($VC = 8.74\%$). The mean size of spermatophores in Atlantic specimens was 18.28 ± 1.45 mm ($15.63 \pm 0.8\%$ ML). This was somewhat less than in squid from the Indian Ocean: 24.8 ± 2.85 mm ($16.9 \pm 2.1\%$ ML) ($t = 3.14$; $p < 0.01$ for absolute sizes and $t = 0.711$; $p > 0.01$ for relative sizes) (Table 1). The weights of spermatophores in specimens from the Atlantic and the Indian Oceans differed sharply: they were 2.0–12.0 mg and 35.0–39.6 mg, respectively. So, during sexual maturity, the total weight of spermatophores in males from the Atlantic was approximately 1.5 g, and from the Indian Ocean approximately 4 g.

The head of the spermatophore is massive (3.6–7.7% SL) with relief bends, and contains the twisted end of the ejaculatory tube (Figure 4A,C,E). A thin thread with a length about half that of the SL is attached to the head. The ejaculatory tube is

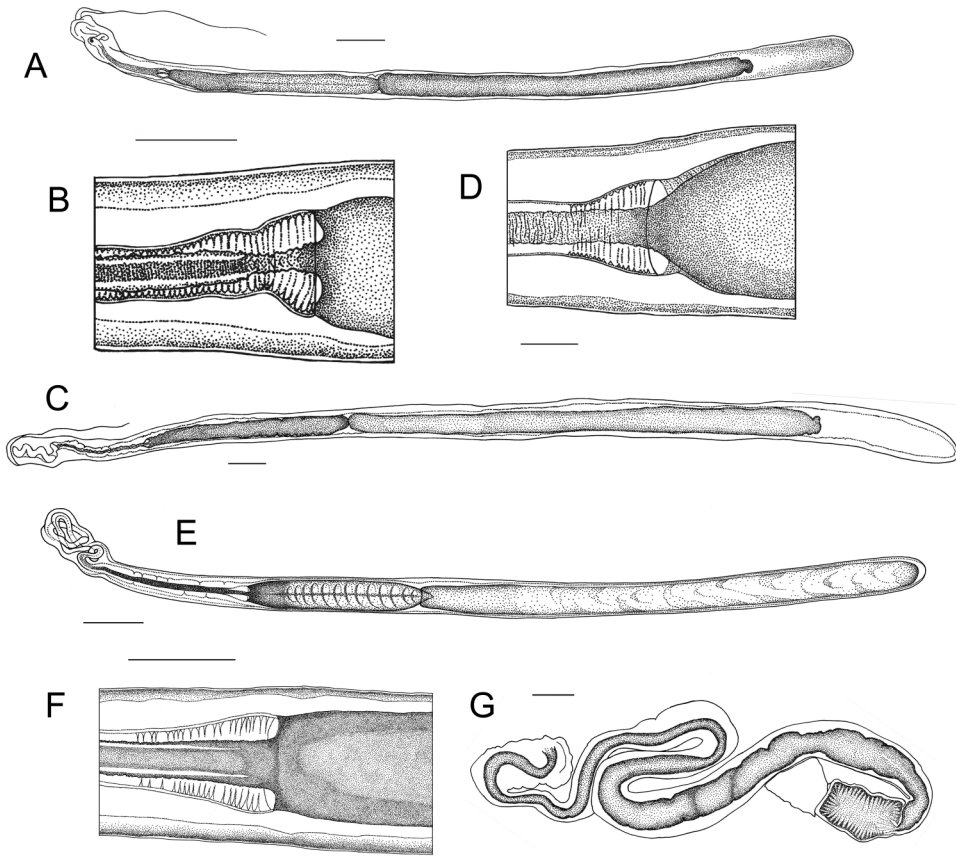


Figure 4. The spermatophores of *Todaropsis eblanae* from (A, B) the shelf of Mauritania [mantle length (ML) 12.1 cm, V₂ maturity stage]; (C, D) the Mascarene ridge (ML 14.2 cm, V₂ maturity stage); and (E–G) the Barents Sea (ML 9.2 cm, V₁ maturity stage). B, D, F, anterior part of cement body; G, tentative spermatophore. Scale bars: 1 mm (A, C, E), 0.5 mm (B, D, F, G).

distinctively short (6.8–15.5% SL). Its mean size differs insignificantly in specimens from the Atlantic and Indian Oceans: $8.05 \pm 0.94\%$ SL and $10.6 \pm 1.9\%$ SL, respectively ($t = 1.51$; $p > 0.1$). The ejaculatory tube directly passes into the cement body. *Todaropsis eblanae* is the only species among Ommastrephidae that has no tip to the cement body. The connection of the ejaculatory tube with the cement body differs in specimens from the Atlantic and the Indian Oceans. In the first, the ejaculatory tube is pressed into the cement body, forming a peculiar circular invagination on its anterior end (Figure 4B). In the second, such invagination is absent, and the anterior end of the cement body is narrowed and smoothly passes into the ejaculatory tube (Figure 4D). The cement body is large (19.0–29.1 % SL), and contains a small granulated secretion, brown in colour. The anterior quarter of the cement body is coloured more intensely. In squid from the Indian Ocean, the mean length of the cement body ($21.2 \pm 1.6\%$ SL) is significantly less in comparison with the Atlantic specimens ($26.0 \pm 2.5\%$ SL; $t = 2.51$; $p < 0.05$).

Table 1. Sizes and morphometric indices of *Todaropsis eblanae* spermatophores from different parts of the range.

Measurements	Units	The location of sampling			
		The Barents Sea	Central Eastern Atlantic (Morocco – Mauritania coasts)	Southeastern Atlantic (Angola – Namibia Coasts)	The Indian Ocean (the Mascarene ridge)
Number		1	11	5	3
Mantle length (ML), cm		9.2	11.6–16.5	7.6–13.7	14.2–15.0
Maturity stage		V ₁	IV–V ₂	V ₁ –V ₂	V ₂
Spermatophores number in sac		29	19–232	19–83	70–95
Spermatophore total length (SL)	mm	13.5–15.5	15.0–31.5	11.6–23.5	19.1–30.5
Length of head	% of ML	14.7–16.8	12.4–19.0	14.6–17.4	12.7–21.1
	mm	0.9–1.1	1.0	0.7–1.4	0.9–1.7
	% of SL	6.7–7.1	4.6–5.6	4.9–7.7	3.6–5.8
Length of ejaculatory tube	mm	1.9–2.3	1.5–2.0	1.1–2.3	2.2–3.6
	% of SL	13.3–14.8	6.8–9.1	7.4–15.5	8.2–12.0
Length of cement body	mm	2.1–2.5	5.0–6.1	2.8–6.1	5.7–6.7
	% of SL	15.6–16.8	23.8–29.1	22.8–28.4	19.0–23.4
Length of seminal reservoir	mm	8.5–9.5	9.0–12.9	6.1–12.0	12.5–16.0
	% of SL	61.3–63.0	43.2–61.4	46.1–61.5	49.0–55.7
Volume of seminal reservoir, mm ³		1.13–1.86	2.14–5.38	0.43–6.52	4.38–13.96
Maximum total volume of spermatophores, packed in all spermatophores, cm ³		≈ 0.05	≈ 0.45	≈ 0.35	≈ 0.95
Length of posterior part	mm	0.1–0.2	1.4–3.8	0.1–2.7	2.2–4
	% of SL	0.65–1.4	7.6–18.1	0.9–15.6	8.1–13.3
Spermatophore width	mm	0.5–0.6	0.65–0.9	0.5–0.95	1.1–1.7
	% of SL	3.5–4.4	3.4–4.3	3.3–4.9	4.2–5.7
Spermatophore weight, mg		2.5–4.0	2.0–12.0	6.0–11.0	35.0–39.6
Total weight of all spermatophores, g		≈ 0.1	≈ 1.0	≈ 1.0	≈ 4.0

The seminal reservoir is single, in the form of a cylinder with a light-cream coloured sperm mass. A small lump is usually present on its posterior end. The length of the seminal reservoir varies from 43.2 to 61.5% SL. Its mean values are practically coincident in different parts of the species range within 52.4–53.0% SL ($t = 0.8$; $p > 0.1$). However, the absolute sizes of the seminal reservoir in larger squid from the Indian Ocean (12.5–16.0 mm) are considerably larger than in Atlantic specimens (6.1–12.9 mm). Accordingly, the volume of the seminal reservoir of these male squid differs: 4.38–13.96 mm³ in the Indian Ocean specimens and 0.43–6.52 mm³ in the Atlantic ones. Total volume of spermatophoric mass in squid from the Indian Ocean (about 1 cm³) is almost twice that of the Atlantic specimens (less than 0.5 cm³). The size of the posterior hollow part is the same ($10.6 \pm 2.35\%$ SL) in squid from different parts of the range (Table 1).

A typical curve of spermatophore size frequency distribution is unimodal (Figure 5). During the mature stage of ontogenesis, an insignificant increase of spermatophore length is observed: “new” spermatophores are 0.3–1.0 mm (1.6–5.5% SL) longer than “old” ones. This process is accompanied by a minor change in proportions of spermatophore inner components. Both absolute and relative dimensions of seminal reservoirs (0.6 ± 0.16 mm; $3.3 \pm 0.9\%$ SL), ejaculatory tubes and

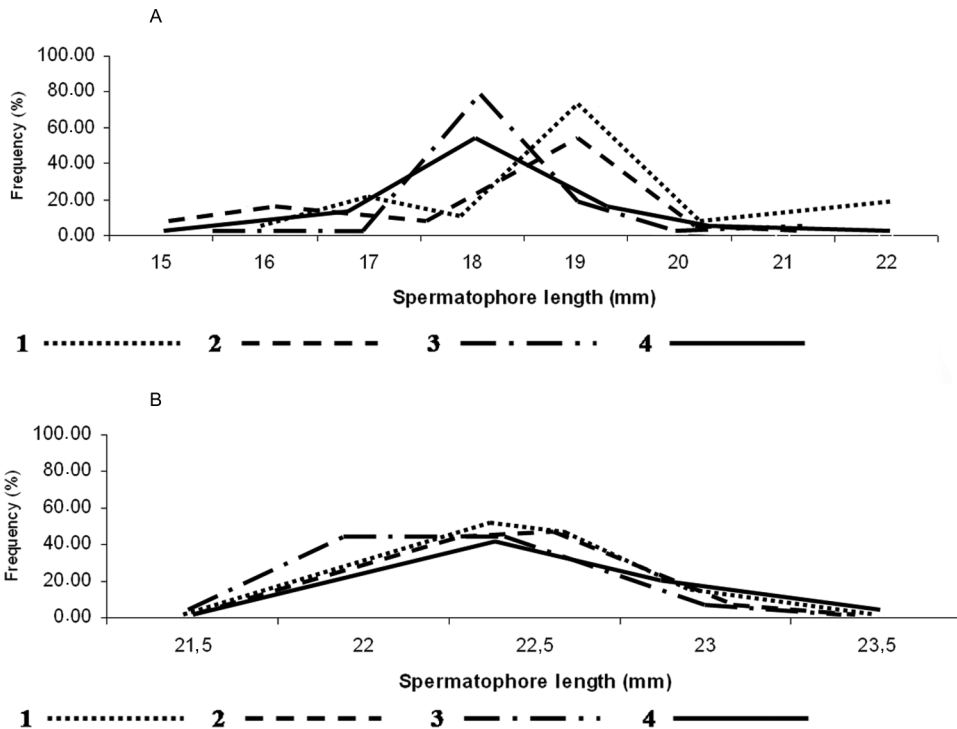


Figure 5. Ontogenetic variability of spermatophore length in *Todaropsis eblanae* (V₂ maturity stage) from (A) the shelf of Mauritania [mantle length (ML) 12.1 cm, 113 spermatophores]; and (B) the shelf of Angola (ML 13.5 cm, 79 spermatophores). 1, spermatophores from the anterior part of the spermatophoric sac; 2, from the main part; 3, from the posterior part; 4, all spermatophores.

cement bodies are slightly increased. The length of the posterior hollow part is slightly decreased by 0.4 ± 0.11 mm ($2.2 \pm 0.6\%$ SL).

Tentative spermatophores, up to six to eight in number, were found in the penis and anterior part of the spermatophoric sac of all maturing male squid of IV and V₁ maturity stages. They were usually intricately twisted, and their internal content was presented as a single continuous mass (Figure 4G). The length of the tentative spermatophores is 4.0–7.6 mm, i.e. three to four times less than those of normal ones.

The maximum number of spermatophores recorded in the spermatophoric sac is 232. This was found in the male of ML 13.5 cm from the Mauritania shelf. Larger squid from the Indian Ocean produce larger spermatophores. Their numbers in studied specimens were not in excess of 100. Males with ML 14.2 cm from the Mascarene Ridge had a maximum number of 95.

The spermatophoric sac of maturing male squid from the Barents Sea contained 29 spermatophores, including eight that were tentative with atypical shape, 11 false ones with typical shape and reservoir without sperm, and the first 10 proper spermatophores. The length of proper ones was 13.5–15.5 mm (14.7–16.9% ML). The outer tunic of these was very thin and fragile, and a thread was absent. The structure of the anterior part of the cement body is typical for the Atlantic specimens (Figure 4F). It was however truncated (15.6–16.8% SL), semi-translucent because of the low concentration of granulated secretion, and in its posterior part the secretion was set into a spiral structure. In contrast, the seminal reservoir was long (61.3–62.9% SL), and translucent with flakes of sperm (Figure 4E). The posterior hollow part was almost absent (0.65–1.4% SL).

The concentration of spermatozoa in the seminal reservoir of the male from the Barents Sea was low (0.89 ± 0.05 million/mm³), whereas in spermatophores in the mature male from the shelf of Namibia (ML 7.6 cm, spermatophore length 11.6–13.0 mm) it reached 2.22 to 2.48 ± 0.07 million/mm³ (Laptikhovsky, personal communication). Hence, the total number of spermatozoa packed into seminal reservoirs was 1.01–1.66 million in the first specimen, and 1.47–2.73 million in the second.

Hectocotylus

In maturing male squid, both ventral arms are modified: the left arm is modified in the proximal part and the right, which is in fact the hectocotylus, is modified along all its length (Figure 6). In Atlantic specimens, the right arm is slightly shorter than the left and varies from 57.9 to 61.8% ML, and the length of the left arm is 61.8–64.9% (Table 2). Proximal parts of both arms are subjected to identical modification and their length is 21.0–36.8% of arm length (AL). Here the suckers are reduced, and their stalks (basal pads – after Naef 1923) are developed into eight coarse crests, which are sharply alternating in twin rows (Figure 6). The dorsal row is displaced to the proximal end of the arm, in comparison to the ventral row. The dorsal crests have two to four blunt tooth-like ledges, while the ventral crests have only two ledges separated by a wide semi-lunar notch. The lateral tooth-like ledges of dorsal crests are closed. The left arm has typical armature in the form of 23 twin rows of suckers in the distal part. The diameter of suckers decreases towards the distal end of the arm, from 1.4 mm to 0.2 mm.

The hectocotylied distal part of the right arm is 48.3–64.9% AL (Table 2). Sucker stalks of the dorsal row are converted into leaf-like plates with an angular edge (serrated papillae – after Naef 1923), and the terminal parts of the sucker stalks remain

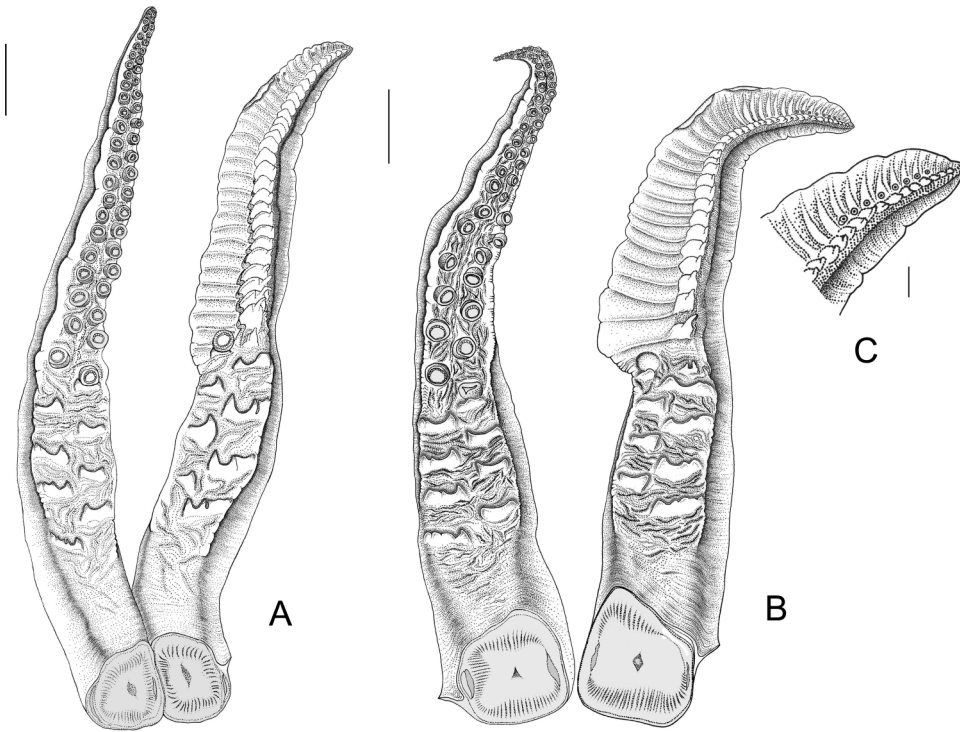


Figure 6. The hectocotylus *Todaropsis eblanae* from (A) the shelf of Namibia [mantle length (ML) 7.6 cm, V_1 maturity stage], and (B, C) the Barents Sea (ML 9.2 cm, V_1 maturity stage). Scale bars: 5 mm (A, B), 1 mm (C).

in the form of cirri. The number of leaf-like plates is 30–32; their width decreases towards the distal end of the arm from 1.85 to 0.26 mm. Suckers of the ventral row are reduced completely. The suckers of the distal tip, with diameter 0.17–0.2 mm, and the suckers of the central part with diameter 1.2–1.4 mm, are the last to reduce. Along the ventral edge of the hectocotylized distal part of the right arm, the wide sail-like membrane with width 5.3–6.4% AL is developed. It is supported by elastic trabeculae growing on the basis of leaf-like plates. Obviously, the trabeculae are formed from the sucker stalks of the ventral row.

The hectocotylus structure of mature males from the Indian Ocean has no significant differences from the typical Atlantic specimens described above (Table 2). The male squid from the Barents Sea have generally the same morphology of modified ventral arms (Figure 6B). However, the ventral sail-like membrane of the hectocotylus is more highly developed (7.7% AL), the number of leaf-like plates reaches 34, and they have a roundish form and shorter cirri. The distal part of the hectocotylus has eight suckers, reduced to a diameter of 0.13 mm. The unmodified part of the left arm has 28 twin rows of suckers.

In the process of male maturation, a significant change in size and structure of the different parts of the hectocotylized arms was observed (Table 3). The ontogenetic development of the hectocotylus in both ventral arms is revealed in the reduction of suckers and the formation of new structures from sucker stalks (crests and leaf-like

Table 2. Sizes and morphometric indices of *Todaropsis eblanae* hectocotylus from different parts of the range.

Measurements	Units	The location of sampling		
		The Barents Sea	The shelf of Morocco	The shelf of Namibia
Mantle length (ML), cm		9.2	11.4	7.6
Maturity stage		V ₁	IV	V ₁
Left arm				
Total length	mm	57	74	47
	% of ML	61.9	64.9	61.8
Length of modified proximal part	mm	19	19	16
	% of AL	33.3	25.7	34.0
Width of modified proximal part	mm	7–8.5	7–7.5	4–6
	% of AL	12.9–14.9	9.5–10.1	8.5–12.8
Coarse crests in proximal part	number	8	8	8
	width, mm	2.7–4.5	1.6–3.0	1.5–2.5
Number of suckers twin rows in proximal part		28	23	23
Diameter of suckers, mm		0.2–1.6	0.2–1.4	0.2–1.4
Right arm				
Total length	mm	52	66	47
	% of ML	56.5	57.9	61.9
Length of modified proximal part	mm	18	18	16.5
	% of AL	34.6	27.3	35.1
Width of modified proximal part	mm	7–9.5	6–7	5–6
	% of AL	13.5–18.3	9.1–10.6	10.7–12.7
Coarse crests in proximal part	number	8	7	8
	width, mm	2.9–4.5	1.6–3.2	1.5–3
Length of hectocotylized distal part	mm	34	38	30.5
	% of AL	65.4	57.6	64.9
Maximum width of ventral membrane of distal part	mm	4	3.5	3.0
	% of AL	7.7	5.3	6.4
Leaf-like plates lengthwise of distal part	number	34	32	30
	width, mm	0.16–1.56	0.26–1.8	0.28–1.85
Suckers in middle part	number	1	2	1
	diameter, mm	1.2	1.2–1.4	1.2
Suckers in tip of distal part	number	8	2	4
	diameter, mm	0.13–0.2	0.17	0.17–0.2

plates). This process is most intensive during the period of physiological maturation from stages III to V of maturity.

Evidence of copulation was found in 12 maturing and mature specimens of 21 studied females (ML 4.7–11.5 cm) sampled from the shelf of Morocco. From one to 67 fresh spermatangia (mainly 16–32) were found on their buccal membranes. The buccal membrane of these females holds 16–46 (on average 27) seminal receptacles.

Table 3. Sizes of different parts of hectocotylyzed arms in *Todaropsis eblanae* during maturation in the Eastern Atlantic from Morocco to Namibia.

Stage of maturity	Number of studied males	Mantle length (ML), cm	Length of modified proximal part, % of AL	Length of sucker bearing part, % of AL	Length of hectocotylyzed distal part, % of AL
Right arm					
III	10	7.0–11.0	12.2–26.0/23.0	13.5–40.6(23.8)	22.6–50.0(42.0)
IV	5	10.0–13.0	18.4–34.0/28.0	0.0–13.2(3.0)	44.0–60.0(52.0)
V	9	13.0–16.7	23.0–36.8/29.0	0.0–14.0(1.5)	48.3–59.1(54.0)
Left arm					
III	10	7.0–11.0	11.2–28.6(19.7)	57.1–72.7(64.0)	–
IV	5	10.0–13.0	20.0–31.0(25.0)	52.0–64.0(57.2)	–
V	9	13.0–16.7	21.0–33.0(26.0)	48.2–59.5(56.0)	–

Discussion

In males of *T. eblanae*, the SCO has the typical structure for Teuthida. Its parts are well-separated. There was no evidence for variability in the structure of the SCO in specimens from different parts of the species range. The growth of the parts of the SCO is characterized by isometry, after which followed the insignificant increase of size of forming spermatophores during the mature period of ontogenesis.

The spermatophores of *T. eblanae* have peculiar features sharply distinguishing them from other Ommastrephidae. It is the only species of the family which has no tip to the cement body, which passes directly into the ejaculatory tube. The distinction between spermatophores in specimens from the Atlantic and the Indian Oceans was established. The latter have larger absolute and relative total length, total weight, length of cement body and volume of seminal reservoir. However, because of the small number of squid studied, not all of these findings are significant. Also, the connection of the cement body and ejaculatory tube differs. *VC* in spermatophores of Atlantic specimens is generally higher (11.2% versus 6.6%). This morphological variability is in agreement with the data on genetic variability in *Todaropsis* from the Atlantic Ocean (Dillane et al. 2005). Moreover, the squid from the Indian Ocean obviously produce lower numbers of spermatophores. The maximum number of spermatophores (269) was discovered in male squid caught near the north-west coast of Africa (Hernandez-Garcia 2002). Their mean number in specimens from the Atlantic part of the range is 200 (Roper et al. 1984).

Superficially, these differences appear marked, and it may be that they have taxonomic significance. However, the study size is too small to draw final conclusions, and further studies with larger sample sizes are necessary. This demonstrates that the problem of intraspecific divergence is especially important for *T. eblanae* investigations.

The concentration of spermatozoa in the seminal reservoir of the first proper spermatophores of maturing male squid from the Barents Sea were low, half as much relative to data from mature males from the shelf of Namibia (2.15–2.55 million/mm³). Final values are within the typical limits for other ommastrephid squid (*Ornithoteuthis*

and *Hyaloteuthis*), at 1.39–3.4 million/mm³ (Laptikhovsky 1990). Low concentrations of spermatozoa in the specimen from the Barents Sea may be connected with abnormal development of the SCO and dilution of its secretion.

During sexual maturation in males, both ventral arms are modified, but only the right arm is actually a functional hectocotylus. The suckers of the dorsal row in the right hectocotylized arm modify into leaf-like plates, and those in the ventral row are reduced from the central part in both proximal and distal directions. It is possible that the basal and proximal modified parts of the arms with coarse crests carry out an additional function during mating. Naef (1923) indicated that at mating these parts of the arms slightly open the mantle cavity. We suppose that, using proximal coarse crests, the male holds the female by the outer side of her ventral arms. At the same time, spermatophores are transferred to the female between the leaf-like plates of the hectocotylus. Using the wide sail-like membrane of the hectocotylus, spermatophores are passed on to the buccal sperm receptacle.

The hectocotylus of the ommastrephid squid *Nototodarus gouldi* has a similar structure, but several suckers survive in its central part (Dunning and Forch 1998). In males of *Todarodes*, only the right ventral arm is hectocotylized. The dorsal row of leaf-like plates also form in the distal part of the hectocotylus. However, the ventral sail-like membrane is narrow and the suckers in the proximal part of the hectocotylus are not modified (Roper et al. 2010). The hectocotylus in Ommastrephinae have no structures for holding the females and transferred spermatophores (Wormuth 1976; own data), and it is more likely it acts as a “conductor’s baton” for directing spermatophores from the funnel to buccal sperm receptacle. Hectocotyli of *T. eblanae* and *Nototodarus* (Dunning and Forch 1998), therefore are the most aberrant among Ommastrephidae.

The data on maturing males of *T. eblanae* obtained from the Barents Sea are of greatest interest. The most eastern location where they were caught is situated at a distance of more than 2000 km to the north-east from the previous northern limit of the species. The water temperature of the Barents Sea is thought too low for habitation and reproduction of *Todaropsis* (Zumholz and Piatkowski 2005). Hence, the specimens caught in the Barents Sea most probably belong to the nearest population which inhabits the shelf of the North Sea. *Todaropsis eblanae* could be transferred from the North Sea to the Barents Sea along the eastern branch of the Norwegian current, and then by the southern and northern branches of the Nordcap current (Sabirov et al. 2009). Since 1999, increased water temperature has been observed in the Barents Sea (Boitzov 2006; Walczowski and Piechura 2006), which is the terminal point of the Gulf Stream system. Evidently, the spreading of this species to the Barents Sea has happened periodically in recent years. However, because of the specific low abundance of lesser flying squid (Nigmatullin 2000; Lordan 2001; Roper et al. 2010), it is seldom caught in the Barents Sea. Investigated males of *T. eblanae* belong to the seasonal-spawning group that reproduced in June–November on the North Sea shelf. Male size of maturity in this group is 92–140 mm of ML; they have up to 130 spermatophores of length 10.9–18.1 mm (Hastie et al. 1994). Taking into account the speed of spermatophore formation of about five per day (Sabirov 1995), the specimen from the Barents Sea could reach functional maturity in October. The structure of its reproductive system was partly abnormal. This abnormality in hectocotylus structure included the highly developed ventral sail-like membrane and the increased number of specialized appendages. The SCO had an underdeveloped sperm duct, the tissues of the SCO

were swollen, and the produced secretion evidently was much diluted. As a result, the spermatophores were fragile. Hence, the Barents Sea is assumed to be a zone of non-reproductive spreading of *T. eblanae*.

Todaropsis, together with *Illex*, are the most primitive ommastrephids. From a phylogenetic point of view, *Todaropsis* (Todaropsinae) occupies an intermediate position between Illicinae and Todarodinae (Nigmatullin 2007; Roper et al. 2010). The aberrant unique traits are predominant in the functional morphology of its male reproductive system and hectocotylus. However, the pattern of spermatophorogenesis is plesiomorphic.

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