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Spermatozoa of *Conger myriaster* Observed by Electron Microscopy

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ABSTRACT—We examined the morphological features of spermatozoa from *Conger myriaster* by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Spermatozoa consisted of three regions, a head, neck and flagellum. Total length was approximately 40 μm, with the head 3 μm, neck 0.5 μm and flagellum about 37 μm. The head was crescent-shaped, with a mitochondrion on the concave surface. The neck, with an attached rootlet, consisted of two constrictions close to the base of the flagellum. Two bundles extending from the proximal centrioles existed on both the convex and concave surfaces of the sperm head. The flagellum showed a 9+0 axonemal pattern. Some flagella showed a coiled form, the developmental mechanism of which was unclear. These features were compared with those of other Anguilliformes.

INTRODUCTION

Spermatozoal ultrastructures of sixteen species of the order Anguilliformes (teleost fish) have been investigated to date. Studies on those of the family Anguillidae have been performed for *Anguilla anguilla* (Ginsburg and Billard, 1972; Billard and Ginsburg, 1973; Gibbons *et al*., 1983), *A. japonica* (Çolak and Yamamoto, 1974; Gwo *et al*., 1992) and *A. australis schmidtii* and *A. diffenbachii* (Todd, 1976). Mattei and Mattei (1974) have reported those of family Congridae, *Congermuraena bertini*, *Paraconger notialis* and an undetermined congridae. Nine other species belonging to the families Echelidae, Heterenchelyidae, Muraenesocidae, Muraenidae and Ophichthyidae have also been described (Mattei and Mattei, 1974).

According to these results, common features of spermatozoa of Anguilliformes (except for Muraenidae) are a crescent-shaped head, a mitochondrion located in the concave surface of the head, a rootlet attached to the neck region, a flagellum of the 9+0 pattern, and, in common with other teleosts, the absence of the acrosome.

The conger eel, *Conger myriaster* (Congridae), is widely distributed in the coastal waters of Japan and Korea, and in the East China Sea (Takai, 1959; Asano, 1984). Recently, we obtained sexually mature males of *C. myriaster* from which fresh spermatozoa could be milked by squeezing. Here, we examined the spermatozoa of *C. myriaster* by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to clarify its morphological characteristics, and compared results with those of other Anguilliformes.

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MATERIALS AND METHODS

Fishes used

Parent *C. myriaster* were caught with trap pots in October 1996 in Mikawa Bay, Aichi Prefecture, Japan. They were reared in an open-air tank for 19 months. Annual water temperature was controlled from a low of 10°C in May to a maximum of 20°C in September. They were fed a commercial eel diet (Nisshin Flour Milling Co., Ltd.). By May 1998, average body weight of males (approx. 200 individuals) was 200 g. Milt could be obtained by light pushing on the abdomen. Age of the fishes was 2.5 years judging from body length at the time they were caught.

Preparation for SEM

Milt of *C. myriaster* was squeezed from three matured males anesthetized with 2-phenoxyethanol. The collected fresh milt was dropped into a fixative of a 2.5% glutaraldehyde (GA) solution in M/10 phosphate buffer (pH 7.4). After fixation for two days the materials were rinsed in distilled water and stained with a 3% platinum blue solution. The stained specimens were treated with a 20% dimethylsulphoxide (DMSO) solution to avoid ice crystal damage and observed in the hydrous state with a low vacuum SEM (Hitachi S-2360N) equipped with a cooling stage at –10°C and 130–270 Pa (Tanaka *et al*., 1997, 1998).

Some materials were adsorbed on a specimen stub (Jeol, SEMpore) and dehydrated through a graded ethanol series. The specimens were then transferred to isoamyl acetate and dried in a critical point dryer (Hitachi HCP-1). The dried specimens were coated with Au in an ion coater (Jeol JFC-1200) and observed in the SE mode of a SEM (Jeol 8500 LV).

Preparation for TEM

Some materials were post-fixed in 1% osmium tetroxide in M/10 phosphate buffer (pH 7.4) for 1 hr. These were then dehydrated through a graded ethanol series and embedded in Quetol 812 resin. Ultrathin sections (8 nm) were cut with an ultra-microtome UCT (Leica), double stained with uranyl acetate and lead citrate, and observed with a TEM (Jeol JEM1220).

RESULTS

Observations by SEM

On low vacuum SEM, spermatozoa of *C. myriaster* consisted of three regions, namely a head, neck and flagellum. Total length was approximately 40 μm. The head was gently curved and the neck was narrowed, from which a short rod-like structure (rootlet) approximately 0.7 μm long projected. The flagellum was about 37 μm long and 0.1 μm in diameter. The tip of the flagellum was slightly fanned (Fig. 1).

On ordinary SEM, the head was 3 μm long by 1 μm wide and curved like a crescent or bowl, with the cephalad portion pointed sharply. The caudal portion of the head tapered off to the slender neck (Fig. 2). On the convex surface of the head, four striae running from the caudal portion to the cephalad were seen (Fig. 3A). On the concave surface, a spherical body (0.5–0.6 μm in diameter) was located at the cephalad end and five striae running from the caudal portion to the side of the spherical body were seen. The ends of the five striae seemed to be connected to the spherical body surface (Fig. 3B). The neck was somewhat constricted and measured roughly 0.5 μm long (Fig. 3C). The rootlet was approximately 50 nm in diameter and projected from the end of the neck close to the origin of the flagellum (Fig. 3D). The flagella of most spermatozoa were elongated, but around 10% showed a coiled appearance. In these cases the flagella were coiled tightly with about 7-8 strata as counted from the center to the periphery (Fig. 2).

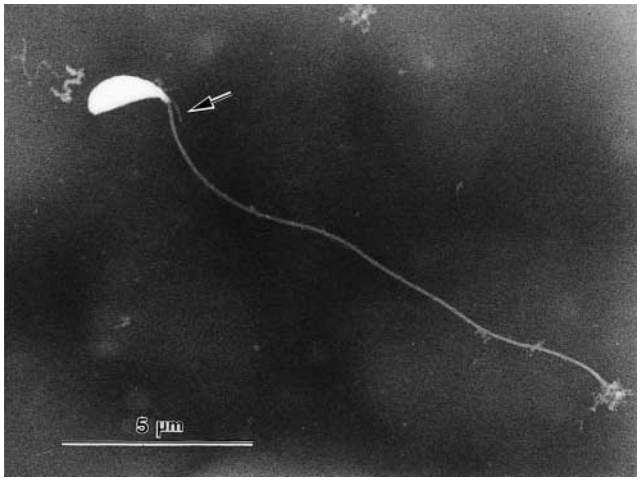

Fig. 1. Whole spermatozoon of *Conger myriaster* observed by low vacuum SEM. This observation was performed with hydrous specimens. Arrow shows rootlet projecting from the neck. ×5,800.

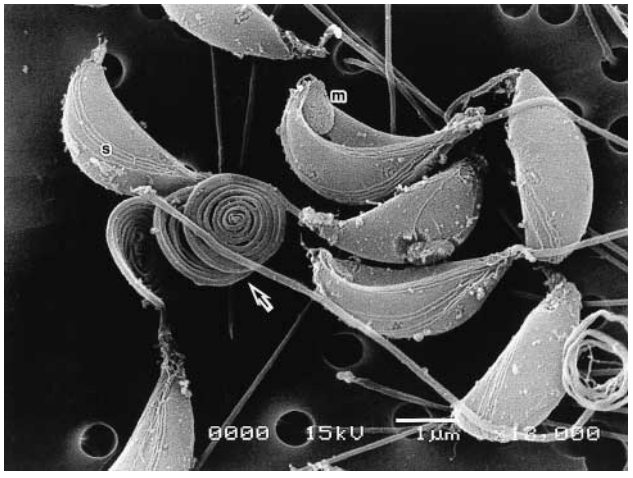

Fig. 2. Scanning electron micrograph of spermatozoa from *Conger myriaster*. Four striae (s) are seen on the convex surface of the head and a spherical body (m) is seen on its concave surface. Coiled flagella (arrow) are seen. ×14,000.

Observations by TEM

The head of the spermatozoon was electron dense and contained chromatin material forming the nucleus. A wall-less vacuole was present within the nucleus (Fig. 4A). This was randomly distributed in the nucleus and sometimes contained cytoplasmic material (Fig. 4D). Some nuclei contained up to three wall-less vacuoles. The spherical body on the head showed a crystae structure, indicating it to be a mitochondrion (Fig. 4B). The whole head including the mitochondrion appeared to be covered with a plasma membrane (Fig. 4A, B, C). The neck was formed of two constrictions close to the base of the flagellum (Fig. 4D). Microfibrils on the nuclear surface extending from the proximal centriole into the cephalad end of the head were seen (Fig. 4A, C).

The proximal centriole was gradually transformed into two bundles on the nuclear surface of the spermatozoa (Fig. 5A–D), being close together posteriorly and separated anteriorly (Fig. 5B–D). The two bundles consisted of a five triplet and a four doublet A-C that lacked middle subfibrils B (Fig. 5C, D), but in contrast sometimes showed a four triplet and a five doublet pattern (Fig. 5B). In most cases the five triplets extended along the concave surface of the nucleus and the four doublets extended along the convex side.

The flagellum consisted of a 9+0 axonemal pattern (Fig. 6A–C). In transverse sections of main flagellum, the inner dynein arms on the two subfibrils of each filament were seen but outer dynein arms were not seen (Fig. 6B). In the flagellum near to the distal centriole, the two subfibrils were linked to the plasma membrane by radial Y-shape electron dense bodies (Fig. 6A). Near the distal end of the flagellum, the two subfibrils became unclear (Fig. 6C). Figure 6D shows that coiled flagella were also seen in TEM observation. In this case the coil was sectioned obliquely.

A schematic drawing of a spermatozoon of *C. myriaster* is given in Fig. 7.

DISCUSSION

Comparison of the morphological features of a spermatozoon from *C. myriaster* with those of other Anguilliformes showed them to be closely similar in overall shape. A few differences from the previous descriptions are seen in size and location of the organelle.

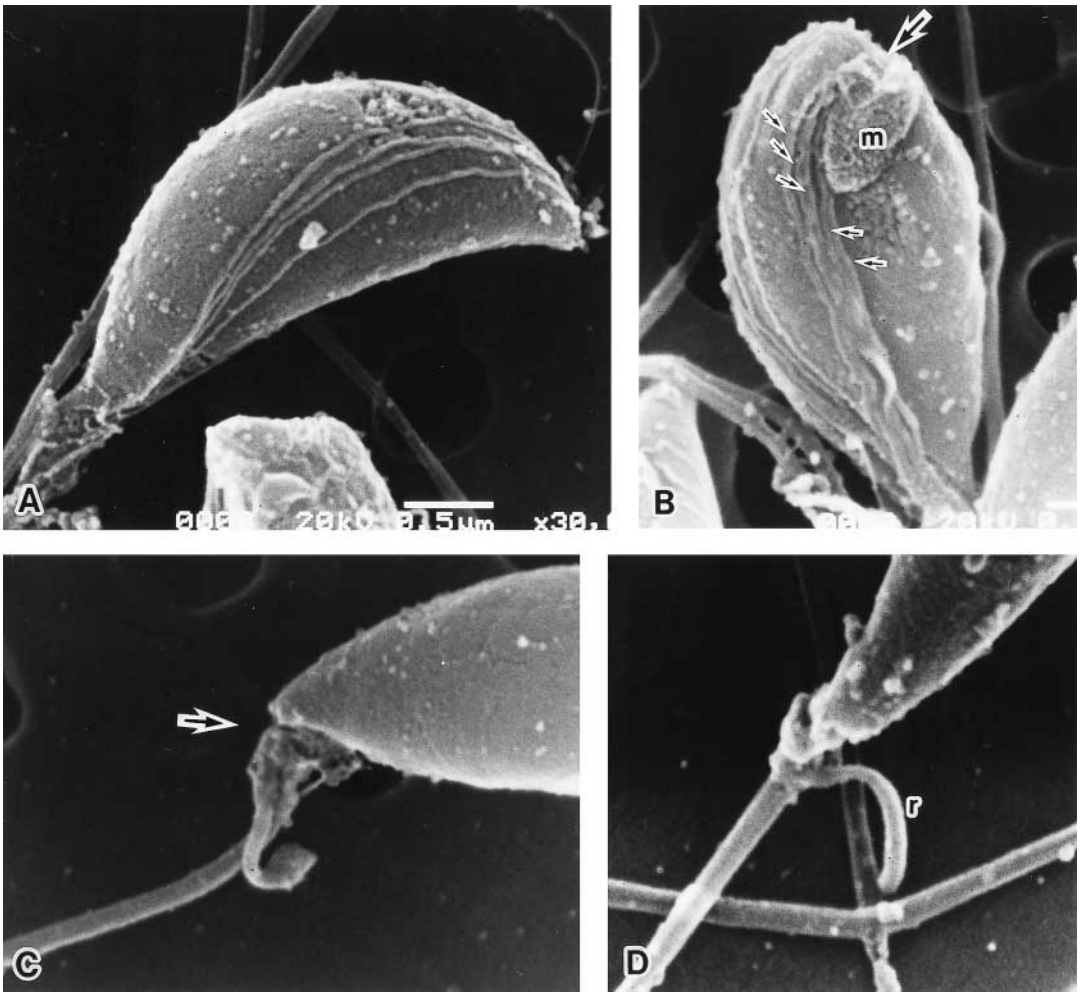

Fig. 3. Scanning electron micrographs of the heads and necks of spermatozoa from *Conger myriaster*. (A) Magnification of the concave side of the head. Four striae are seen. ×24,000. (B) Magnification of the convex side of the head. Five striae (small arrows) and a spherical body (m) are seen. The tips of striae are connected to the spherical body (big arrow). ×23, 000. (C) Magnification of the neck (arrow). ×30,000. (D) Magnification of rootlet (r). ×30,000.

Head length of other anguilliform spermatozoa varied from about 6 to 20 μm (summarized by Jamieson, 1991), indicating that of *C. myriaster* (3 μm) to be the smallest. The head width in *C. myriaster* (1 μm) is close to that of *A. japonica* (Çolak and Yamamoto, 1974), but the head shape seems to be somewhat bulbous compared to other Anguilliformes.

In *A. australis schmidtii*, *A. dieffenbachii* (Todd, 1976), *A. anguilla* (Billard and Ginsburg, 1973), *Paraconger notialis*, *Pythonichthys microphthalmus* and an undetermined Congridae (Mattei and Mattei, 1974), the mitochondrion is located at the cephalad end of the sperm head. In contrast, it reported to be located in the posterior sleeve of the head in *A. japonica* (Çolak and Yamamoto, 1974) and *Congermuraena bertini* (Mattei and Mattei, 1974). However, Miura *et al*. (1991) and Gwo *et al*. (1992) subsequently noted that the location of mitochondrion in *A. japonica* was on the cephalad portion of the head. Furthermore Çolak and Yamamoto (1974) reported the existence of some mitochondria in *A. japonica* but this observation differs from that subsequently given by Miura *et al*. (1991) and Gwo *et al*. (1992), in which single mitochondrion were seen. In the present study, a single spherical mitochondrion was seen in the spermatozoa of *C. myriaster*. Together, these findings indicate that a single mitochondrion located at the cephalad end of the head seems to be a common characteristic of the spermatozoa of Anguilliformes.

The rootlet at the neck is approximately 0.7 μm long in *C. myriaster*, but 1–1.8 μm in *A. australis schmidtii*, 1.6–2.0 μm in *A. dieffenbachii* (Todd, 1976), 1–2 μm in *P. notialis* and *C. bertini* (Mattei and Mattei, 1974) and 4 μm in an undetermined Congridae (Mattei and Mattei, 1974), and is thus the shortest among these Anguilliformes. As described by Gibbons *et al*. (1983), the function of rootlet is still uncertain in the present study.

All Anguilliformes investigated to date (Ginsburg and Billard, 1972; Billard and Ginsburg, 1973; Çolak and Yamamoto, 1974; Todd, 1976; Mattei and Mattei, 1974) have a flagellum of the 9+0 type, which lacks the central pair of subfibrils. *C. myriaster* is also of this 9+0 type. Length of the flagellum of *C. myriaster* is closely similar to that of four Anguillidae (Ginsburg and Billard, 1972; Çolak and Yamamoto,

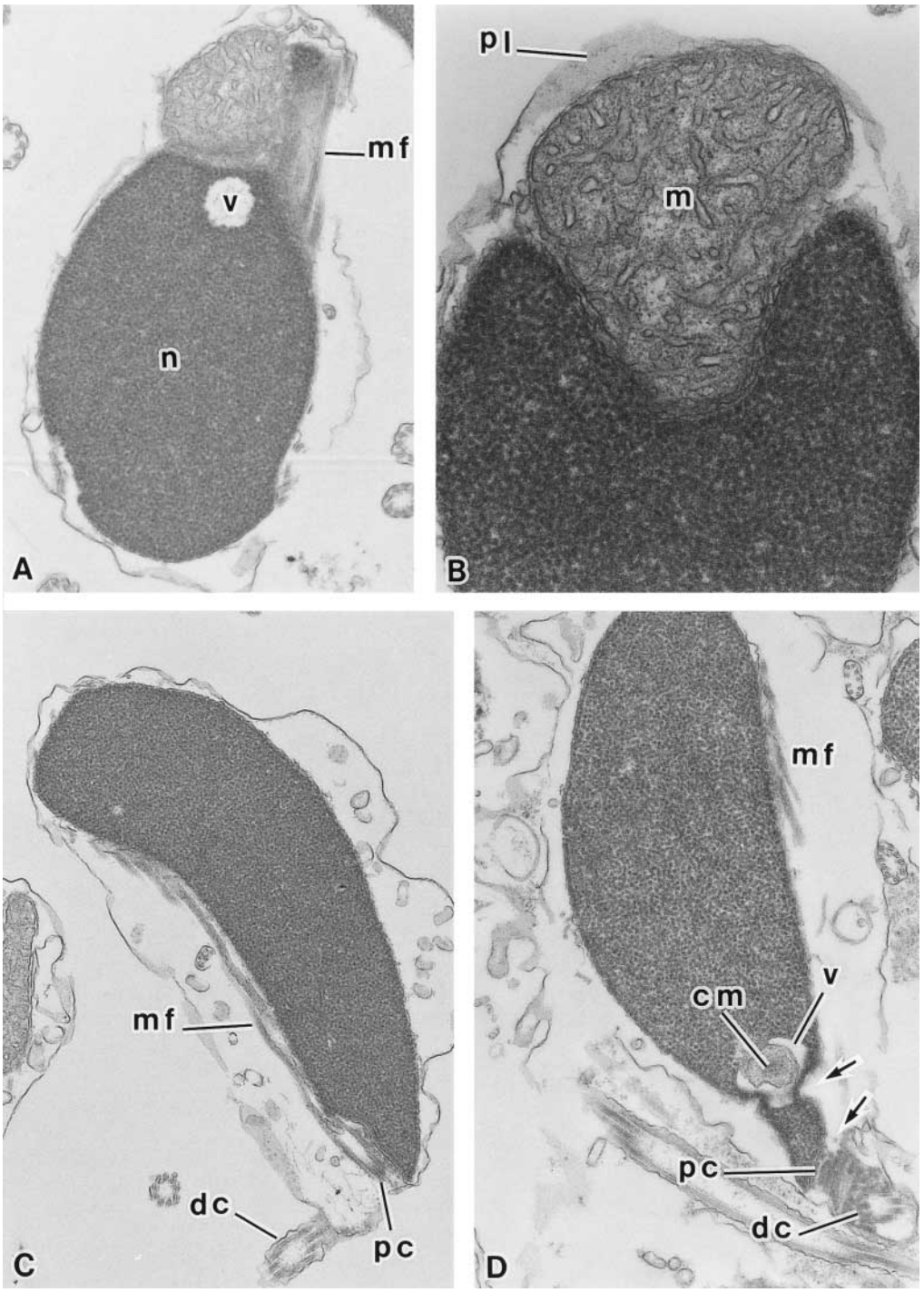

Fig. 4. Transmission electron micrographs of spermatozoa from *Conger myriaster*. (A) Longitudinal section of the head (nucleus). Chromatin material is condensed in the nucleus (n). A wall-less vacuole (v) is present within the head. Microfibrils (mf) are seen beside the mitochondrion (m). ×40,000. (B) Transverse section of the head region. Crystae in the spherical body (m) are seen. The head including the spherical body (mitochondrion) is covered with a plasma membrane (pl). ×71,000. (C) Longitudinal section of the head. The distal centriole (dc) and microfibrils (mf) extending from the proximal centriole (pc) are seen. ×25,000. (D) Longitudinal section of the head. Two constrictions of the neck are seen (arrows). Cytoplasmic material (cm) is seen in the wall-less vacuole (v). (pc); proximal centriole, (dc); distal centriole, (mf); microfibrils. ×32,000.

1974; Todd, 1976).

The four striae found on the concave surface of the sperm head of *C. myriaster* and the five on its the convex side are apparently the same as the two bundles (microfibrils) seen in TEM observations extending from the proximal centriole. Billard and Ginsburg (1973) suggested that this extension of the proximal centriole in *A. anguilla* spermatozoa exists as temporary structures playing a stabilizing role in joining the centrior-flagellar complex to the sperm head during elongation. However, on the basis of the present observations that

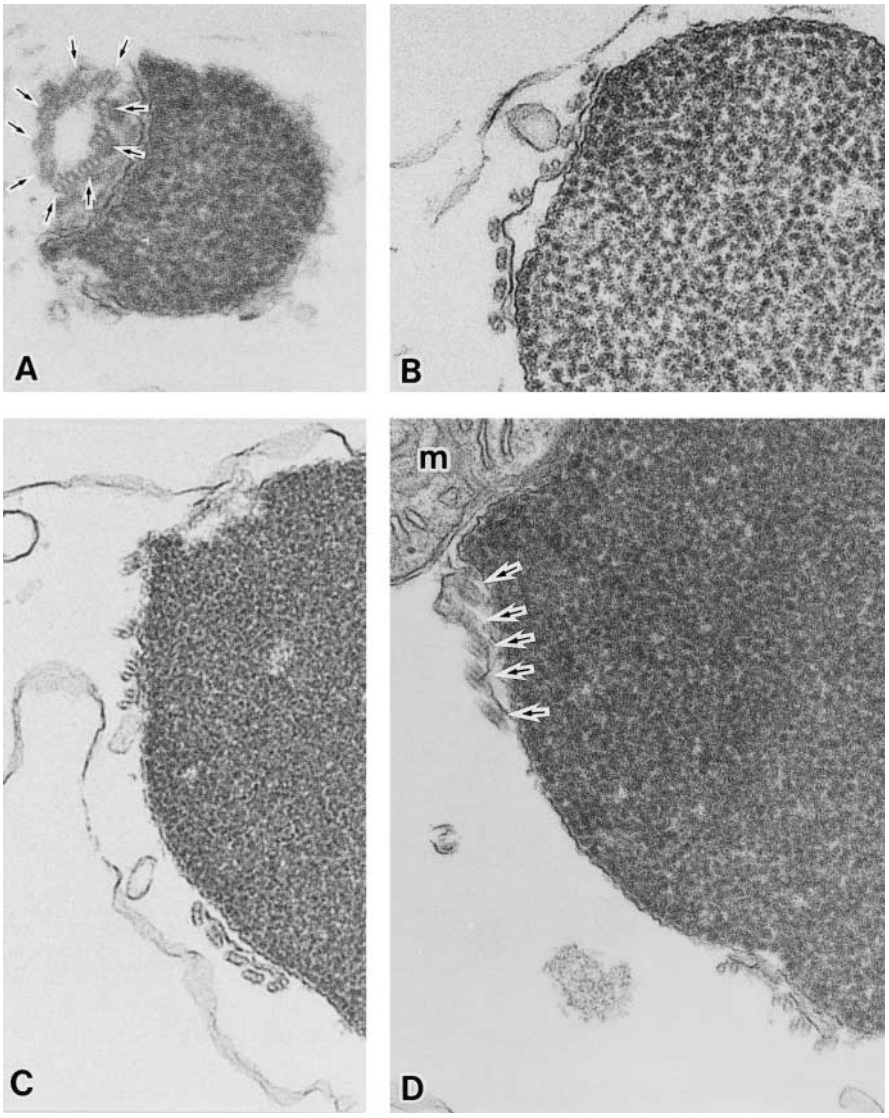

Fig. 5. Transmission electron micrographs of bundles extended from the proximal centriole in spermatozoa from *Conger myriaster*. (A) Transverse section of the proximal centriole located at the caudal portion of the sperm head. Nine microfibril triplets (arrows) are seen. ×82,000. (B) Transverse section of the middle portion of the head. Bundles gradually dissociate from the proximal centriole. ×80,000. (C) Transverse section of the upper side of that shown in Fig. 5(B). (D) Transverse section of the cephalad portion of the head. Five triplet bundle (arrows) shown in the side of the mitochondrion (m). ×80,000.

the end of the inner bundle extended from the proximal centriole connected to the mitochondrion surface, we suggest that these structures play an important role in transportation of ATP as energy to the flagella.

A conspicuous finding in the present study was the observation of coiled flagella (Fig. 2). We first considered this as due to osmotic incompatibility with the fixative used, as occurs in the case of mammalian spermatozoa (Drevius and Eriksson, 1966). However, these were still seen on preparation with fixative in M/15, M/10, and M/7 buffer solutions. Further, flagella showing this coiled shape were seen even in spermatozoa discharged naturally in seawater. We then considered that these might be immature spermatozoa, on the basis that their more compact shape made them more easily accommodated in the limited space of the testis. Observation of testicular tissue from matured *C. myriaster*, however, showed almost all spermatozoa in the testis with extended flagella and the same frequency (approx. 10%) of coiling as in discharged spermatozoa. Thus, we were unable to clarify the developmental mechanism of these coiled flagella in this study. Coiled flagella have also been found in spermatozoa of *Muraenesox cinereus* and artificially matured *A. japonica* prepared by the same method (Okamura *et al*., unpublished observations).

It has been argued that the order Anguilliforemes together with Elopiformes and Notacanthiformes belong to the same super-order Elopomorpha (Greenwood, 1977; Patterson and Rosen, 1977). Such classification has been defined on the basis of anatomic and osteological characters, with the most remarkable feature being the occurrence of leptocephalus

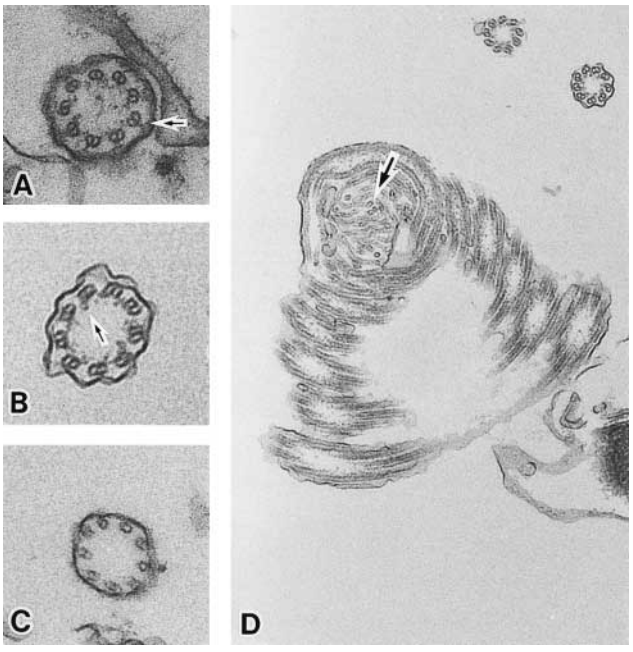

Fig. 6. Transmission electron micrographs of the flagella of spermatozoa from *Conger myriaster*. (A) Transverse section through the flagellum near to the distal centriole. The two subfibrils were linked to the plasma membrane by radial Y-shape electron-dense bodies (arrow). ×120,000. (B) Transverse section through the main flagellum. The inner dynein arms (arrow) are seen. ×136,000. (C) Transverse section through the flagellum near the distal tip. ×136,000. (D) Cross section of coiled flagella. The flagellum is coiled tightly with about 7–8 strata as counted from the center (arrow) to the periphery. In this case the coil is somewhat bent. ×55,600.

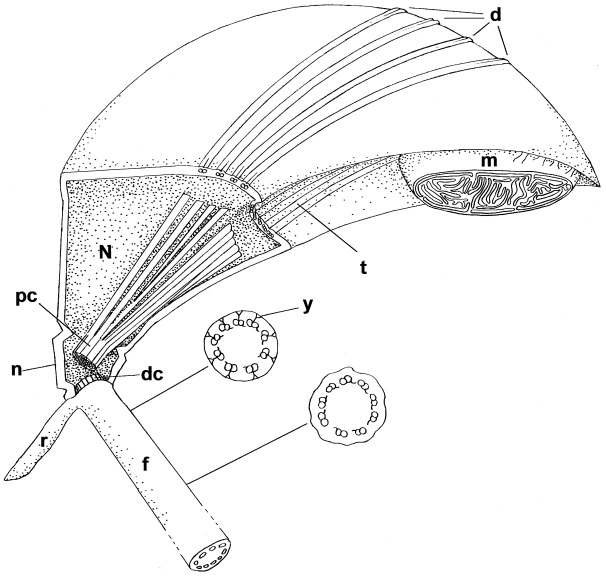

Fig. 7. Schematic drawing of a spermatozoon from *Conger myriaster*. N; nucleus, m; mitochondrion, d; four doublet bundle, t; five triplet bundle f; flagellum, r; rootlet, pc; proximal centriole, dc; distal centriole, n; constricted neck, y; radial Y-shape electron dense bodies.

larva (Lauder and Leim, 1983; Smith 1989). Further, the major features of the spermatozoa, namely a crescent-shaped head with a mitochondrion on the concave face, a rootlet and a flagellum of the 9+0 type, have been found not only in Anguilliformes but also in Elopiformes (Mattei and Mattei, 1972, 1973, 1974) and Notacanthiformes (Mattei, 1988). On this basis, Mattei and Mattei (1974) noted that the similarity among spermatozoa may be significant to the evolution of elopomorph fishes. Jamieson's (1991) subsequent superimposition of the morphologies of spermatozoa on a phylogenetic tree of Elopomorpha showed good agreement with these previous classifications. These findings indicate that, in addition to the traditional indices of anatomic and osteological characters, the morphological characteristics of spermatozoa are increasingly relevant to the divergence of species in Elopomorpha. Our present study provides the seventeenth reference for the order Anguilliformes, and may be of significance in further studies on the evolution and systematics of Anguilliformes or elopomorph fishes. Further studies on the ultrastructure of spermatozoa in other Anguilliformes or elopomorph fishes are now in progress.

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