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Ultrastructure of *Thunnus thynnus* and *Euthynnus alletteratus* spermatozoa

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The spermatozoa of *Thunnus thynnus* and *Euthynnus alletteratus* consist of an acrosome-less head (comprising the ovoid nucleus and the short midpiece) and a long flagellar tail that contains the conventional 9+2 axoneme and lacks lateral fins. The centrioles are arranged at approximately right angles and lie outside of a shallow nuclear groove. The flagellum inserts laterally on the nucleus, therefore the spermatozoon is asymmetrical. The midpiece contains a few mitochondria which are separated from the axoneme by the cytoplasmic canal; they are spherical in *T. thynnus* and elongate, somewhat irregular in *E. alletteratus*. Although the main ultrastructural characteristics of the spermatozoa appear to indicate a great homogeneity in the sperm morphology within the family Scombridae, small species-specific divergences may be of use in systematics.

Key words: sperm ultrastructure; Scombridae; *Thunnus thynnus*; *Euthynnus alletteratus*.

INTRODUCTION

In spite of an evident overall simplicity, the teleost spermatozoa exhibit a broad range of varying structural features that makes it impossible to depict a common sperm type (Mattei, 1970). Morphological differences between species can be used in taxonomy, although the ultrastructural characterization of the spermatozoon is not as useful in solving phylogenetic relationships as in other animal groups (Jamieson, 1991; Mattei, 1991). Among the Teleostei, Mattei (1970) distinguished two main morphological sperm types. The type II spermatozoon, which is also referred to as the perciform-type sperm because of its widespread occurrence among the perciforms, differs from the plesiomorphic type I spermatozoon in that the centriolar complex lies outside of the nuclear fossa and the flagellar axis is parallel to the nuclear base.

Within the family Scombridae the spermatozoal ultrastructure has been examined in only three mackerel species, *Cybium tritor* Cuvier (Mattei, 1991), *Scomber japonicus* Houttuyn (Mattei, 1991; Hara & Okiyama, 1998) and *Scomber australasicus* Cuvier (Hara & Okiyama, 1998). Tunas are large scombrids that have a high commercial value [in particular the bluefin tuna *Thunnus thynnus* (L.)] and hence are subject to high fishing pressure, which is resulting in a serious depletion of the stocks (Sissenwine *et al.*, 1998). Despite the importance of tuna species, very little is known about their reproductive biology, in particular about the structure and physiology of their sperm. Doi *et al.* (1982)

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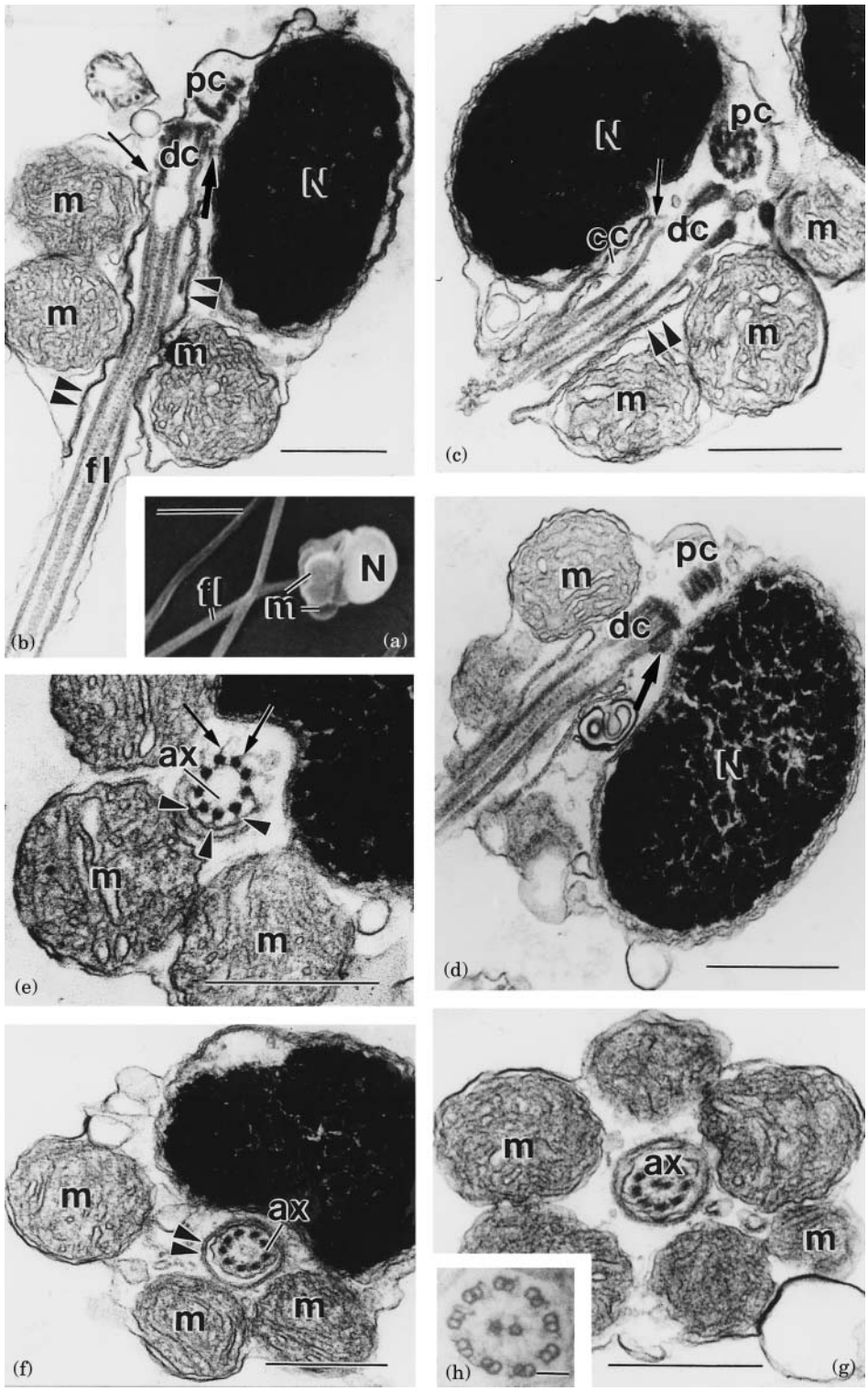
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studied the activity of *T. thynnus* sperm but did not provide a structural description of the gametes. The present paper describes the sperm ultrastructure of two tuna species with the purpose of increasing the current knowledge of Scombridae spermatozoa.

MATERIALS AND METHODS

Adult male *T. thynnus* and *Euthynnus alletteratus* (Rafinesque) were fished by trap in Barbate de Franco (Cádiz, southern Spain) in May–July 2000. Immediately after the fishes were hauled on board, samples of semen and small pieces of testes (*c.* 1 mm^3 in size) were fixed for 3–4 h in 2·5% glutaraldehyde buffered with 0·1 M sodium cacodylate (pH 7·2) containing 10% sucrose. Following two 30 min washes in cacodylate buffer for 1 h, the samples were postfixed for 1 h at 4° C in cacodylate-buffered osmium tetroxide, and rinsed several times in the buffer. Afterwards, the sperm samples were processed for scanning (S.E.M.) or transmission (T.E.M.) electron microscopy, and the testis samples prepared solely for T.E.M.. For S.E.M., spermatozoa were attached to coverslips previously coated with 0·1% poly-L-lysine. After dehydration through an ascending series of acetones, the sperm cells were critical-point dried in a Balzers critical point dryer CPD 030, coated with gold in a Balzers sputter coater SCD 004, and viewed in a Jeol JSM 820 electron microscope. For T.E.M., either the tissue blocks or pellets of sperm obtained by gentle centrifugation (2500 *g*, 10 min) were dehydrated in acetone and embedded in Spurr's epoxy resin. Thin sections (*c.* 80 nm thick) were picked up on copper grids, doubly stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200 EX electron microscope.

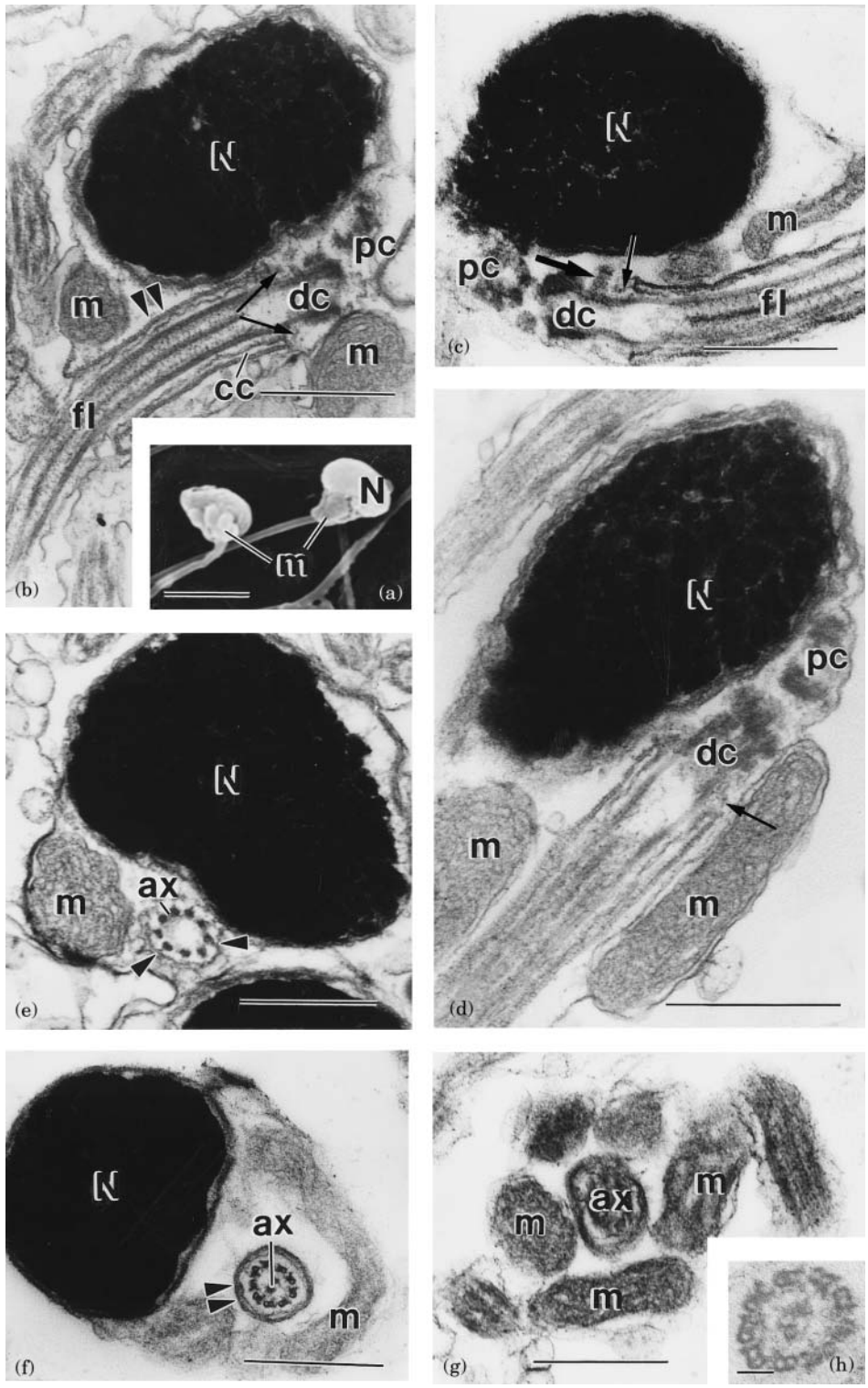
RESULTS

THUNNUS THYNNUS SPERM MORPHOLOGY

The spermatozoon of *T. thynnus* has an acrosome-less head and a long flagellar tail. The whole head measures *c.* 1·9 μm in length and 1·8 μm in width, and comprises the apical nucleus and a few spherical mitochondria located at its base [Fig. 1(a), (b)]. The sperm tail is cylindrical and smooth throughout its whole length, thus lacking lateral fins or ribbons.

The ovoid nucleus contains an electron-dense chromatin that condenses with a granular pattern. At its base, the pore-less nuclear envelope invaginates slightly and forms a shallow groove to accommodate the proximal segment of the axoneme. Thus, the nuclear depression is easily distinguished in transverse [Fig. 1(e),(f)] but not in sagittal [Fig. 1(b)–(d)] sections. The two centrioles are approximately perpendicular to each other, consist of nine peripheral triplets and lie at the base of the nucleus outside of the basal invagination [Fig. 1(b)–(d)]. The distal centriole, which is much longer (*c.* 300 nm in length) than the proximal one (*c.* 150 nm in length), becomes the basal body from which the

FIG. 1. *Thunnus thynnus* sperm. (a) Scanning electron micrographs (S.E.M.) of spermatozoa showing the head, comprising the nuclear and mitochondrial regions, and the proximal part of the tail. (b)–(h) Transmission electron micrographs (T.E.M.) of sagittal (b), (d), frontal (c), and transverse (e)–(h) sections at various levels of the sperm cell, including the nucleus (e), (f), mitochondrial ring (g), and flagellum (h). ax, Axoneme; cc, cytoplasmic canal; dc, distal centriole (basal body); fl, flagellum; m, mitochondria; N, nucleus; pc, proximal centriole; ►, Y-shaped bridges at the proximal region of the axoneme; ▻, dense layer underlying the cytoplasmic canal membrane; ➡, lateral plate; ➡, radial fibres projecting from the base of the distal centriole. Scale bars: 0·5 μm, except in (a) 2 μm, and (h) 50 nm.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axoneme emerges to form the sperm flagellum [Fig. 1(b),(d)]. Anteriorly, the distal centriole is surrounded by a dense material [Fig. 1(c),(d)], and laterally an electron-dense plate projects from one side of the basal body to the nuclear envelope [Fig. 1(b),(d)]. The distal centriole and the axoneme are oriented parallel to the basal surface of the nucleus, and the insertion of the flagellar apparatus is clearly asymmetrical as corresponds to the type II teleostean sperm morphology [Fig. 1(b)–(d)]. Nine radial fibres project from the basal body to the plasma membrane at the bottom of the cytoplasmic canal [Fig. 1(b)–(e)]. The axonemal pattern is the typical 9+2 [Fig. 1(h)], though the central singlet microtubules are absent at the transition between the basal body and the axoneme [Fig. 1(e),(f)]. In this short region, the lumen of the peripheral microtubules appear filled with osmiophilic material and Y-shaped bridges attach each doublet to the flagellar membrane [Fig. 1(e)]. Transverse sections at different levels of the flagellum show that microtubules A and B of the axonemal doublets are both hollow [Fig. 1(h)].

Up to six spherical mitochondria, measuring *c*. 550 nm in diameter, can be found around the flagellum at the midpiece region [Fig. 1(g)], but frequently only four or five mitochondria are seen in cross sections of the midpiece, probably because the mitochondria are not all at the same plane [Fig. 1(b)]. The mitochondria have irregular cristae and a moderately electron-dense, finely granular matrix; they are separated from the axoneme by the cytoplasmic canal, which is formed by a deep invagination of the plasma membrane [Fig. 1(b)]. In the canal, the space between the flagellum and plasma membrane is narrow [Fig. 1(e) and (f)]. A dense layer is observed beneath the plasma membrane in the region of the cytoplasmic canal [Fig. 1(b)–(f)].

EUTHYNNUS ALLETTERATUS SPERM MORPHOLOGY

In general, the above descriptions for *T. thynnus* are applicable to the sperm of *E. alletteratus* (Fig. 2). The similarity between the spermatozoa of both species reflects their close phylogenetic proximity. Thus, in *E. alletteratus* the location of the centrioles is external to the groove-shaped basal depression of the nucleus, and the flagellum inserts asymmetrically in the sperm body [Fig. 2(b),(d)]. The structural pattern of the centrioles and axial filament are identical to those observed in *T. thynnus* [Fig. 2(b)–(d),(f),(h)]. Also, as in *T. thynnus*, intratubular differentiations (ITDs) are not visible in the axonemal doublets, since cross sections at various levels of the axoneme show the doublet microtubules to be hollow. Furthermore, accessory structures similar to those described for *T. thynnus* are present in *E. alletteratus*, such as the lateral plate that connects the distal centriole to the nucleus [Fig. 2(b),(c)], the radial fibres projecting from the basal body to the plasma membrane at the anterior end of the cytoplasmic canal [Fig. 2(b),(c)], and the bridges connecting the axonemal doublets to the flagellar

FIG. 2. *Euthynnus alletteratus* sperm. (a) S.E.M. of two spermatozoa. (b)–(h) T.E.M. of sagittal (b), (d), frontal (c), and transverse (e)–(h) sections at various levels of the spermatozoon, including the nuclear (e), (f), mitochondrial (g), and flagellar (h) regions. ax, Axoneme; cc, cytoplasmic canal; dc, distal centriole (basal body); fl, flagellum; m, mitochondria; N, nucleus; pc, proximal centriole; ►, Y-shaped bridges at the proximal region of the axoneme; ▸, dense layer underlying the cytoplasmic canal membrane; ➡, lateral plate; →, radial fibres projecting from the base of the distal centriole. Scale bars: 0·5 μm, except in (a) 2 μm, and (h) 50 nm.

membrane at the commencement of the flagellum [Fig. 2(e)]. The only feature that appears to denote an interspecific difference is the dissimilar shape of the mitochondria, which in *E. alletteratus* are somewhat more elongate and irregular in shape [Fig. 2(d),(f),(g)].

DISCUSSION

The spermatozoa of *T. thynnus* and *E. alletteratus* exhibit the configuration of the uniflagellate anacrosomal aquasperm typically found in externally fertilizing fishes (Jamieson, 1991). Specifically, the sperm ultrastructure of *T. thynnus* and *E. alletteratus* conform to the perciform sperm type or teleostean type II spermatozoon of Mattei (1970), which occurs in as many as 29 out of 41 families of Perciformes studied (Mattei, 1991), and is the result of a simplified (apomorphic) spermiogenic process in which rotation of the spermatid nucleus has been lost (Mattei, 1970; Mattei *et al*., 1979).

In the Percomorpha, the morphology of spermatozoa shows considerable variation in nuclear shape, number of periflagellar mitochondrial layers, and orientation of the centrioles in relation to each other (Mattei, 1991). This variability extends to lower taxonomic levels; in Perciformes, for instance, the spermatozoon is not always uniform even at the the family level (Mattei, 1991). In comparison to the relatively simple aquasperm of the percomorphs, the most complex neopterigian spermatozoa are present in the primitive teleostean groups. Therefore, according to Mattei (1991), it is in these ‘primitive’ groups that the spermatozoon could be useful in systematics, whereas within the Perciformes the evolved perciform-type sperm would be the only form taxonomically useful. The perciform-type spermatozoon was originally described as possessing intratubular differentiations (ITDs) in the A microtubules of doublets 1, 2, 5 and 6 (Mattei *et al*., 1979; Jamieson, 1991; Mattei, 1991). ITDs have been clearly identified in many teleostean species (Mattei *et al*., 1979; Afzelius, 1981; Mattei, 1991; Eiras-Stofella *et al*., 1993), but appear to be absent in perciform-type spermatozoa of other teleosts belonging to different families (Mattei, 1991; Gwo *et al*., 1994; Lahnsteiner *et al*., 1994), including *T. thynnus*, *E. alletteratus* (present study) and two other species of the family Scombridae, *S. japonicus* and *S. australasicus* (Hara & Okiyama, 1998). Therefore, the presence or absence of ITDs does not appear to be a key feature in the characterization of the perciform-type sperm, though it might well define evolutionary affinities within the Perciformes.

There appears to be a consistent homogeneity in the sperm ultrastructure throughout the family Scombridae. The spermatozoa of the tunas examined in this study are very similar to those of other scombrid species previously investigated (Mattei, 1991; Hara & Okiyama, 1998). They share the following chief characteristics: insertion of the flagellum tangential to the base of the nucleus, shallow nuclear fossa forming a groove over the proximal segment of the axoneme (Hara & Okiyama, 1998), centrioles located outside of the nuclear fossa and arranged perpendicularly to each other, deep and narrow cytoplasmic canal, and flagellum lacking ITDs and lateral fins. This homogeneity makes it difficult to define distinct interspecific differences within the family. Between *T. thynnus* and *E. alletteratus*, for instance, the only apparent dissimilarity lies in

the shape and arrangement of the mitochondria, which are more irregular in the latter species. The sperm mitochondrial number also seems to be slightly variable within the family Scombridae: four spherical mitochondria are present in *S. japonicus*, five in *S. australasicus* (Hara & Okiyama, 1998), and six in *T. thynnus* (present study). This character could be useful in scombrid taxonomy as has been shown by Baccetti *et al.* (1984) for Cyprinidae.

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