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Comparative ultrastructure of spermatozoa of three marine teleosts of the genus *Serranus*: *Serranus atricauda*, *Serranus cabrilla* and *Serranus scriba*

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SUMMARY - Spermatozoa of the three species *Serranus atricauda*, *Serranus cabrilla* and *Serranus scriba* were investigated by transmission and scanning electron microscopy. In all three species the spermatozoon is of the primitive type with ovoid head, short midpiece and a long flagellum. The spermatozoa of *Serranus atricauda* and *Serranus cabrilla* are similar, they have no acrosome, their morphology is symmetric with central insertion of flagellum and contain five and four spherical mitochondria around the flagellum, respectively. The sperm of *Serranus scriba* presents stack of membranes apposed to the nuclear envelope, its structure is asymmetrical due to the lateral insertion of the flagellum and it has four spherical mitochondria: three appear to form a rectangular triangle, and one is closer to the centriolar complex. The spermatozoa size suggests a relation of these with the habit of the species, because smaller size and more rounded morphology are observed in deeper species. Morphology shows a close affinity between *Serranus atricauda* and *Serranus cabrilla*, *Serranus scriba* presenting less developed features. This study contributes to existing knowledge of comparative spermatology and may provide a useful systematic and phylogenetic character.

KEY WORDS *spermatozoa - ultrastructure - environmental - taxonomy - phylogeny - Serranus*

INTRODUCTION

Organization of spermatozoa of many teleosts, especially of marine species, is known only from brief characterisations or completely unknown. Both light and electron microscopy of a spectrum of teleost spermatozoa have demonstrated that important morphological differences can be found between different species and can be used for taxonomic purposes (Jamieson, 1991; Gwo and Gwo, 1993; Afzelius and Mims, 1995; Yao *et al.*, 1995; Lahnsteiner and Patzner, 1995, 1996). However, comparative studies have generally been carried out in species of very unlike genera (*e.g.*, Mattei, 1969, 1970; Lahnsteiner and Patzner, 1990; Gwo *et al.*, 1994).

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The species of the genus *Serranus* show synchronous hermaphroditism, so that male and female tissues are functional at the same time, and this has been considered as the most primitive pattern of reproduction within Serranidae (Smith, 1965). The most common mating is serial monogamy, in which individuals are solitary during the day, and pair up and spawn in the late afternoon (see Fischer, 1986). In these cases, one fish functions as male and the other as female so that a cross-fertilization takes place. This special characteristic and the possibility of self-fertilization have led to describe in detail the gonad morphology of genus *Serranus* (Reinboth, 1962, 1970; Atz, 1965; Fishelson, 1970; Febvre *et al.*, 1975; Zanuy, 1977; Bruslé, 1983; Abd-el-Aziz and Ramadan, 1990; García-Díaz *et al.*, 1997), but in any species of *Serranus* the spermatozoon ultrastructure has been examined.

Three species of genus *Serranus* (Serranidae) inhabit in the Eastern Atlantic and Mediterranean. Blacktail comber *Serranus atricauda* Günther, 1874 is a demersal species found on rocky, rocky-sandy or coralline bottoms, to depth up 150 m. Comber *Serranus cabrilla* (Linnaeus, 1758) and painted comber *Serranus scriba* (Linnaeus, 1758) are demer-

sal species found mainly on rocky, mud and algal or seagrass substrates, between 5 and 500 m and 5 to 150 m, respectively (Smith, 1981, 1990; Tortonese, 1986; Bauchot, 1987; Brito, 1991; Franquet and Brito, 1995).

The objective of the present study was *a*) to examine the spermatozoon ultrastructure of the species of genus *Serranus* present in the Canary Islands (Central-Eastern Atlantic), using transmission and scanning electron microscopy, and *b*) to determine if it shares important differences among species of the same genus.

MATERIALS AND METHODS

Mature individuals of *Serranus atricauda*, *Serranus cabrilla* and *Serranus scriba* were caught in two surveys conducted around the island of Gran Canaria (Canary Islands) in June 1998 for ultrastructural analysis. Fishes were anaesthetised with 1,1,1, trichloro-2methyl-2-propanol prior to handstripping. Small pieces of the gonads were fixed in Karnovsky's fixative for 2-4 h and then washed several times in 0.1 M phosphate buffer (for approximately 68-70 h) to 4 °C, for scanning (SEM) and transmission electron microscopy (TEM).

Samples were dehydrated in alcohol, dried from liquid carbon dioxide in a critical-point drier (Tousimis Autosamdri 814), coated with gold-palladium by a sputter coater (Biorad SC 500) and examined with a scanning electron microscope (Hitachi S-4100).

Other samples were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 3 h and dehydrated in acetone for transmission electron microscopy. The tissue was embedded in araldite (Durcupan, Fluka), ultrathin sections were obtained with an ultramicrotome (Ultracut-E, Reichert-Jung) and examined by transmission electron microscopy (Philips CM 10). Measurements were performed on electron micrographs and are expressed as mean ± standard deviation (n = 10).

RESULTS

Spermatozoa of *Serranus atricauda* as well as of *Serranus cabrilla* and *Serranus scriba* are of the primitive type. In all three species the spermatozoon has an ovoid shaped head, a short midpiece consisting of several mitochondria, a long flagellum and has no acrosome.

The head and midpiece are not clearly separated and contain only very small amounts of cytoplasm, which have a granular appearance. The nucleus contains highly electron dense granular chromatin. The nuclear envelope lacks pores. The nuclear invagination is not deep and its dimensions are similar to the centriolar complex.

In the midpiece is found the centriolar complex, consisting of a proximal and distal centriole, and is separated from the flagellum by a cytoplasmic canal. The two centrioles are orientated perpendicularly to each other and arranged at a right angle to the base of the head. The proximal centriole is formed by nine peripheral pairs of microtubules, has no central microtubules, and it houses in the nuclear fossa. The distal centriole consists of nine pairs of peripheral microtubules and of two central microtubules, which form the basal body of the axoneme. Although the size and the number of the mitochondria vary among the three spermatozoa, mitochondria exhibit certain common features and a generalized organelle can be described. The mitochondrion is enclosed by two distinct membranes. The inner membrane separates the organelle volume into two phases: the electron lucent matrix and the intermembrane space. The inner membrane displays numerous infoldings called cristae which are irregular or laminar shaped, and extend into the matrix.

The flagellum shows a typical structure of nine pairs of peripheral microtubules and two central microtubules.

Serranus atricauda and Serranus cabrilla

The general organization of spermatozoa of both species is similar, presenting symmetrical morphology with central insertion of flagellum (Fig. 1).

The head and midpiece together measure 2.33 ± 0.25 and 1.58 ± 0.10 µm in length, with a maximal diameter of 1.92 ± 0.15 and 1.52 ± 0.15 µm in *Serranus atricauda* and *Serranus cabrilla* (Fig. 2), respectively. The ovoid nucleus is 1.19 ± 0.12 µm in longitudinal axis and 1.39 ± 0.13 µm in lateral axis for *Serranus atricauda*; it is 0.98 ± 0.09 µm in length and 1.34 ± 0.11 µm in width for *Serranus cabrilla*. The nuclear fossa is 0.28 ± 0.04 µm long and has a diameter of 0.39 ± 0.05 µm in *Serranus atricauda*; it reaches values of 0.27 ± 0.06 µm in length and 0.37 ± 0.04 µm in width for *Serranus cabrilla* (Figs. 1 and 2).

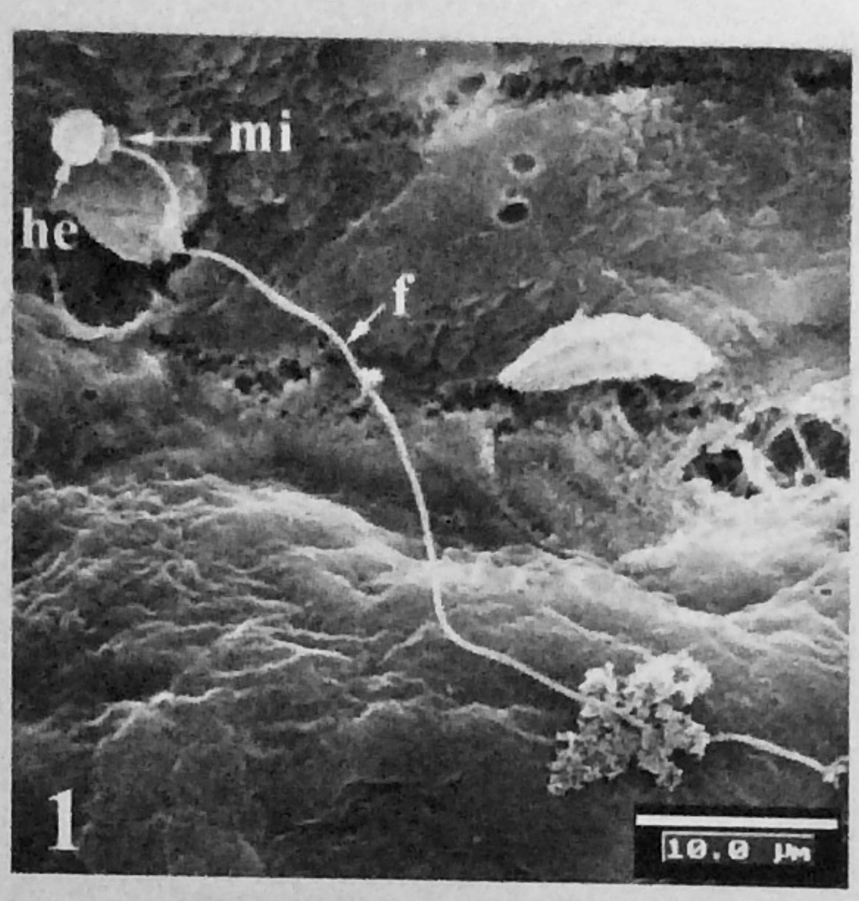
The midpiece contains five equal-size spherical mitochondria with a diameter of 0.54 ± 0.08 µm in *Serranus atricauda*, and four mitochondria with a diameter of 0.57 ± 0.06 µm in *Serranus cabrilla* (Fig. 3). The cytoplasmic canal is 0.32 ± 0.02 µm in width and 0.41 ± 0.04 µm in length in *Serranus atricauda*; it is 0.37 ± 0.05 µm long and 0.21 ± 0.03 µm wide in *Serranus cabrilla*. The proximal centriole presents a diameter of 0.21 ± 0.02 µm and a length of 0.18 ± 0.03 µm in *Serranus atricauda*, while it is 0.21 ± 0.03 µm in length and 0.19 ± 0.03 µm in width for *Serranus cabrilla* (Fig. 4). The distal centriole measures 0.20 ± 0.02 µm and 0.17 ± 0.02 µm in diameter for *Serranus atricauda* and *Serranus cabrilla*, respectively.

The flagellum has a length of about 43 µm and a diameter of 0.19 ± 0.03 µm in *Serranus atricauda*; it has values of about 40 µm in length and 0.18 ± 0.05 µm in width in *Serranus cabrilla* (Figs. 5 and 6).

Serranus scriba

The spermatozoa morphology is asymmetrical due to the lateral insertion of the flagellum (Figs. 7 to 9).

The head and midpiece together have a length of 2.83 ± 0.20 µm and a width of 1.71 ± 0.15 µm. The ovoid nucleus is asymmetrical and measures 1.39 ± 0.15 µm maxi-



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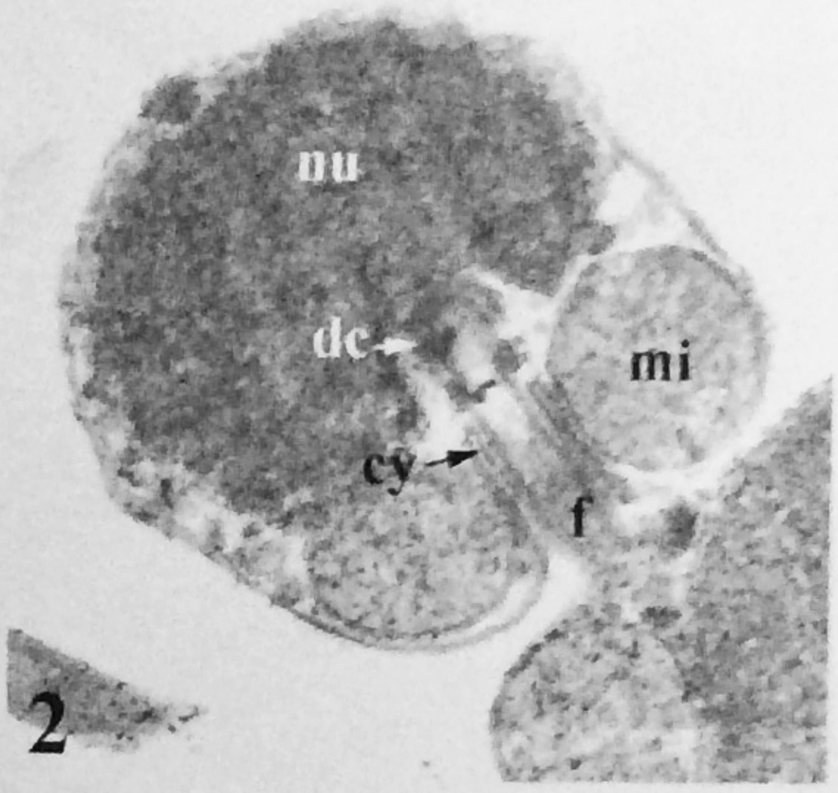

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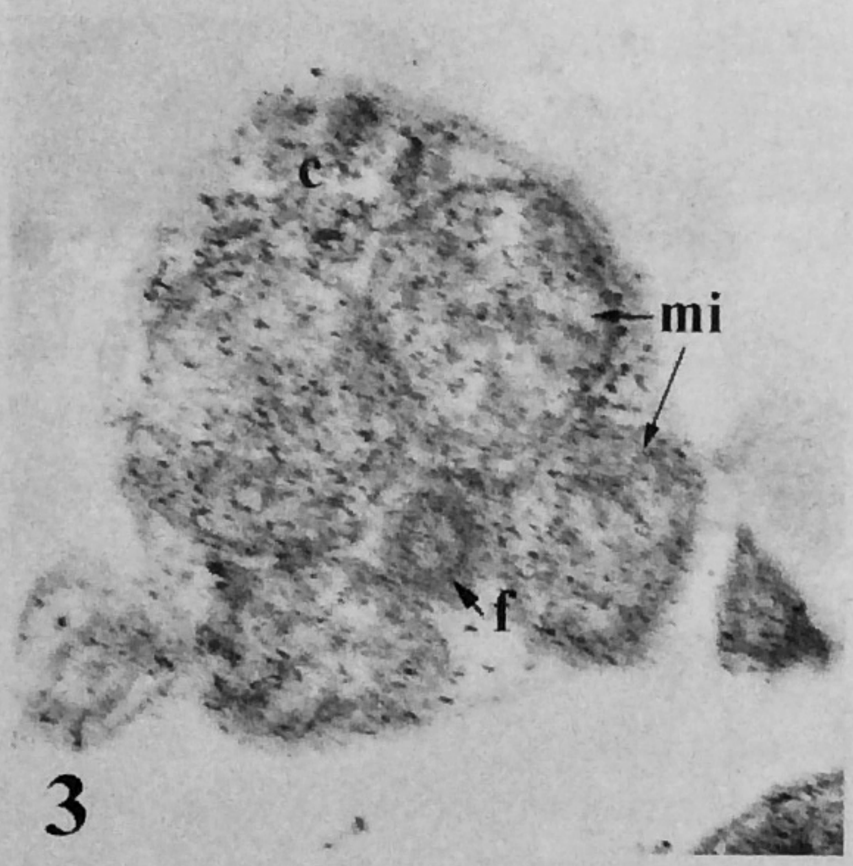

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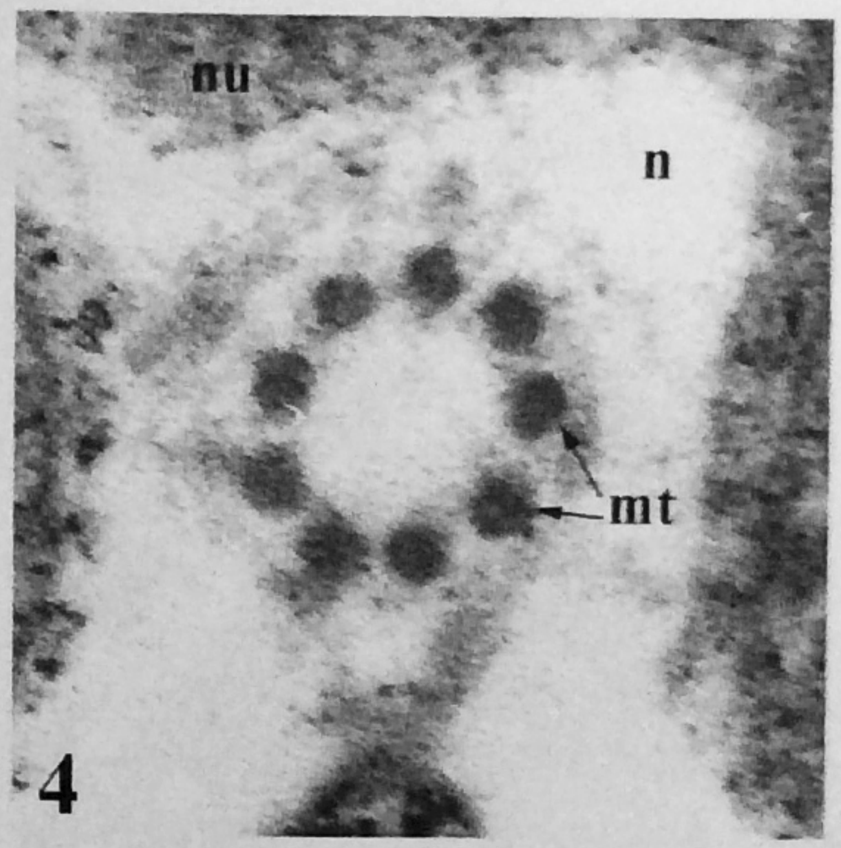

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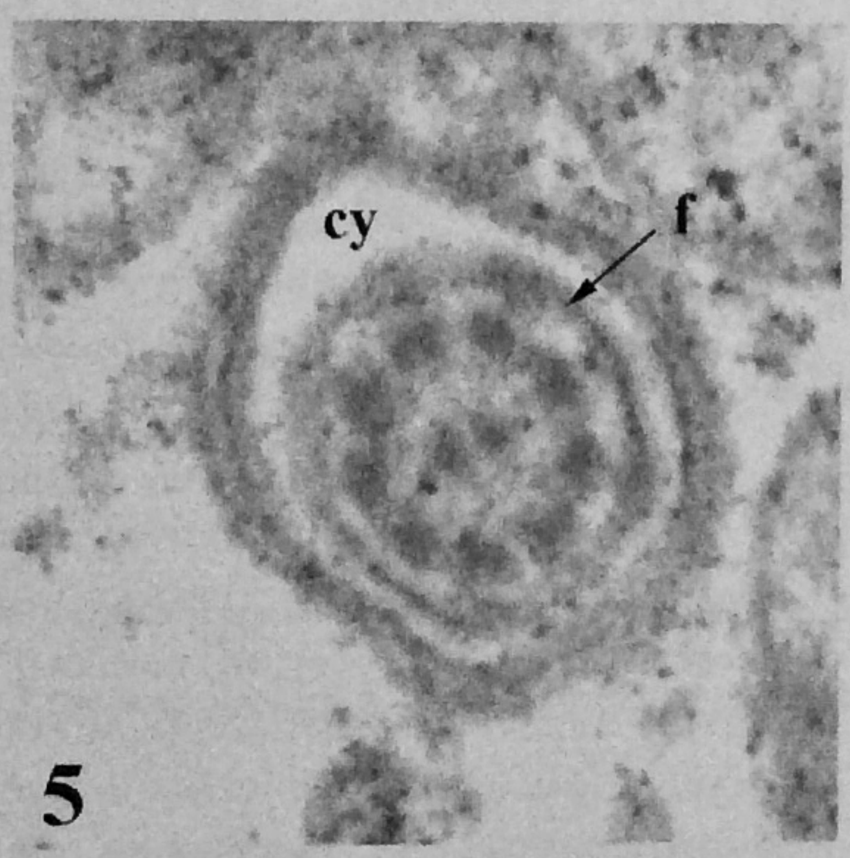

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FIGURE 1 Spermatozoon of *S. atricauda*. SEM. he: head; f: flagellum; mi: mitochondrion.

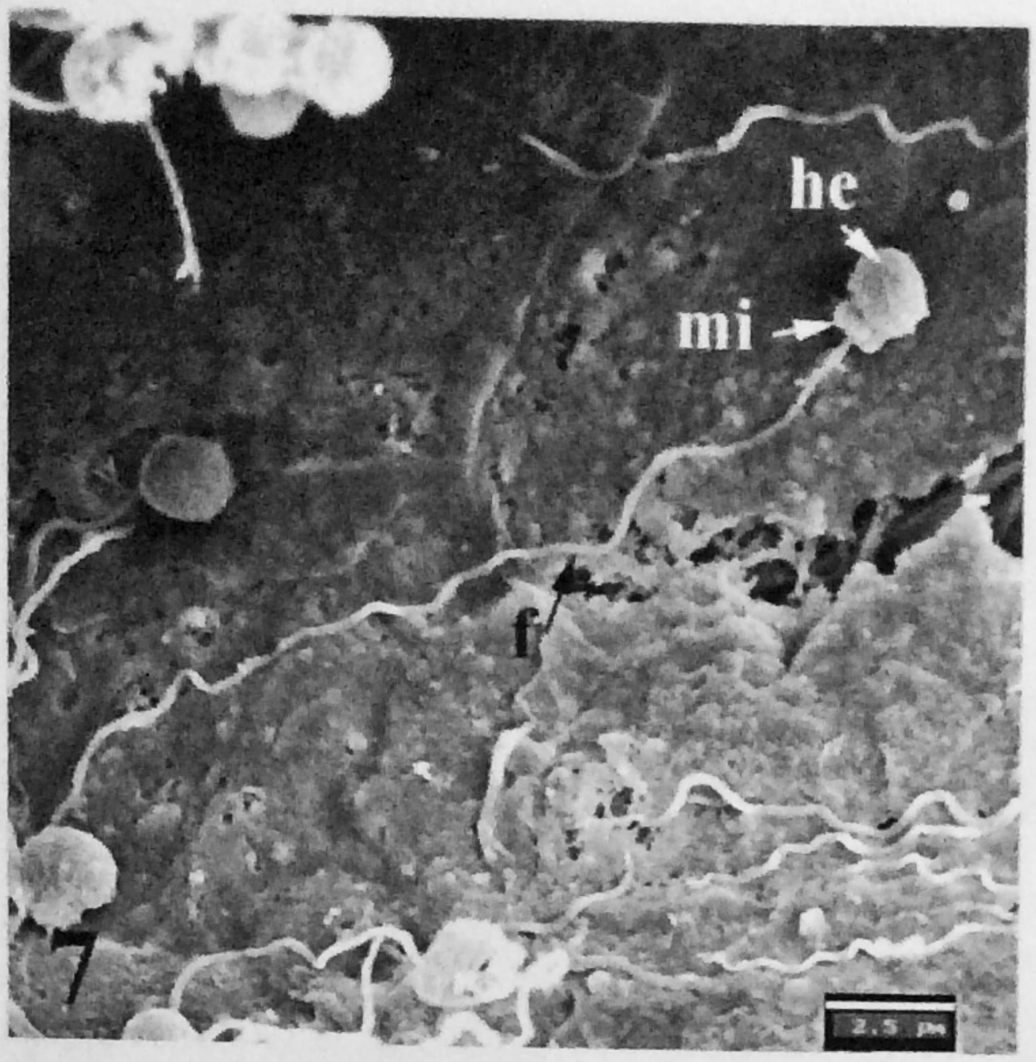
FIGURE 2 Spermatozoon of *S. cabrilla*. TEM. Longitudinal section. cy: cytoplasmic channel; dc: distal centriole; f: flagellum; mi: mitochondrion; nu: nucleus. × 27,500.

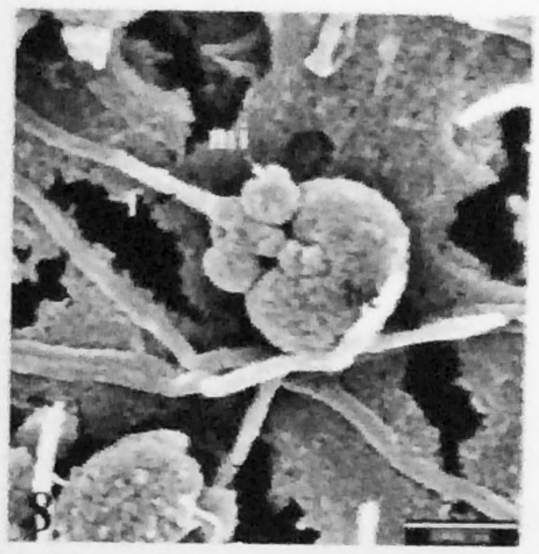
FIGURE 3 Midpiece of the spermatozoon of *S. cabrilla*. TEM. Longitudinal section. c: cytoplasm; f: flagellum; mi: mitochondrion. × 15,500.

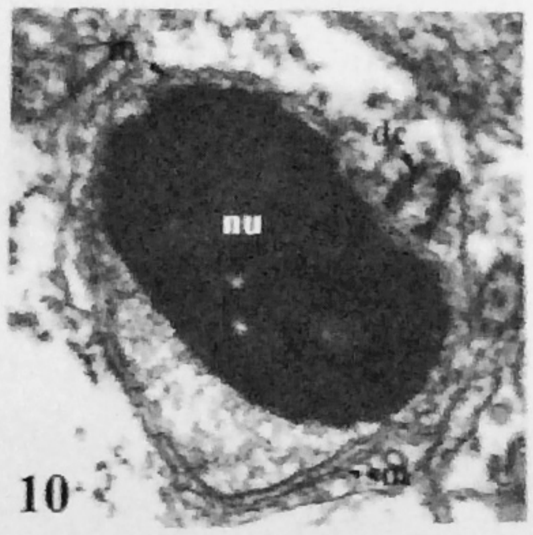
FIGURE 4 Proximal centriole of the spermatozoon of *S. cabrilla*. TEM. n: nuclear fossa; nu: nucleus; mt: microtubules. × 27,500.

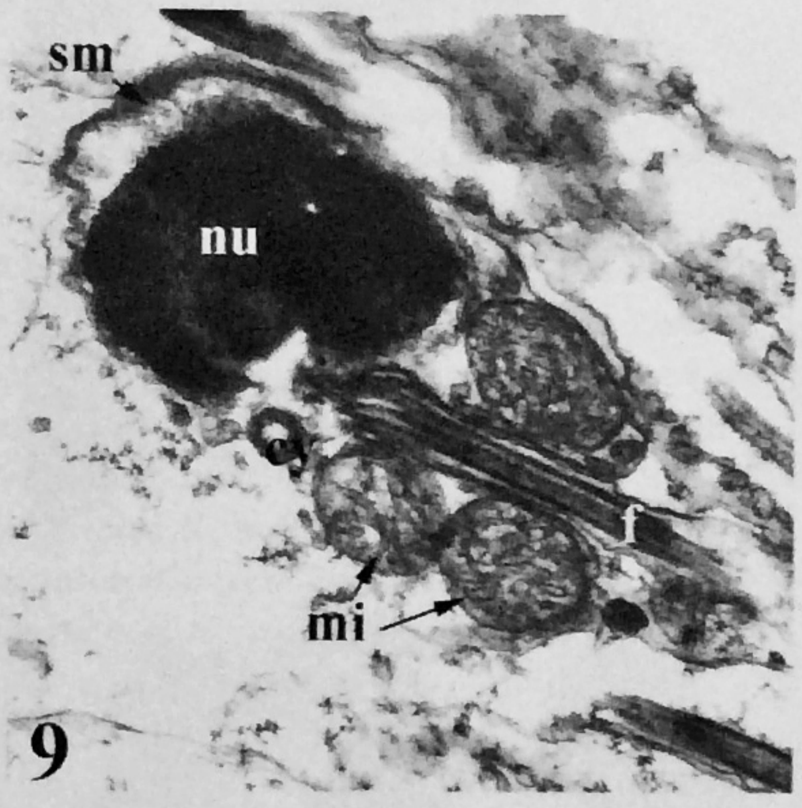
FIGURE 5 Flagellum of the spermatozoon of *S. cabrilla*. TEM. Cross section. cy: cytoplasmic channel; f: flagellum. × 46,000.

FIGURE 6 Flagella of the spermatozoa of *S. atricauda*. TEM. Cross section. × 39,000.









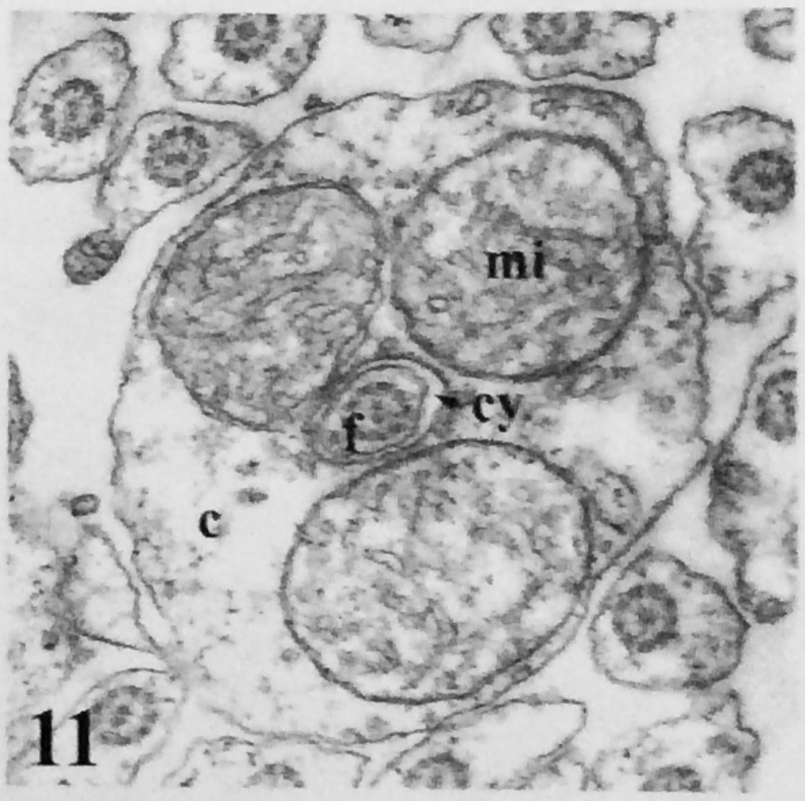


FIGURE 7 Spermatozoon of *S. scriba*. SEM. he: head; f: flagellum; mi: mitochondrion.

FIGURE 8 Insertion of the flagellum of the spermatozoon of *S. scriba*. SEM. he: head; f: flagellum; mi: mitochondrion.

FIGURE 9 Spermatozoon of *S. scriba*. TEM. Longitudinal section. cy: cytoplasmic channel; mi: mitochondrion; nu: nucleus; sm: stacked membranes. × 18,000.

FIGURE 10 Anterior nuclear surface of the spermatozoon of *S. scriba*. TEM. dc: distal centriole; m: nuclear membrane; nu: nucleus. × 27,500.

FIGURE 11 Midpiece of the spermatozoon of *S. scriba*. TEM. Longitudinal section. c: cytoplasm; cy: cytoplasmic channel; f: flagellum; mi: mitochondrion. × 18,000.

mum in length and 1.22±0.13 µm in width; it consists of densely packed granular chromatin material (Fig. 10). Although the acrosome is missing, the spermatozoon exhibits stack of membranes (one or two) apposed to the nuclear envelope in its anterior region. It consists of two membranes separated by a distinct perinuclear cisterna, which contains electron lucent material. The nuclear fossa is located in the longitudinal axis of the spermatozoon and has a length of 0.34±0.07 µm and a diameter of 0.21±0.05 µm (Figs. 9 and 10).

The midpiece contains four spherical mitochondria with a diameter of 0.71±0.18 µm: three appear to form a rectangular triangle (Fig. 11), and one is closer to the centriolar complex (Fig. 9). The cytoplasm shows several lipid droplets. The cytoplasmic canal has a diameter of 0.33±0.03 µm and its length is 1.13±0.08 µm. The proximal centriole is 0.25±0.02 µm long and 0.30±0.02 µm wide. The distal centriole has a diameter of 0.30±0.02 µm.

The flagellum presents a length of about 47 µm and a diameter of 0.30±0.04 µm.

DISCUSSION

Sperm morphology reflects the mode of individual fertilization. The so-called primitive type of sperm belongs to aquatic species with external fertilization; these sperms typically have a spheroidal nucleus with a short midpiece (Grier, 1981). Spermatozoa of the three species examined in this study exhibit a primitive morphology as that of other external fertilizing teleosts (Koch and Lambert, 1990; Jamieson, 1991; Gwo, 1995; Lahnsteiner and Patzner, 1996), differentiating in an ovoid head, smaller midpiece and a tail region. However, the comparison of spermatozoa in the genus *Serranus* reveals important morphological differences among species in relation with its morphology, sperm size, dimensions of the organelles and mitochondria number and position. The spermatozoon of *Serranus scriba* is the most different being its specific characteristics the stack of membranes apposed to the nuclear envelope, asymmetrical figure and localization of mitochondria in two planes. These characteristics indicate that its morphology is more primitive than its congenera. This observation is based on the fact that spermatozoa of teleost fish do not present an acrosome because it is missing during the evolution, this structure being considered as an ancestral feature (Afzelius, 1978; Koch and Lambert, 1990). Moreover, the lateral insertion of the flagellum, involving an asymmetrical lateral ribbon and a loss of the efficiency of flagellar undulations and stabilization structures, is not common in teleosts species (Lahnsteiner and Patzner, 1995). This type of insertion may explain the mitochondria distribution in different planes. Consequently, during the spermatozoon evolution in this genus two events occur: 1) the change in position of the flagellum insertion causes the moving of the mitochondrion closer to the centriolar complex towards the plane occupied by another mitochondrion; 2) the loss of stack of membranes apposed to the nuclear envelope.

The differences found in the spermatozoa size could be related with environmental aspects. *Serranus scriba* is the shallowest species and it presents the biggest head, mitochondria and flagellum, *Serranus cabrilla* inhabits in depth waters and has a smaller spermatozoon, while *Serranus atricauda* occurs in medium waters and shows an intermediate size. Moreover, the relation length/width of the head and midpiece together is greater in *Serranus scriba* (more elongated) and minor in *Serranus cabrilla* (more rounded). These results suggest that the decrease in spermatozoa size is directly related with the depth at which species inhabit. In deeper waters the pressure is greater than in shallower waters (Brown *et al.*, 1989), and so a smaller structure and a more rounded morphology may swim easier. Jamieson (1991) and Lahnsteiner and Patzner (1995) argued that the differences in the shape of spermatozoa, the number of mitochondria, the arrangement of centrioles as well as the occurrence of lateral flagellar ribbons among different teleost species may have consequences in the swimming behaviour as sperm velocity, swimming types and head detachment.

In the last years, the divergencies in sperm structure are considered to be mainly phylogenetic, although the morphology of spermatozoa is influenced by the reproductive mode (Gwo and Gwo, 1993). Spermatozoa of teleosts are very variable and there are conspicuous differences between those of different families, while they are very similar within families (Mattei, 1969, 1970; Bruslé, 1981; Jamieson, 1991; Gwo *et al.*, 1994). The studies within genus have not shown high variations; *e.g.*, Bruslé (1981) observed a very similar spermatozoa morphology in *Liza* spp., with the exception of a few details. However, in this study we have demonstrated that important differences exist among species of the genus *Serranus*, being sperm morphology of *Serranus atricauda* and *Serranus cabrilla* similar. Although environmental factors as pressure may influence the shape of spermatozoa, this morphological affinity may have phylogenetic implications, as a close relationship between these species is observed, considering that *Serranus scriba* is the less developed species. In this respect, Tuset (unpublished results), in otolith morphological studies on this species, found identical phylogenetic relationships. Consequently, morphological differences in *Serranus* spermatozoon indicate that the ultrastructure of these cells can be useful as additional characters in taxonomic classification, determining phylogenetic relations and interpreting evolutionary changes.

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