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Morphology and ultrastructure of pink cusk-eel (*Genypterus blacodes*, Schneider 1801) spermatozoa by scanning and transmission electron microscopy

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ABSTRACT

In this study, the morphology and ultrastructure of *Genypterus blacodes* spermatozoa were characterized through scanning and transmission electron microscopy. Findings revealed that the *G. blacodes* spermatozoa can be differentiated into three major parts: a spherical head without an acrosome (typical for externally fertilizing fish), a short mid-piece, and a long flagellum. The mean length of the spermatozoa was 57.6 ± 6.08 µm, with flagella accounting for 56.2 ± 7.2 µm. The head was 1.47 ± 0.2 µm long, and 0.89 ± 0.06 µm wide. The mid-piece had a total dimension of 0.72 ± 0.16 µm, and was 0.31 ± 0.02 µm in length and 0.6 ± 0.05 µm in width. It was located lateral to the nucleus and contained 4 or 5 spherical mitochondria. The mitochondria were separated from the axoneme by a cytoplasmic canal. The main piece of the flagellum had short irregular side-fins, and the axoneme was composed of the typical 9 + 2 microtubular doublet structure enclosed by a cell membrane. The present study reveals that *G. blacodes* sperm can be categorized as a primitive type. This study is the first to provide comprehensive details on the morphology and ultrastructure of spermatozoa in *G. blacodes*.

1. Introduction

Descriptions of the morphology and ultrastructure of fish sperm provide information to guide our understanding of taxonomic classifications, including relationships at the family, subfamily and species levels, and to establish phylogenetic relationships among fish species (Lahnsteiner and Patzner, 2008; Mattei, 1991). This information also allows for better *in vitro* management of the gametes of each species. In the teleost species studied to date, a large diversity in spermatozoa structure has been observed, according to whether a species adopts internal or external fertilization. In particular, this diversity is seen in the head shape; the number, shape and location of mitochondria; and the length and structure of the flagella (Guo et al., 2016).

Research and development of *G. blacodes* aquaculture has been initiated in over six countries including in Chile where the *G. blacodes* fishery has been developed in Chilean waters between Talcahuano (36° 44′S) and south of Cabo de Hornos (57° 00′S) (Wiff et al., 2011). *G. blacodes* is a species that has great farming potential in Chile, due to the exceptional quality of its flesh and high commercial value (Vega et al., 2012). Despite the importance of *G. blacodes*, very little is known about its reproductive biology. In Chile, breeding in captivity is currently in the initial stages and hatchery production has yet to be developed for large-scale farming. In addition, *G. blacodes* is considered an endangered species in Chile. Considering their potential value and the importance of protecting these fish, it is essential to study and understand their reproductive biology. Therefore, the present study aimed to investigate the ultrastructure and morphology of *G. blacodes* spermatozoa using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

2. Materials and methods

This study was carried out in the Laboratory of Engineering, Biotechnology and Applied Biochemistry (LIBBA) at the Frontera

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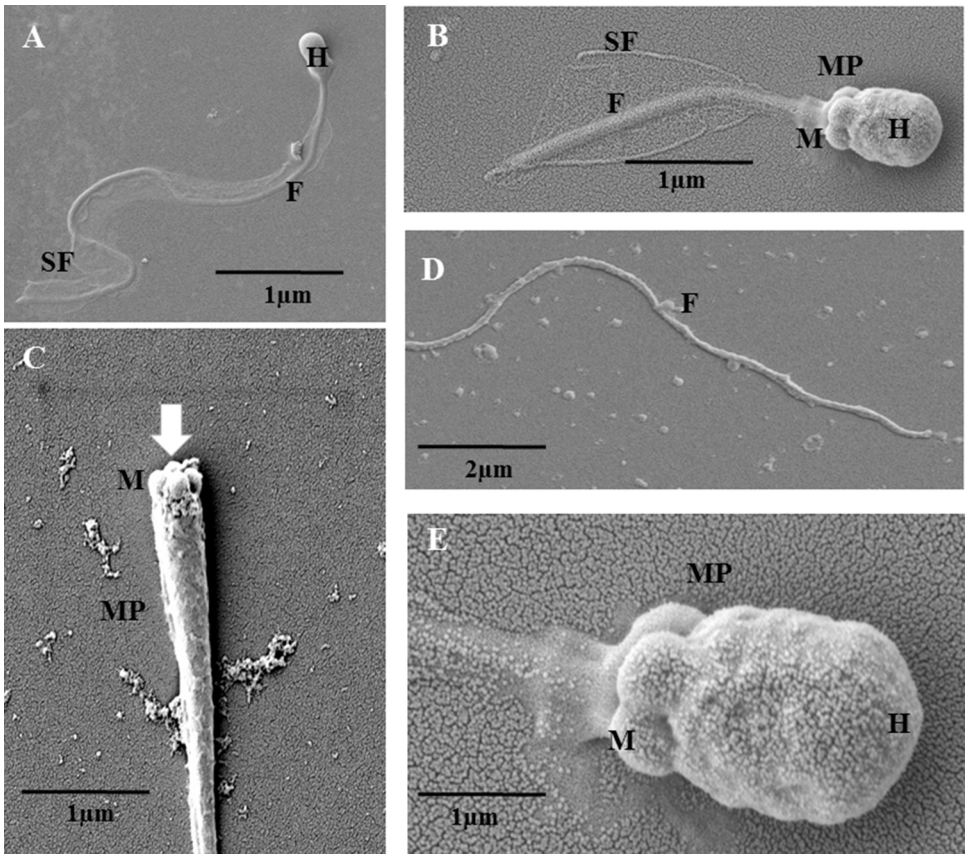

Fig. 1. Scanning electron microscopy (SEM) micrographs of *Genypterus blacodes*. A) General views of different rotations of the sperm with mitochondrial regions. H, head; MP, mid-piece; F, flagella; SF, side-fins. B) Spermatozoa with their characteristics. H, head; MP, mid-piece; M, mitochondria; F, flagella; SF, side-fins. C) The mid-piece with 4 or 5 mitochondria. D) Flagellum without head and mid-piece. E) Magnified view of a spermatozoon with prominent features. H, head; MP, mid-piece; M, mitochondria. Scale bars: 1 µm (A, B, C and E), 2 µm (D).

Table 1
Ultrastructural and morphological variables of *Genypterus blacodes* spermatozoa.

Spermatozoa	Variables			
Sperm size (µm)	Total length			
	57.6 ± 6.08			
Head (µm)	Length	Width	Nucleus length	Nucleus width
	1.47 ± 0.2	0.89 ± 0.06	0.52 ± 0.08	± 0.06
Mid-piece (µm)	Length	Width	Mitochondria diameter	Mitochondria number
	0.31 ± 0.02	0.6 ± 0.05	0.42 ± 0.04	4-5
Flagellum (µm)	Length	Width	Flagellum diameter	
	56.2 ± 7.2	0.14 ± 0.02	0.14 ± 0.02	
Axoneme (nm)	PDM	CDM	Microtubule diameter	Axoneme pattern
	31.35 ± 5.13	38 .42 ± 3.21	15.25 ± 1.05	9 + 2

Data are mean ± SD (n = 20), PDM: Peripheral doublets of microtubules width; CDM Central doublets of microtubules width.

University in Temuco, Chile. The specimens were caught in the wild with traps at Piedra Azul (41° 56′40. 86″ S and 72° 73′46. 62″ W), which is located near the city of Puerto Montt in the Los Lagos Region of Chile. Specimens had an average body weight of 1 960 ± 1.06 g, and total body length of 62.6 ± 4.45 cm ($n = 10$). This study was carried out with intratesticular spermatozoa because it is difficult to find fully sexual mature male individuals of this species in the wild, and sexual maturation in captivity has not yet been reported. *G. blacodes* males were anaesthetized by immersion with AQUI-S® (BAYER S.A. Animal Health, Chile) and decapitated. The testicles were surgically extracted and carefully cleaned according to the procedure described by Cabrita et al. (2005). The testes were cut into pieces directly in an Eppendorf tube (on ice) using a scalpel, and sperm were collected by directly dripping them into dry, sterile, graduated disposable plastic containers, after which they were kept at 4 °C. The samples of spermatozoa were then fixed for SEM and TEM analysis with 1 ml of 2.5% glutaraldehyde and cacodylate buffer following the methodology of Luo et al. (2011). Briefly, the samples were fixed in 0.1 M cacodylate buffer (pH 7.5) containing 2.5% glutaraldehyde for 2 h at 4 °C (Karnovsky, 1965). For SEM, the samples were dehydrated through an ascending series of ethanol, after which the dehydrated samples were critical-point dried. Spermatozoa were coated with gold/palladium at approximately 20 nm and observed and photographed on an SEM (ZEISS 409 DMS, Germany). For TEM, the samples were dehydrated as described above and embedded in epoxide resin.

Then, ultra-thin sections of 60–100 nm thickness were collected using glass knives. Sections were then placed on copper grids, stained with uranyl acetate and lead citrate, and a TEM (Hitachi H700 TE) was used for screening the ultracellular structure of sperm samples. All SEM and TEM measurements were evaluated using Prisma® software version 6.0. (Version 4.0.1 for Windows, Olympus Optical Co., Hamburg, Germany). Spermatozoa morphological characteristics were assessed and expressed as numbers with ranges (mean ± standard deviation). The SEM and TEM experiments were repeated four times.