

# Ultrastructural study of spermatozoa of the paddlefish, *Polyodon spathula*

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## Summary

Paddlefish, *Polyodon spathula*, spermatozoa were examined by transmission electron microscopy. Their structure has the same characteristic architectural features as sturgeon spermatozoa. Paddlefish spermatozoa are of the primitive type and consist of a rod-shaped head, a midpiece and a long flagellum. The head is about 5.15  $\mu\text{m}$  in length and contains the nucleus and an apical acrosomal complex. Inside the nucleus there are three nuclear channels that begin in the subacrosomal area and have a triple helical arrangement. An nuclear fossa is present centrally, at the posterior end of the nucleus. The midpiece contains a pair of centrioles in a perpendicular arrangement, mitochondria and a narrow cytoplasmic sleeve. The flagellum has a central axoneme with a 9 + 2 pattern and two lateral projections or fins.

Keywords: Acipenseriformes, Paddlefish, Spermatozoon, Ultrastructure

## Introduction

North American paddlefish (*Polyodon spathula*, Walbaum) belongs to the order Acipenseriformes, which consists of the families Acipenseridae, with 25 sturgeon species, and Polyodontidae. Paddlefish is one of the two living species of Polyodontidae, the other being the Chinese paddlefish, *Psephurus gladius* (Graham, 1997).

Transmission electron microscopy investigations of Acipenseriformes sperm have been performed only in sturgeons, including Russian sturgeon (*Acipenser gueldenstaedti*) (Ginsburg, 1968), stellate sturgeon (*Acipenser stellatus*) (Ginsburg, 1977), white sturgeon (*Acipenser transmontanus*) (Cherr & Clark, 1984, 1985), Atlantic sturgeon (*Acipenser oxyrinchus*) (DiLauro *et al.*, 1998), shortnose sturgeon (*Acipenser brevirostrum*) (DiLauro *et al.*, 1999), lake sturgeon (*Acipenser fulvescens*) (DiLauro *et al.*, 2000) and pallid sturgeon (*Scaphirhynchus albus*) (DiLauro *et al.*, 2001).

Most of the work on paddlefish spermatozoa has been restricted to light microscopy (Weldon Larimore, 1950; Mester *et al.*, 1997), analysis of motility (Mims, 1991; Linhart *et al.*, 1995; Cosson & Linhart, 1996;

Cosson *et al.*, 2000; Linhart *et al.*, 2002) and acrosin-like activity (Ciereszko *et al.*, 2000).

The aim of the present work was to investigate at the electron microscopic level the spermatozoon of paddlefish (*Polyodon spathula*). To the best of our knowledge no ultrastructural studies of Polyodontidae sperm have been published.

## Materials and methods

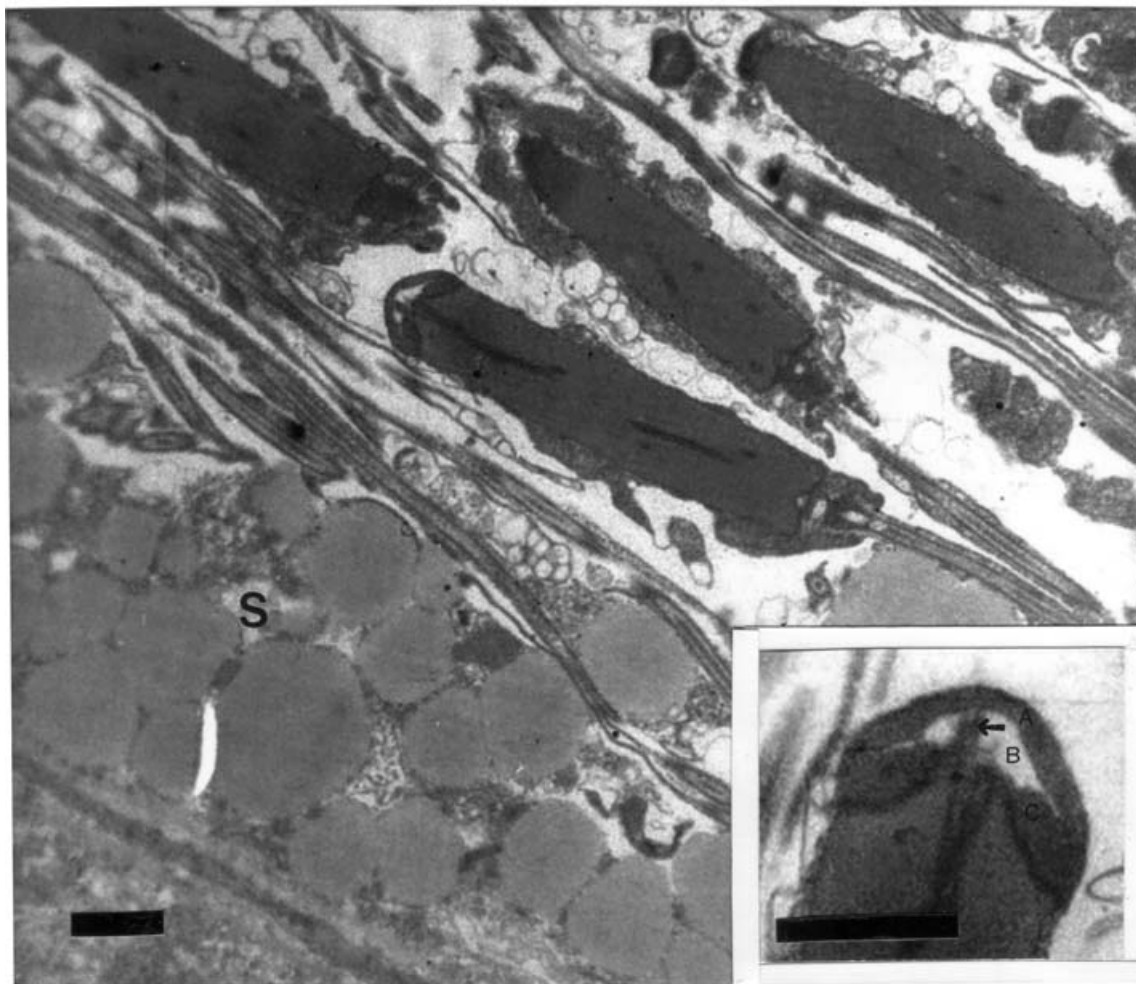
*Polyodon spathula* 4 summers old that had been reared in captivity ( $4.475 \pm 0.125$  g) were purchased from Nucet Station. The fish were brought to Romania from the USA as larvae. Following capture, the fish were killed by medullary transection and testis fragments were removed and fixed in 2.5% glutaraldehyde, buffered at pH 7.4 in 0.1 M sodium cacodylate, then postfixed in 1% OsO<sub>4</sub>, in the same cacodylate buffer, dehydrated and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, then studied with a Philips 206S electron microscope operating at 80 kV.

## Results

In the Osteichthyes, the spermatogenic unit of the testis is the spermatocyst. Germ cells reside within spermatocysts and Sertoli cell processes are restricted

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**Figure 1** Spermatozoa in the lumen of the spermatocyst. S, Sertoli cell. Scale bar represents 1  $\mu\text{m}$ . Inset: Acrosomal complex. A, acrosomal vesicle; B, subacrosomal space; C, subacrosomal material; arrow, nuclear channels that extend in the subacrosomal area. Scale bar represents 0.5  $\mu\text{m}$ .

to the periphery of the spermatocysts. At the end of spermiogenesis, the spermatocyst opens and sperm are released into the lumen of the germinal compartment (Grier, 1993). In the mature testis of *Polyodon spathula* spermatozoa are arranged in parallel in the lumen of the cyst (Fig. 1).

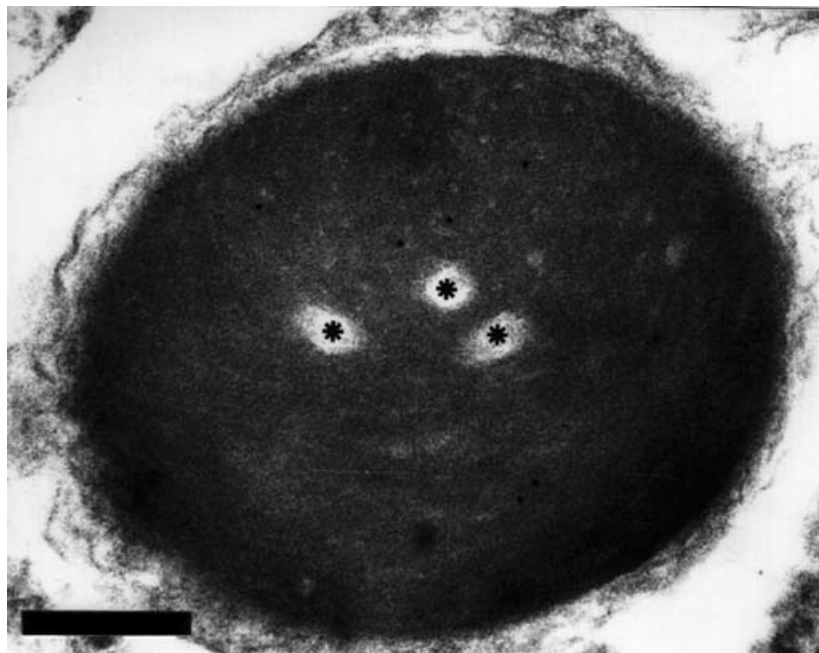
Paddlefish spermatozoa are of the primitive type and consist of a rod-shaped head, a midpiece and a long flagellum. The **head** is about 5.15  $\mu\text{m}$  long and 1.05  $\mu\text{m}$  wide and contains the nucleus surrounded by a little cytoplasm and an apical acrosomal complex. The dome-shaped *acrosomal complex* is composed of the acrosomal vesicle, a subacrosomal space and subacrosomal material, and lies above the nucleus (Fig. 1, inset). The acrosomal complex is surrounded by the sperm plasma membrane. The subacrosomal space is electron-translucent and contains central electron-dense structures that connect the acrosomal vesicle with the nuclear channels (Fig. 1, inset). Subacrosomal material

is electron-dense and has a granular appearance. The *nucleus* consists of homogeneous highly compacted chromatin. Inside the nucleus there are three nuclear channels that extend all the way to the base of the nucleus (Figs 2, 3). These central nuclear channels have a triple helical arrangement and begin in the subacrosomal area (Fig. 1, inset). At the base of the nucleus there is a nuclear fossa, centrally located, that houses dense material extending from the proximal centriole (Fig. 4).

The **midpiece** contains a pair of centrioles, mitochondria and a narrow cytoplasmic sleeve. The pair of centrioles are oriented at right angles to each other, with the distal centriole aligned in continuity with the axoneme of the tail (Fig. 4). Lateral to the centrioles there is a fibrous body with a striated appearance in longitudinal section. It is composed of discs and lies close to the nucleus (Figs 4, 5). Around the centrioles are a few mitochondria. At the posterior end of the

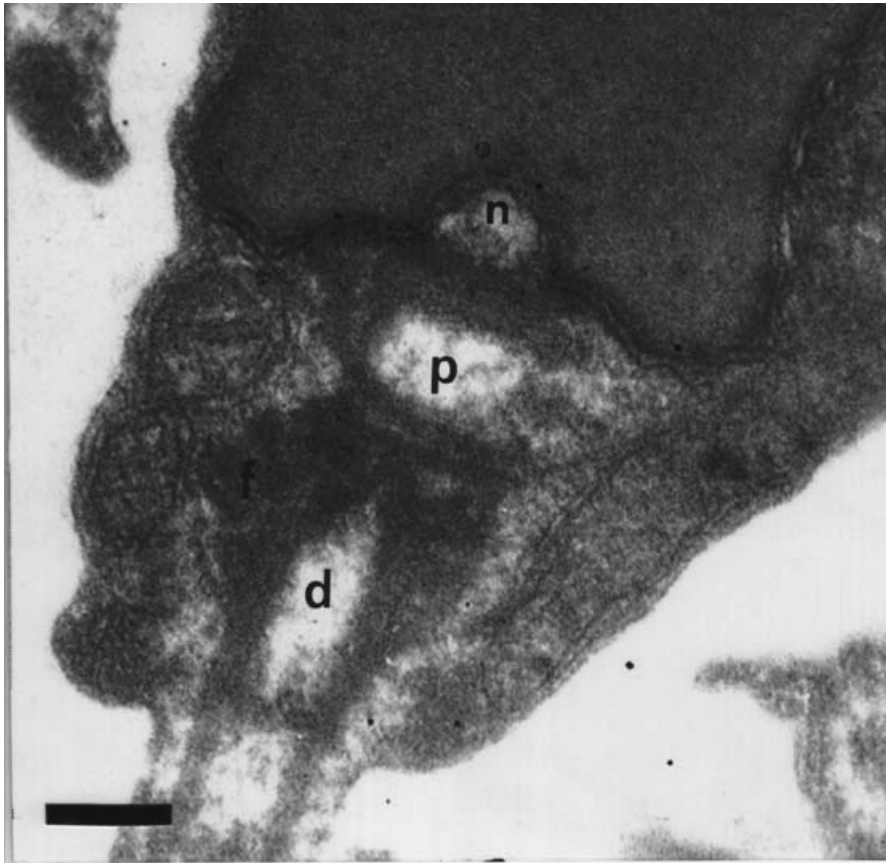


**Figure 2** Longitudinal section of the acrosome and nucleus. Note the triple helical arrangement of the nuclear channels (N). Scale bar represents 0.3  $\mu\text{m}$ .

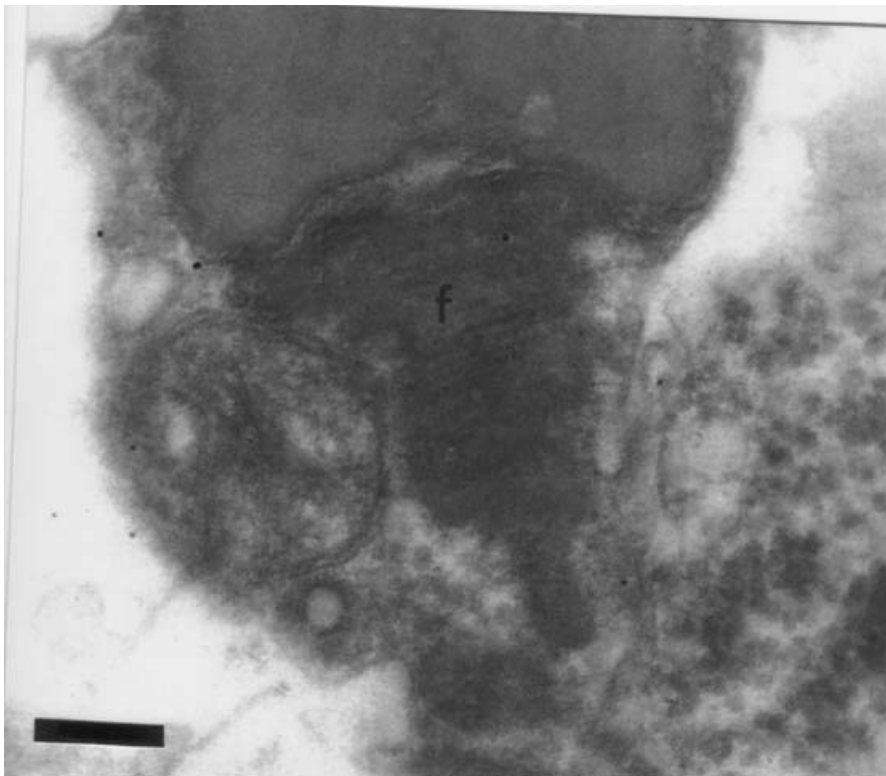


**Figure 3** Transverse section of the nucleus. Asterisks, nuclear channels. Scale bar represents 0.3  $\mu\text{m}$ .





**Figure 4** Longitudinal section of the centriolar apparatus at the base of the nucleus. n, nuclear fossa; p, proximal centriole; d, distal centriole; f, fibrous body. Scale bar represents 0.2  $\mu\text{m}$ .



**Figure 5** Fibrous body (f) with a striated appearance in longitudinal section. Scale bar represents 0.2  $\mu\text{m}$ .



**Figure 6** Longitudinal section of the midpiece with thin cytoplasmic projections (asterisks). Scale bar represents 0.3  $\mu\text{m}$ .

midpiece there is a thin cytoplasmic projection or sleeve (Fig. 6).

The **tail** is composed of a long, thin flagellum (Fig. 7) with a central axoneme with a 9 + 2 pattern. Scant cytoplasm containing glycogen granules may be present surrounding the axoneme. Along the main part of the sperm tail the plasma membrane forms two lateral projections or fins (Fig. 8). These extensions are filled with granular material and are in line with the two central microtubules. Sometimes, only one lateral fin is visible in cross-sectioned tails.

## Discussion

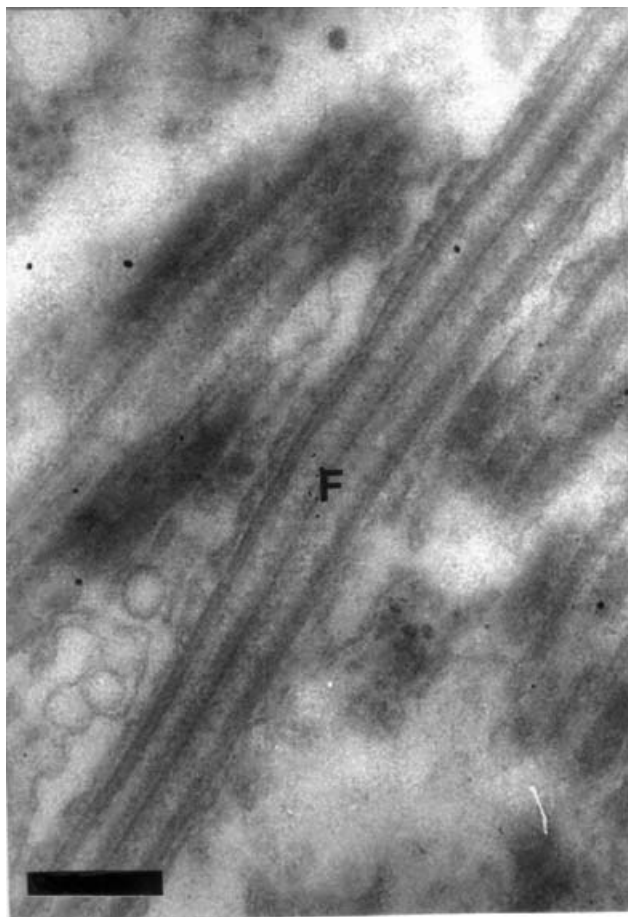
Morphologically, the spermatozoon of the paddlefish has a same characteristic architectural features as sturgeon spermatozoa. Spermatozoon ultrastructure has most recently served as a criterion for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991; Mattei, 1991).

There are several similarities and dissimilarities in the morphologies of paddlefish and sturgeon sper-

matozoa. The total head length (the sum of acrosome length, nuclear length and midpiece length) of paddlefish spermatozoa is about 5.85  $\mu\text{m}$ . In the sturgeons the longest spermatozoal head is that of the white sturgeon (10.52  $\mu\text{m}$ ) (Cherr & Clark, 1984), while that of the Atlantic sturgeon is shorter (3.98  $\mu\text{m}$ ) (DiLauro *et al.*, 1998). In terms of total length *Polyodon spathula* spermatozoa most closely resemble those of the *Acipenser sinensis* (Xu & Xiang, 1988) and *Acipenser gueldenstaedti* (Ginsburg, 1968).

In the paddlefish the acrosomal complex is composed of the acrosomal vesicle, subacrosomal space and a subacrosomal material, similar to those of the white sturgeon (Cherr & Clark, 1984) and Russian sturgeon (Dettlaff *et al.*, 1993). Acrosomes have been described in the primitive sperm of lampreys (Stanley, 1967), sharks (Stanley, 1971), lungfish (Jespersen, 1971) and sturgeons (Ginsburg, 1968, 1977; Cherr & Clark, 1984; DiLauro *et al.*, 1998, 1999).

As in the majority of sturgeons, paddlefish sperm have three central endonuclear channels that traverse the entire nucleus in a helical arrangement. *Acipenser oxyrinchus* is the only sturgeon species that has two endonuclear channels (DiLauro *et al.*, 1998). One

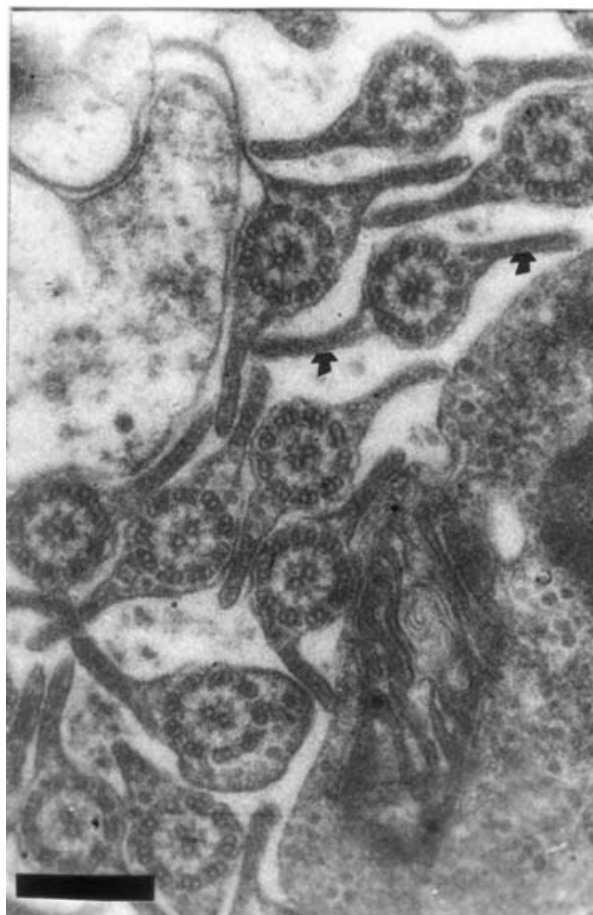


**Figure 7** Longitudinal section of the flagellum (F). Scale bar represents 0.3  $\mu\text{m}$ .

endonuclear channel is present in *Lampetra planeri* (Stanley, 1967) and *Latimeria chalumnae* (Mattei *et al.*, 1988). In the acrosomal reaction the material contained in the three intranuclear channels is ejected to form a long, fine acrosomal process (Ginsburg, 1977). Moreover, the material of the subacrosomal region appears to be involved also in the formation of the acrosomal process, since its amount decreases after the acrosomal reaction is completed (Dettlaff *et al.*, 1993).

The nuclear fossa is present at the base of the nucleus and centrally located in the paddlefish spermatozoa. Centrally located nuclear fossae were found in sperm nuclei of white (Cherr & Clark, 1984), stellate (Ginsburg, 1977), Atlantic (Dilauro *et al.*, 1998) and shortnose (Dilauro *et al.*, 1999) sturgeons and garfish (Afzelius, 1978). On the other hand, the lamprey nuclear fossa is eccentric (Stanley, 1967) and in the coelacanth there is an apparent nuclear fossa resulting from enlargement of the endonuclear canal (Mattei *et al.*, 1988).

The midpiece of paddlefish spermatozoa contains a centriolar system and mitochondria. A fibrous body with a striated appearance lies lateral to the centrioles.



**Figure 8** Transverse section of the flagellum with 9 + 2 microtubular organization and lateral projections (arrows). Scale bar represents 0.3  $\mu\text{m}$ .

No such structure has been described in the white or stellate sturgeons. Among primitive fish, the fibrous body has been described in *Acipenser gueldenstaedti* (Dettlaff *et al.*, 1993), *Acipenser brevirostrum* (DiLauro *et al.*, 1999) and *Lepisosteus osseus* (Afzelius, 1978).

Paddlefish midpiece shows in its posterior part a cytoplasmic collar surrounding the flagellum base. This structure is present not only in primitive fish, such as garfish (Afzelius, 1978) and sturgeons (Dettlaff *et al.*, 1993; DiLauro *et al.*, 1998, 1999) but also in teleosts such as *Cichla intermedia* (Quagio-Grassiotto *et al.*, 2003).

*Polyodon spathula* sperm tail is similar to that of sturgeons regarding the classical 9 + 2 axoneme and two lateral extensions (sidefins). The sidefins are present in several fishes such as *Lepisosteus osseus* (Afzelius, 1978), *Cymatogaster aggregata* (Gardiner, 1978), *Oncorhynchus mykiss* (Billard, 1983), *Oreochromis niloticus* (Lou & Takahashi, 1989) and *Plecoglossus altivelis* (Gwo *et al.*, 1994). They are varied in size and number, and their presence is independent of the mode of reproduction (internal or external) and number of flagella (Mattei, 1988).



The ultrastructural study presented here attempts to extend the ultrastructural analysis on living “fossil” fishes not previously studied from this point of view.

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