

MORPHOLOGY OF THE SPERM OF TWO WRASSES, *THALASSOMA BIFASCIATUM* AND *LACHNOLAIMUS MAXIMUS* (LABRIDAE, PERCIFORMES)

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The sperm of fishes are morphologically diverse, but broad-level relationships between morphology and mating pattern are apparent (Jamieson, 1991; Stockley et al., 1996, 1997). Significant questions about the extent and functional importance of this morphological diversity remain, however. The wrasses (Labridae) are a widely distributed, diverse group of fishes with an array of mating systems both among and within species, making them good subjects for the study of sperm morphology. The morphology of the sperm of labrids is poorly known, however, except for a few species (Lahnsteiner and Patzner, 1997; Schärer and Robertson, 1999). The bluehead wrasse, *Thalassoma bifasciatum* (Bloch), and the hogfish, *Lachnolaimus maximus* (Walbaum), are two relatively common wrasses living on and near the coral reefs of Florida and the Caribbean. These fishes are broadcast spawners that rush into the water column to release gametes. We describe the ultrastructure of the sperm of these two species.

METHODS

Divers collected six mature male *T. bifasciatum* with handnets on the coral reefs of south Florida and speared four mature male *L. maximus* on the western coast of Florida near Tampa. We collected sperm either by milking the abdomen (i.e., *T. bifasciatum*) or removing whole gonads (i.e., *L. maximus*). We then fixed the samples immediately in 2.5% glutaraldehyde in full strength Millonig's phosphate buffer on ice. For transmission electron microscopy (TEM), we processed samples following a modification of the procedure of Coelho et al. (1998). Briefly, the steps involved: post-fixation of samples in 1% osmium in buffer; dehydration in an ethanol series with uranyl acetate enblock staining in 50% ethanol; embedding portions of each sample in LR White resin; and staining thin sections with ethanolic uranyl acetate and Reynolds lead citrate. We used a Philips 300 transmission electron microscope at 60 kV to view the sections. We examined at least 25 mature sperm from each individual of both species. For scanning electron microscopy (SEM), we gravity filtered (0.45 μm micropore filter) the remaining portions of the fixed and dehydrated samples of *T. bifasciatum* sperm followed by critical point drying and sputter coating. We examined the external morphology of a minimum of 50 sperm from the six males of *T. bifasciatum* with a Jeol 5600 LV SEM. We used SigmaScan software to quantify morphometrics from digital TEM and SEM micrographs.

RESULTS

The sperm of both wrasses are morphologically simple. They consist of a round head, a short midpiece and a single, long flagellum that penetrates the posterior region of the sperm body through a relatively short cytoplasmic canal (Figs. 1E,F,2). No accessory structures were apparent on the flagellum (Figs. 1A,2). Both species have heads of approximately 2 μm in diameter and the length of the entire sperm is approximately 20–25 μm . Assuming a perfectly spherical head, this results in an estimated volume of 4.19 mm^3 .

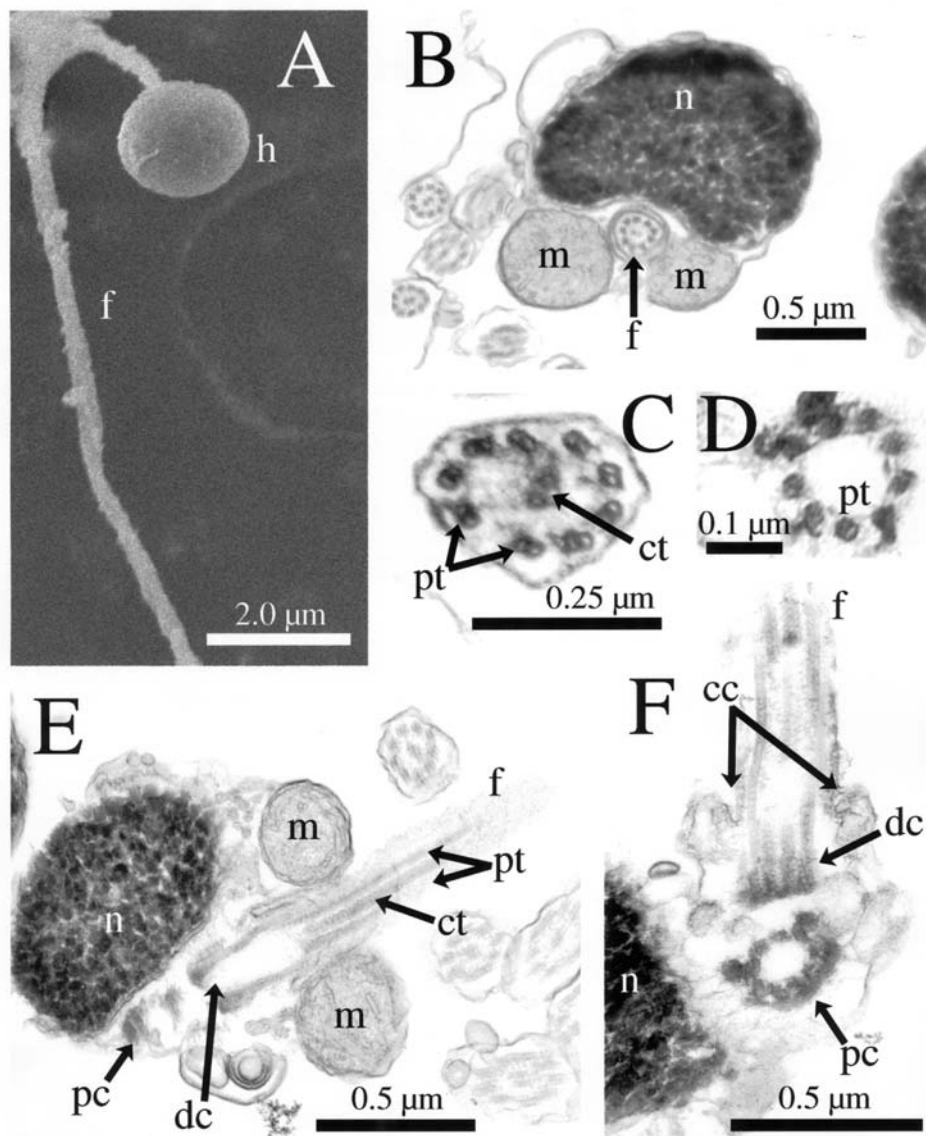


Figure 1. Sperm of *Thalassoma bifasciatum*. (A) SEM of a representative sperm. (B) Ultrastructure of head, midpiece and base of flagellum. (C) Cross section of flagellum. (D) Cross section of distal centriole. (E) Section through head, midpiece and a portion of the flagellum. (F) Detail of cytoplasmic canals and centriolar complex. Symbols: cc—cytoplasmic canal; ct—central microtubule; dc—distal centriole; f—flagellum; h—head; m—mitochondrion; n—nucleus; pc—proximal centriole; pt—peripheral microtubule.

The ultrastructures of the sperm of both species are similar and relatively simple. The head consists mostly of a nucleus containing dense, osmophilic material (Figs. 1B,E,2). There are no acrosomes. This otherwise spherical nucleus is slightly indented adjacent to the centrioles and mitochondria (Figs. 1B,2). Two (i.e., *T. bifasciatum*) or four (i.e., *L.*

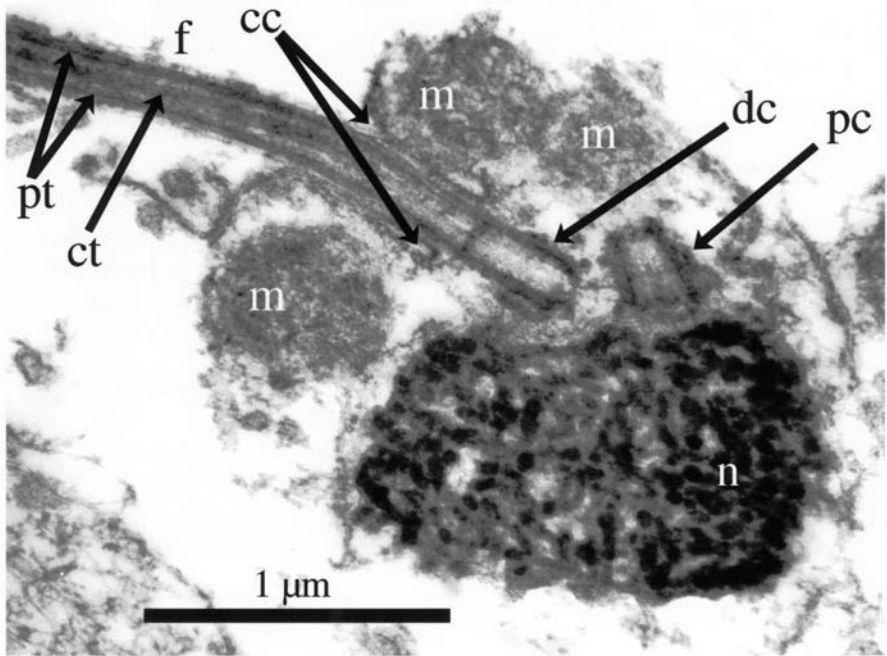


Figure 2. Section through the head, midpiece and flagellum of a sperm of *Lachnolaimus maximus*. Refer to Figure 1 for explanation of symbols.

maximus) mitochondria surround the basal portion of the flagellum and centrioles (Figs. 1B,E, 2). The cristae in these mitochondria appear tubular and positioned unevenly. Diameters of the mitochondria are $0.40 \pm 0.09 \mu\text{m}$ (mean \pm SD; *T. bifasciatum*) and $0.45 \pm 0.10 \mu\text{m}$ (*L. maximus*). The distal centriole is parallel to the flagellum whereas the proximal is oriented either perpendicularly (i.e., *T. bifasciatum*) or at approximately 135° (i.e., *L. maximus*) to the distal (Figs. 1E,F,2). The centrioles have a $2 \times 9 + 0$ arrangement of microtubules. The insertion of the flagellum into the head is asymmetric as indicated by the orientation of the centrioles and the nucleus (Figs. 1B,E,2). Flagella have the typical $2 \times 9 + 2$ microtubule organization (Fig. 1C) and diameters of $0.27 \pm 0.03 \mu\text{m}$ (*T. bifasciatum*) and $0.27 \pm 0.04 \mu\text{m}$ (*L. maximus*). Schematic representations of these sperm are presented in Figure 3.

DISCUSSION

This study describes the morphology of the sperm of two labrids, *T. bifasciatum* and *L. maximus*. The sperm of these fishes are morphologically simple and are typical of derived, externally fertilizing teleosts (Jamieson, 1991; Mattei, 1991). The sperm of such fish, termed aquasperm, typically have a round head without an acrosome, a single, generally unadorned flagellum and a short midpiece with a prominent cytoplasmic canal (Jamieson, 1991). The sperm of these wrasses share these traits. Among fishes, the more structurally complex sperm are generally restricted to ancestral species and internal fer-

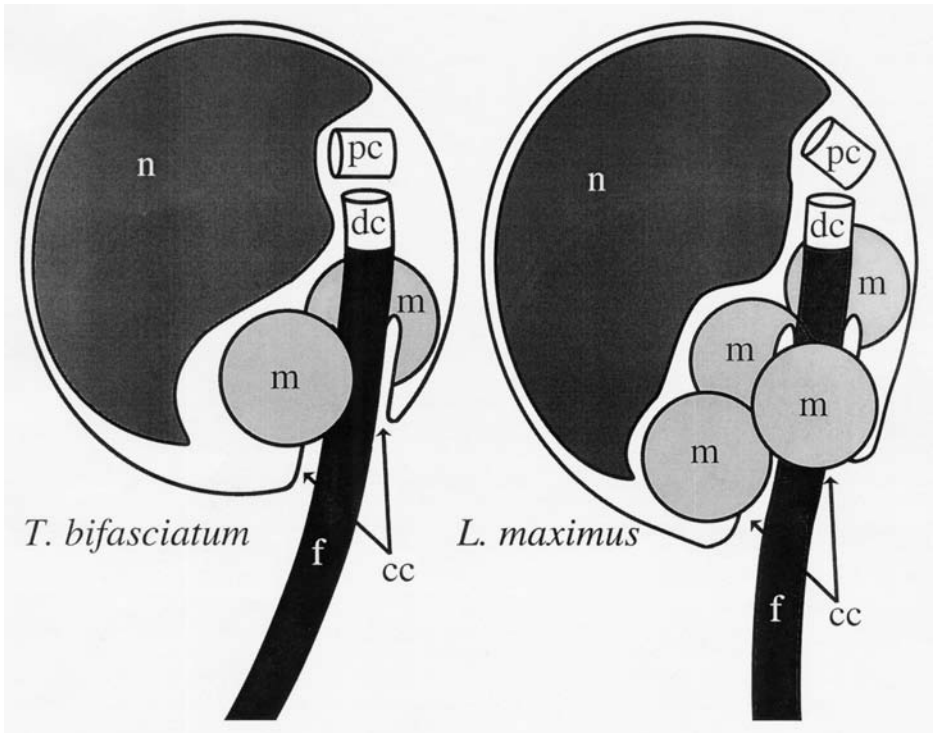


Figure 3. Schematic representations of sperm of *Thalassoma bifasciatum* and *Lachnolaimus maximus*. Refer to Figure 1 for explanation of symbols.

tilizers (Jamieson, 1991). Thus, the sperm morphologies of *T. bifasciatum* and *L. maximus* are characteristic of their positions as derived, externally fertilizing teleosts.

Two types of fish aquasperm are recognized. Type I aquasperm have the flagellum and centrioles inserted directly into the nucleus resulting in a nucleus which surrounds the centrioles (Mattei, 1970). In Type II aquasperm, the flagellum inserts parallel to one side of the nucleus, with mature sperm retaining this morphology. The flagellum is oriented parallel to the base of the nucleus in the sperm of these two wrasses suggesting that these fishes have Type II aquasperm (*sensu* Mattei, 1970; 1988).

It appears that, in general, the sperm of *T. bifasciatum* and *L. maximus* are similar to the only other labrid sperm that have been described: *Thalassoma pavo* (L.), *Coris julis* (L.) and *Symphodus ocellatus* (Forsskål) (Lahnsteiner and Patzner, 1997). The general structure of the mitochondria, centrioles and flagella are all the same. The sperm of the two species of *Thalassoma* are similar in that they are essentially the same size and have the same number of mitochondria. All five wrasses also share the same $2 \times 9 + 0$ arrangement of microtubules in their centrioles, a state which differs from some fishes but is not unique to this family (Lahnsteiner and Patzner, 1997). A major difference among these wrasses is that the head of *T. pavo* is reported as ovoid versus the spherical head of *T. bifasciatum* shown here. A second major distinction among them is that the centrioles of *L. maximus* are arranged at an angle of 135° rather than 90° as seen in the four other labrids. Most strikingly, neither of the species examined here has the deep, central nuclear notch result-

ing from the insertion of the flagellum as observed in *S. ocellatus* (Lahnsteiner and Patzner, 1997).

Labrids as a group have evolved a diverse array of spawning styles. Within the wrasses there are pelagic and benthic spawners as well as different levels of sperm competition within and among species (Thresher, 1984; Taborsky, 1994) making diverse sperm morphologies within the family likely. The two main differences between the sperm of *L. maximus* and *T. bifasciatum* are that the sperm of the former have more mitochondria and centrioles that are not oriented perpendicularly to one another. The functional significance of these differences is unknown, but it is possible that the greater number of mitochondria in *L. maximus* allow the sperm to swim farther and/or faster than those of *T. bifasciatum*. This might be important in fertilization, because the larger *L. maximus* are probably not as close to each other during spawning as are individual *T. bifasciatum* with the result that the gametes of *L. maximus* must traverse greater distances. Further research on the sperm of other labrid species with different mating systems and spawning behaviors would provide valuable information regarding the function, evolution and ecology of sperm morphology.

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