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Influences of phylogenetic position and fertilization biology on spermatozoal ultrastructure exemplified by exocoetoid and poeciliid fish

B. G. M. Jamieson [1] & H. J. Grier [2]
[1] *Zoology Department, University of Queensland, Brisbane 4072, Australia;*
[2] *Department of Biology, University of South Florida, Tampa, FL 33620, USA*

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Abstract

In a cladistic analysis, poeciliids and zenarchopterids homoplasically show elongation and flattening of the nucleus at right angles to the plane of the central axonemal singlets; in both the tip of the nucleus appears rounded in the plane of flattening but pointed in the plane at right angles. The two families differ in the distribution of mitochondria in the elongate midpiece: circumferential in poeciliids but bilateral in zenarchopterids. In poeciliid sperm and independently in *Zenarchopterus,* the individual mitochondria are considerably more extensive circumferentially than longitudinally; they differ in poeciliids in being C-shaped. In *Hemirhamphodon* they are moderately elongate. In *Dermogenys* and *Nomorhamphus* they have been modified monophyletically as a pair of elongate mitochondrial derivatives. A wide cytoplasmic periaxonemal sheath (not seen in poeciliids) appears to have developed monophyletically in the ancestry of *Hemirhamphodon, Dermogenys* and *Nomorhamphus* with acquisition of radial rodlets only in *Hemirhamphodon.* A distinctive development in poeciliids is the submitochondrial net. Poeciliids have greatly reduced the axonemal fins which are a synapomorphy of the Actinopteri. Exocoetoids have retained well developed fins in *Arrhamphus, Dermogenys* and *Nomorhamphus* but reduction has occurred in *Zenarchopterus,* in which the fins are small, and, apparently independently, in *Hemirhamphodon* in which fins are absent. A posterior extension of the nucleus over the base of the axoneme is C-shaped and embraces almost the entire circumference of the axoneme in poeciliids but, independently developed, in zenarchopterids is a 'dorsal' plate. Its absence in *Hemirhamphodon* is computed as a loss. These modifications relative to the aquasperm condition are deduced to have been occasioned by the adoption of internal fertilization. To what extent they are constrained by features of the genome peculiar to poeciliids, zenarchopterids or atherinomorphs or are demanded by minute differences in fertilization biology, or by a combination of the two, is not at present determinable.

Abbreviations: a: axoneme; as: central axonemal singlet microtubules; ad: axonemal doublets; cc: cytoplasmic canal (periaxonemal space); cca: centriolar cap; dc: distal centriole; f: flagellum; fi: axonemal fin; m: mitochondrion; n: nucleus; nf: basal nuclear fossa; ps: peri-axonemal cytoplasmic sheath; s: 'dorsal spur' of nucleus; sl: submitochondrial dense layer; sr: satellite rays.

Introduction

Spermatozoal ultrastructure is known to be a valuable indicator of phylogenetic relationship (e.g. Jamieson, 1991). This is here deduced to be due to the generally conservative nature of fertilization biology within given taxonomic groups and to group-specific constraints imposed by the genome on structural variation. If fertilization biology within a group undergoes major change, spermatozoal ultrastructure will be correspondingly altered. However, it may be expected that the new structural forms will retain a group-specificity indicative of genetic (and phylogenetic) constraint. The present study attempts to examine the interplay of fertilization biology and phylogeny, as revealed by sperm ultrastructure, in some atherinomorph fish of the superfamily Exocoetoidea.

Some outline of the classification of the Atherinomorpha is necessary for an understanding of the relationships of the groups under investigation. The Beloniformes, to which exocoetoids belong, is one of the three orders of the Atherinomorpha, a group which is itself the sister-group of the Percomorpha. The other two atherinomorph orders are the Atheriniformes and the Cyprinodontiformes. Atherinomorphs are distinguished and unified by restriction of the spermatogonia to the distal (inner) ends of the testicular lobules. This is a notable contrast with other teleosts, including percomorphs, in which the spermatogonia are distributed along the entire length of the lobules. The telogonic atherinomorph condition is clearly apomorphic relative to the hologonic condition general in teleosts (references in Jamieson, 1991). Within the Exocoetoidea, the Exocoetidae are seen as the sister-group of the Zenarchopterinae + Hemiramphinae by Collette *et al.* (1984) but Tibbetts (1992) pairs the Hemiramphinae with the Exocoetinae, in an enlarged family Exocoetidae, the sister-group of which is the Zenarchopteridae, consisting solely of the former Zenarchopterinae. Both studies recognized the family Belonidae (Needle fish) as the outgroup of these assemblages.

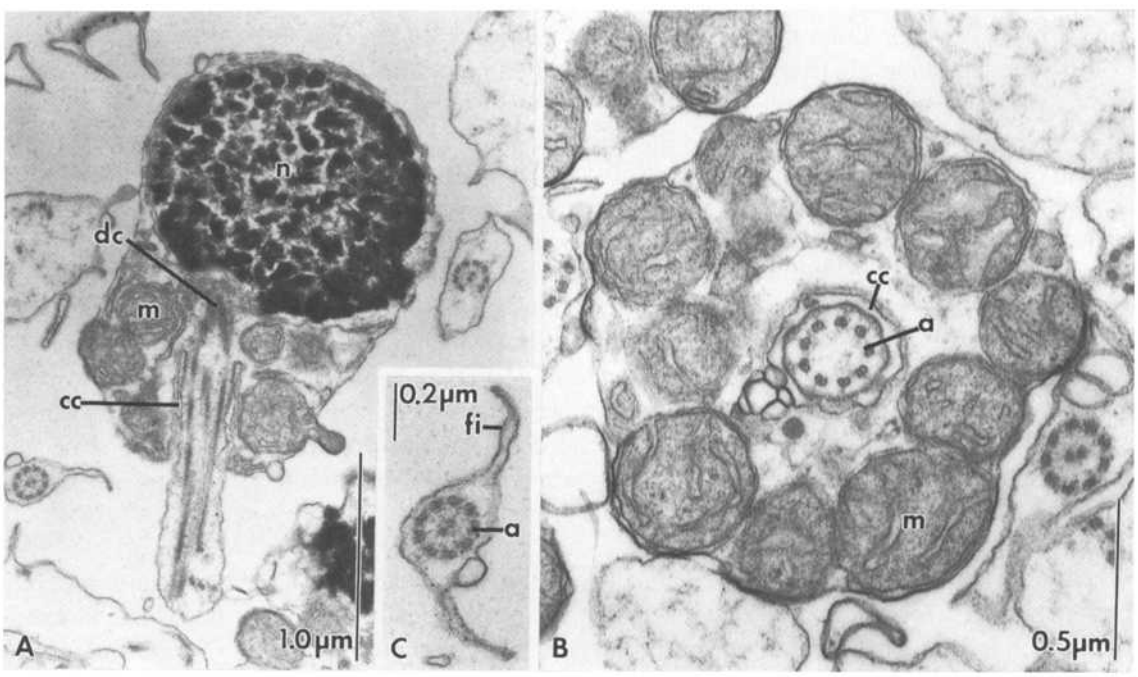


Fig. 1. Arrhamphus sclerolepis. A. Longitudinal section of the spermatozoon. B. Transverse section through the midpiece and the initial 9 + 0 region of axoneme. C. Transverse section of the axoneme, showing the two lateral fins.

Internal fertilization is considered to have evolved from free-spawning independently among several cyprinidontiform families and independently in the Beloniformes (Grier, 1976; Jamieson, 1991). It is attended by production of live young or (*Zenarchopterus)* laying of fertilized eggs. It appears to have arisen in beloniforms once in the ancestry of the family Zenarchopteridae, the subject of this study, viz. the viviparous *Dermogenys pusillus*, *Hemirhamphodon pogonognathus*, and *Nomorhamphus celebensis*, and (Grier & Collette, 1987) at least 11 species of *Zenarchopterus* (of which *Z. dispar* is studied here). As far as is known, all members of the family Exocoetidae are externally fertilizing, like the vast majority of teleosts. An externally fertilizing sperm is represented here by that of *Arrhamphus sclerolepis*. This species is a member of the subfamily Hemiramphinae, the remaining subfamily being the Exocoetinae (including the flying fish).

It is anticipated that comparisons of the sperm of exocoetoids investigated in this study will allow some insights into morphological innovation and variation in sperm ultrastructure which are correlates of internal fertilization relative to those accompanying external fertilization. These modifications for internal fertilization will be compared and contrasted with those in the related family Poeciliidae (Cyprinodontiformes) (described by Jamieson, 1991) which have acquired internal fertilization monophyletically but independently of the Beloniformes. The sperm of *Hemirhamphodon pogonognathus* have previously been described by Jamieson (1989, 1991) and those of *Arrhamphus sclerolepis* by Jamieson (1991), species which will receive less detailed treatment here.

Trends in spermatozoal ultrastructure will be analyzed cladistically using the PAUP program of Swofford (1990).

Materials and methods

Arrhamphus sclerolepis was obtained from the Brisbane River. *Hemirhamphodon pogonognathus* was obtained from aquarium suppliers in Brisbane. *Zenarchopterus dispar* was captured, and *Nomorhamphus celebensis* and *Dermogenys pusillus* were obtained from aquarium suppliers, in Florida. Portions of testes of *A. sclerolepis* and *H. pogonognathus* were fixed in 3% glutaraldehyde in 0.2 M sodium phosphate buffer (pH 7.4) at 4 °C for 2 h.; washed in buffer; post-fixed for 80 minutes in similarly buffered 1% osmium tetroxide; washed in buffer; dehydrated through an ethanol series; and infiltrated and embedded in Spurr's epoxy resin. Testicular portions of *Zenarchopterus dispar*, *Nomorhamphus celebensis* and *Dermogenys pusillus* were fixed in 3% glutaraldehyde overnight postfixed for 1 h in 1% osmium tetroxide with 1.25% $K_4Fe(CN)_6$ (see Russell & Burquet, 1977). Sections were cut with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 500–800 Å thick, were collected on carbon stabilized colloidin-coated 200 mesh copper grids and stained for 40 min in 6% aqueous uranyl acetate (after rinsing in distilled water) and a further 20 min in lead citrate before final rinsing. Specimens were examined with a Hitachi 300 transmission electron microscope operated at 80 kV and a JEOL 100S at 60 kV.

Results

Spermatozoal ultrastructure

An externally fertilizing exocoetoid, Arrhamphus sclerolepis

In the *Arrhamphus* sperm, the nucleus (Fig. 1A) is subspheroidal and 1.6 μm long. Basally it is indented as a poorly defined fossa, sufficient to house only part of the proximal centriole. The chromatin consists of numerous large, separate, electron dense, flocculent masses in a pale matrix. The two nuclear membranes remain separated by a considerable perinuclear cisterna.

The mitochondria are spherical and cristate and are arranged in two tiers longitudinally (Fig. 1A). Ten mitochondria are seen in transverse section of a tier, symmetrically arranged around the central axis (Fig. 1B). Those of the posterior tier lie in the short (0.8 μm long) mito-

chondrial collar, around the periaxonemal invagination (cytoplasmic canal) but those of the anterior tier surround the distal centriole (basal body) anterior to the collar (Fig. 1A). There is some thickening of the wall of the collar surrounding the canal (subplasmalemmal densification) which may be the equivalent of the submitochondrial dense layer of the internally fertilizing, zenarchopterid species.

The proximal centriole (Fig. 1A) is located anteriorly to and slightly to one side of the distal centriole and is tilted at an angle of approximately 45° to its long axis and, therefore, to that of the axoneme. The greater diameter of the nucleus is at right angles to the longitudinal axis of the proximal centriole; the nucleus therefore appears tilted relative to the longitudinal axis of basal body and axoneme.

The plasma membrane investing the 9 + 2 flagellum is extended as a pair of axonemal fins approximately in the plane of the two central singlets and doublet radii 3–4 (Fig. 1C). The fins run longitudinally along a large portion of the flagellum, as indicated by their frequency in transverse sections. The fins show no particular apical differentiation. A region with 9 doublets but no central singlets intervenes, in the collar region, between the basal body and the axoneme proper.

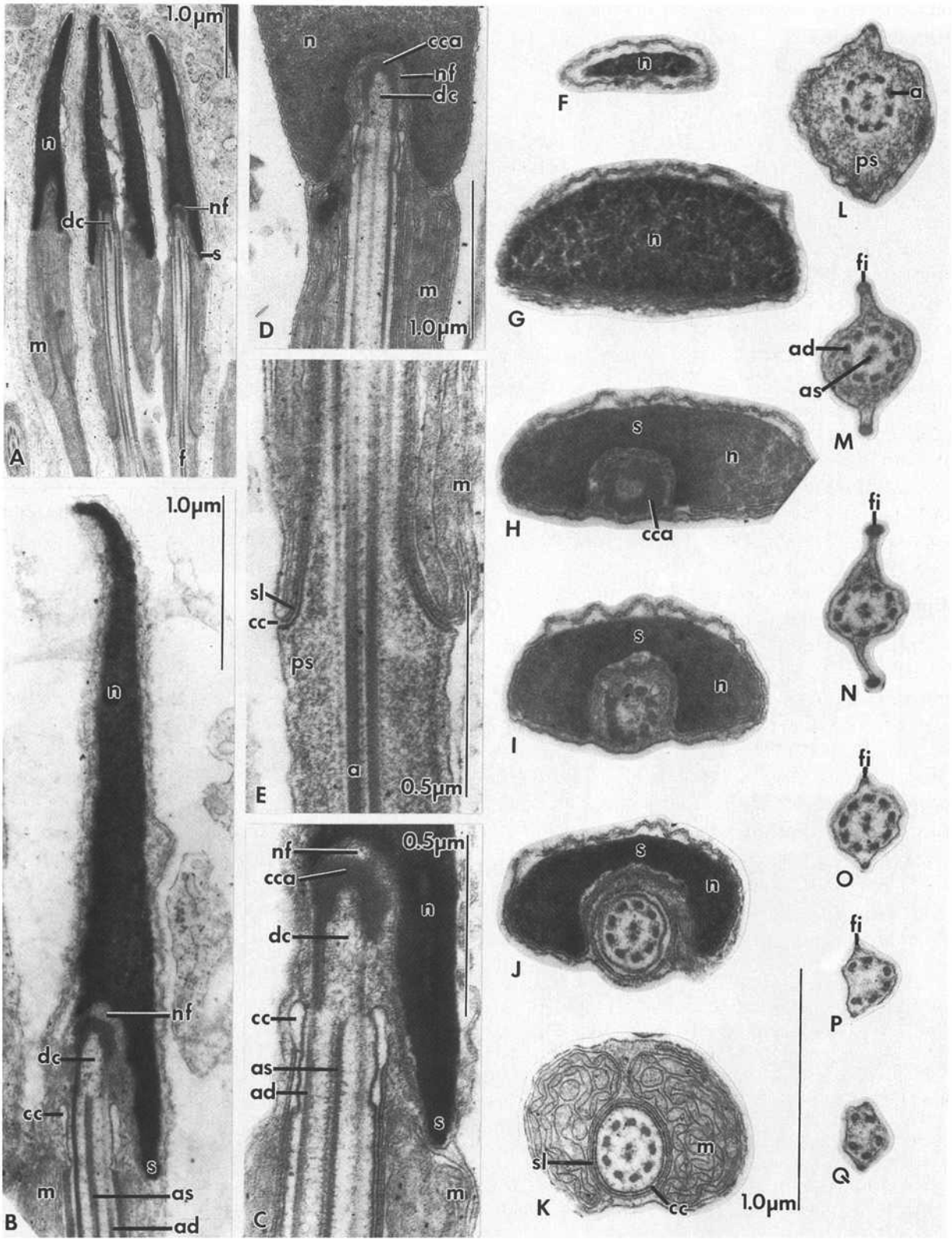
Internally fertilizing exocoetoids

Mature sperm of *Dermogenys pusillus*, *Hemirhamphodon pogonognathus*, and *Nomorhamphus celebensis* were examined. Those of *Zenarchopterus dispar* are very late spermatids.

Nucleus

The sperm nucleus in all four of the investigated zenarchopterid species has the form of an elongate, pointed blade when cut sagittally, *i.e.* in the plane of the central singlets (Figs 2A, B; 3C; 4A, B, both oblique). When cut in a plane at right angles (in what may be termed frontal sections), it appears much wider, almost parallel sided, with broad rounded apex (Figs 2D; 3A; 4C). In the four species, therefore, the sperm nucleus has the form of an approximately parallelsided round-tipped blade. Its strongly depressed form is clearly seen in transverse sections (Figs 2f–J; 3D–I; 4D). Nuclear flattening is always at right angles to the plane of the two central singlets of the axoneme or within a few degrees of this. The base of the nucleus is indented by an implantation fossa. This is least developed in *Hemirhamphodon* in which it is less than one tenth of the length of the nucleus. The fossa is strongly eccentric but its disposition is different in *Hemirhamphodon,* on the one hand, compared with *Dermogenys* (Figs 2B–D), *Nomorhamphus* (Figs 3A–C) and *Zenarchopterus* (Figs 4A–C), on the other. In *Hemirhamphodon* it appears eccentric only in section through the broader, frontal plane, whereas in the other three species its eccentricity is seen in sagittal section of the nucleus where it incises the 'postero-ventral' face of the nucleus (Fig 2A–C; 3B, C; 4AB). Only the anterior half of the distal centriole (basal body of the flagellum) is contained within the fossa in *Hemirhamphodon* and *Zenarchopterus* (Fig. 4A–C) but in *Dermogenys* (Fig. 2B–D) and *Nomorhamphus* (Fig. 3A–C) the initial part of the

Fig. 2. Dermogenys pusillus. Transmission electron micrographs of spermatozoa. A–E. Longitudinal sections. A. Four spermatozoa showing entire nuclear and midpiece regions and anterior region of the axoneme. Note the 'dagger-like' profile of the nucleus when sectioned in or near the plane of the central axonemal singlets. B. Nucleus and adjacent region of midpiece. C. Detail of the nuclear-centriolar junction. D. Base of the nucleus showing flattening apparent when sectioned in the plane at right angles to that of the central axonemal singlets. E. Posterior end of the midpiece and of the two mitochondrial derivatives, showing virtual occlusion of the cytoplasmic canal by widening of the cytoplasmic zone around the anterior region of the axoneme (peri-axonemal sheath). F–Q. Transverse sections in 'anteroposterior' sequence. F–J show the depression of the nucleus in the plane at right angles to that of the central axonemal singlets and (H–J) the basal nuclear fossa and accompanying 'dorsal spur'. K. Shows the bilateral distribution of mitochondria relative to the plane of the axonemal singlets. K, L. Through peri-axonemal sheath. M–Q. Sections though the axoneme, showing posteriorwards reduction of the axonemal fins and progressive disruption of the 9 + 2 pattern of microtubules.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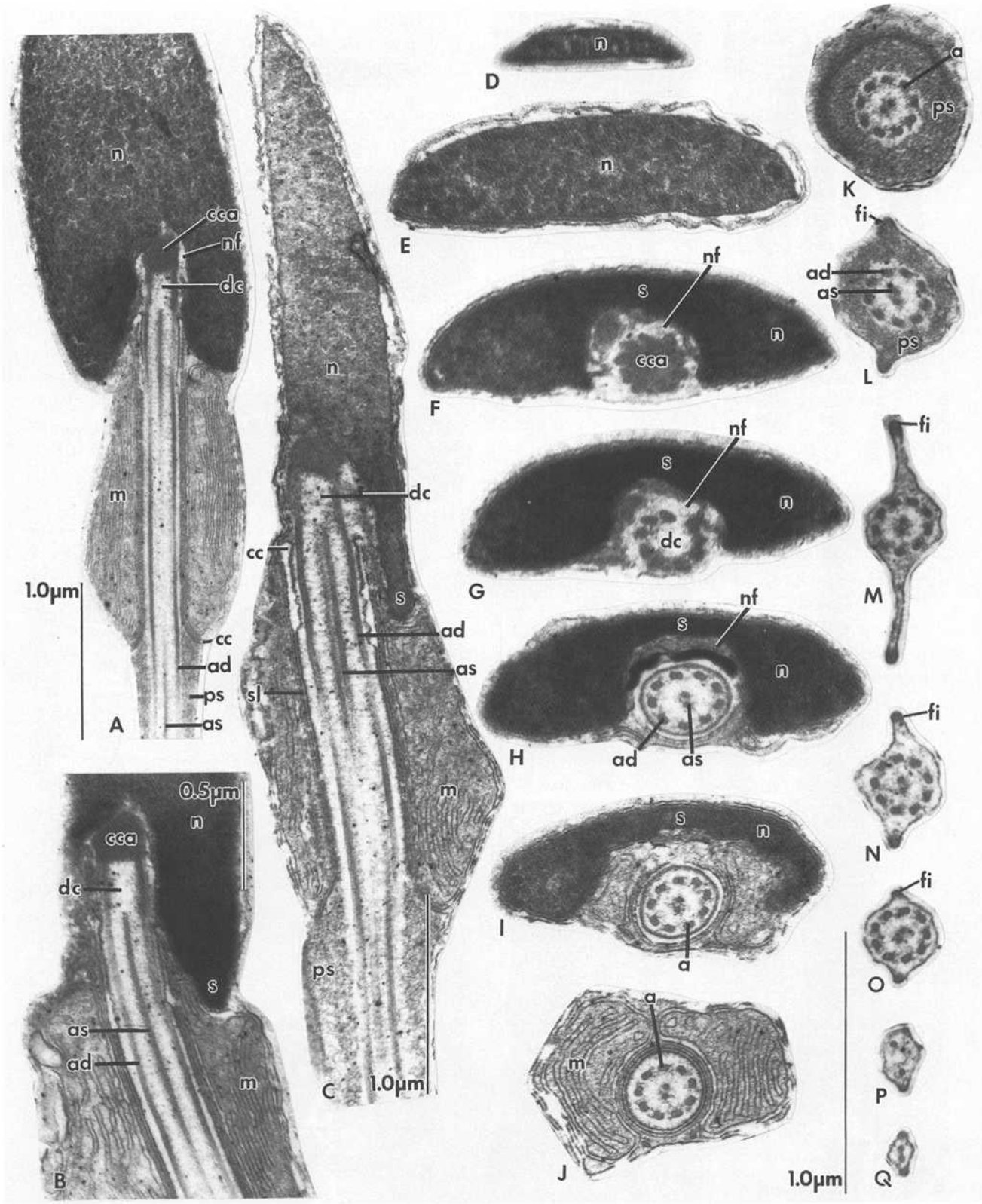
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flagellum is also included. In *Dermogenys* and *Nomorhamphus,* therefore, the nucleus has a long, spur-like continuation behind, and overarching, the anterior region of the flagellum and its basal body. The spur is about 0.23 of the total length of the nucleus in *Dermogenys* and about 0.3 of its length in *Nomorhamphus*. In *Zenarchopterus* this posterior extension is bulkier and less elongate while in *Hemirhamphodon* the nucleus does not extend posteriorly over the axoneme. Although the spur is 'dorsal' in terms of the nomenclature adopted for the nucleus, it is approximately in the radius of axonemal doublet number 3 (in Afzelius notation) and therefore lateral in terms of the usual convention of regarding axonemal doublet number 1 as dorsal (Figs 2IJ; 3H, I; 4D). In this account we will continue to term flattening of the nucleus dorsoventral and the spur as dorsal, however, and to impose this orientation on the sperm as a whole.

The length of the nucleus is 3.13–3.35, mean 3.22 μm (number of sperm = 3) in *Hemirhamphodon*; 3.3–4.1, mean 3.65 μm ($n = 4$) in *Dermogenys*; 3.0 μm ($n = 2$) in *Nomorhamphus;* and 2.4–2.7 μm, mean 2.5 μm ($n = 4$) in *Zenarchopterus*.

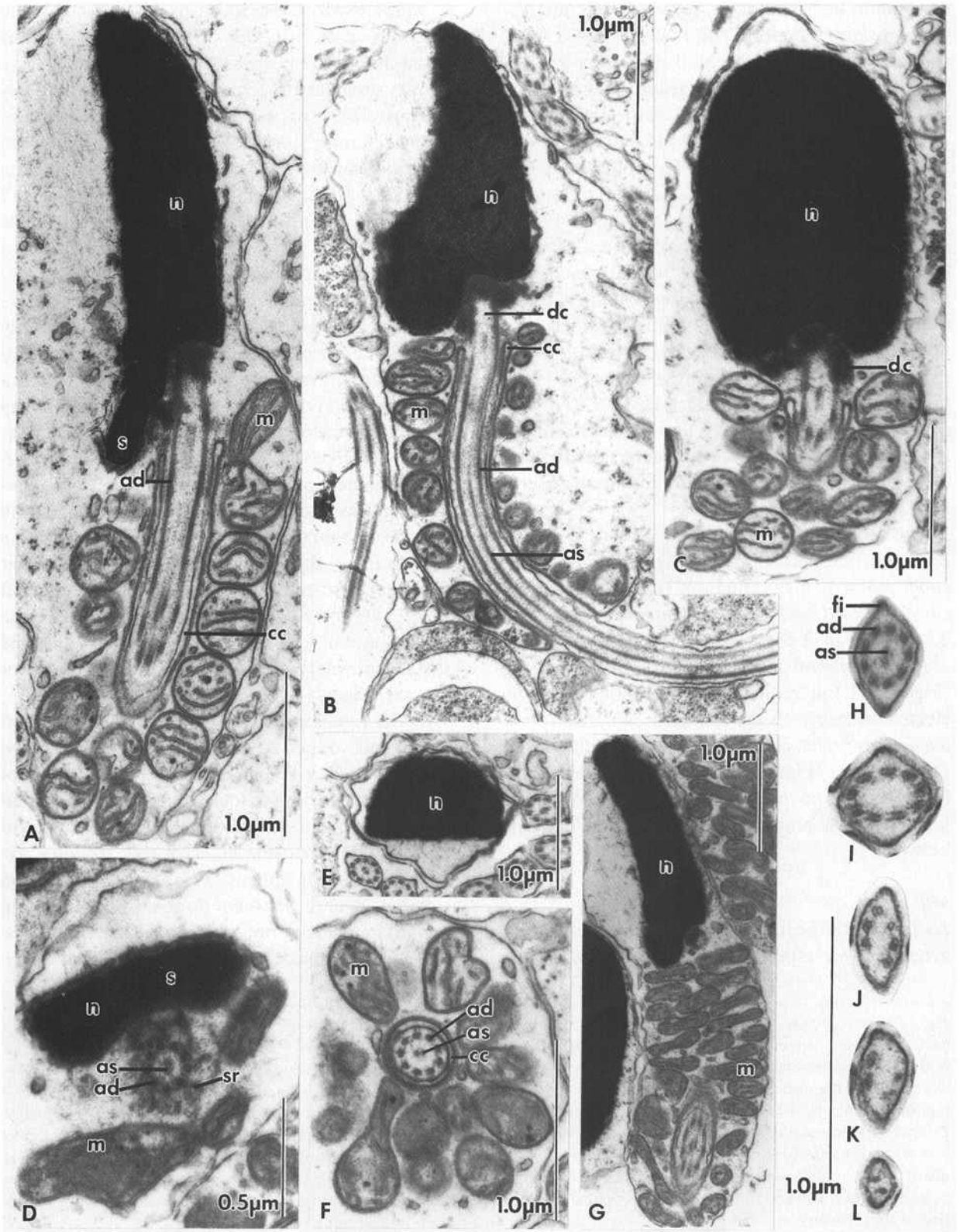
In *Hemirhamphodon* and *Zenarchopterus* (Figs 4A–E), at maturity the chromatin is strongly electron dense with the exception, in *Hemirhamphodon,* of occasional small clear lacunae. In *Dermogenys* (Figs 2A–J) and *Nomorhamphus* (Figs 3A–I) the chromatin consists of closely packed coarse granules with pale matrix material between.

Midpiece

As frequently occurs during teleostean spermiogenesis, the midpiece, grows posteriorwards as a mitochondrial sleeve around the axoneme (Figs 2A–E, K; 3C, J; 4A–C, F). In all four zenarchopterid species the sleeve attains an unusually great length compared with externally fertilizing teleost sperm here exemplified by *Arrhamphus*. The mitochondrial sleeve is separated from the axoneme by a space, the so-called cytoplasmic canal (periaxonemal space). The inner wall of the mitochondrial sleeve, lining the canal, is modified in all species, having the appearance of two parallel membranes, with some densification, and may be termed the submitochondrial dense layer. In *Hemirhamphodon,* but not recognizably in the other three species, this thickening is separated from the mitochondria by a narrow cisterna and has been referred to as the submitochondrial sleeve. The cisterna does not, however, open posteriorly, unlike the cytoplasmic canal, and therefore a single sleeve, the mitochondrial sleeve, including the submitochondrial dense layer, will here be recognized. In the four species the plasma membrane of the head continues posteriorly over the outer surface of the mitochondrial sleeve and turns anteriorly to line the inner surface of the sleeve, as far forward as the basal plate of the distal centriole, before turning posteriorly to cover the axoneme.

In *Hemirhamphodon*, *Dermogenys*, *Nomorhamphus*, and, although the definitive condition is unknown, *Zenarchopterus,* the arrangement of the mitochondria is unique for investigated atherinomorphs. The mitochondria are grouped bilaterally, on opposing sides of the axoneme, on each side of a plane which passes through doublet 3 and between doublets 7 and 8. In *Hemirhamphodon* mitochondrial segregation is extreme and mitochondria, are absent 'dorsally'

Fig. 3. Nomorhamphus celebensis. Transmission electron micrographs of spermatozoa. A–C. Longitudinal sections. A. Base of the nucleus showing flattening apparent when sectioned in the plane at right angles to that of the central axonemal singlets. B. Detail of the nuclear-centriolar junction. C. Entire nuclear and midpiece region and anterior region of the axoneme. Note the 'dagger-like' profile of the nucleus when sectioned in or near the plane of the central axonemal singlets and virtual occlusion of the cytoplasmic canal by widening of the cytoplasmic zone around the anterior region of the axoneme (peri-axonemal sheath). D–Q. Transverse sections in 'anteroposterior' sequence. D–I show the depression of the nucleus in the plane at right angles to that of the central axonemal singlets and (F–I) the basal nuclear fossa and accompanying 'dorsal spur'. J. Shows the bilateral distribution of mitochondria relative to the plane of the axonemal singlets. Cristae are linear in contrast with their zigzagged appearance in *Dermogenys*. L. Through peri-axonemal sheath. M–Q. Sections though the axoneme, showing posteriorwards reduction of the axonemal fins and progressive disruption of the 9 + 2 pattern of microtubules.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and 'ventrally', in this plane. As a result, in *Hemirhamphodon*, mitochondria are fully visible in frontal sections, where the mitochondrial sleeve is wide, but are not seen in sagittal sections, where the sleeve is narrow. In this species, in cross section two to four mitochondria are seen on each side, usually of very unequal sizes. Each is angular in section, approximately triangular to trapezoidal and has well developed irregularly arranged cristae and a moderately dense intercristal matrix. The individuality of each mitochondrion is maintained in longitudinal section of the sperm in which each is usually two or more times longer than wide; there are several discrete mitochondria in longitudinal succession on each side.

In *Dermogenys*, although the mitochondria are bilateral they are contiguous or nearly so dorsally. *Dermogenys* and *Nomorhamphus* differ from *Hemirhamphodon* in having only a single very elongate mitochondrion, which may be termed a mitochondrial derivative, on each side (Figs 2A; 3A, C).

The later spermatids of *Zenarchopterus* resemble *Hemirhamphodon* sperm in having discrete mitochondria in longitudinal succession (Figs 4A–C) but in cross section as many as nine are seen spaced around the axoneme (Fig. 4F), much as occurs in the spermatozoon of *Arrhamphus*. However, in transverse sections in which the nuclear spur is sectioned (Fig. 4E), this is seen to interrupt the circle of mitochondria in the radius passing between doublet 8 and 9, confirming a bilateral arrangement. In some, tangential longitudinal sections (Fig. 4G) individual mitochondria can be seen to have lost their initially spherical form and to have elongate transversely around part of the circumference of the axoneme. It is unlikely from the advanced state of the spermatids that the mitochondria lose their discrete condition.

The arrangement of the cristae in the mitochondria differs remarkably in the four internally fertilizing species. In the numerous mitochondria of *Hemirhamphodon*, the few cristae are chiefly transverse and in transverse section of the midpiece are vertical and parallel; in *Zenarchopterus* three to five or more cristae are chiefly parallel to the long axis of the mitochondrion and therefore transverse relative to the whole spermatozoon. In *Dermogenys* and *Nomorhamphus*, although the sperm are very similar, there are major differences in the cristae. There is no reason at present to believe that the differences are artefacts of fixation. In *Dermogenys*, each mitochondrial derivative shows several (commonly about five) slightly wavy longitudinal cristae, each running most of the length of the derivative (Figs 2A–E), but in transverse section of the midpiece and of the derivative, the mitochondria have the appearance of a continuous zigzag, with rounded angles, meandering through the matrix (Fig. 2K). In *Nomorhamphus*, in contrast, the cristae are almost straight; several run the length of the longitudinal profile of the mitochondrial derivative (Figs 3A–C) and, in transverse section of the organelle, appear as parallel parenthesis-shaped structures, orientated vertically (Fig. 3J).

An 'intermitochondrial link' extending between the mitochondrial masses of opposing sides, near the adaxial border of the mitochondrial sleeve on each side of the axoneme in *Hemirhamphodon* has not been identified in the other species, though a dense horizontal bracket shaped structure seen in transverse section of the nuclear spur in *Nomorhamphus* (Fig. 3H), but anterior to the mitochondrial derivatives, is conceivably equivalent.

In the single longitudinal section of *Hemirhamphodon* sperm in which a complete profile of the midpiece has been obtained, the approximate length of the mitochondrial sleeve (measured

Fig. 4. Zenarchopterus dispar. Transmission electron micrographs of late spermatid. A and B. Longitudinal section (LS) of the nuclear and midpiece regions and anterior region of the axoneme. Note the 'dagger-like' profile of the nucleus when sectioned in or near the plane of the central axonemal singlets. C. LS nucleus and much of midpiece, showing flattening apparent when nucleus is sectioned in the plane at right angles to that of the central axonemal singlets. D. TS through the nuclear spur which interrupts the mitochondrial circlet. E. Transverse section (TS) of the nucleus. F. TS midpiece. G. LS midpiece showing mitochondria wider than long. H–L. TS through the axoneme, showing axonemal fins, reduced relative to *Dermogenys* and *Nomorhamphus*, and progressive disruption of the 9 + 2 pattern of microtubules.

along the mitochondria only) is 3.5 μm. It is 1.8 μm in *Nomorhamphus* ($n = 1$), 2.7–2.9 μm in *Dermogenys* ($n = 2$), and 2.9–3.0 μm in *Zenarchopterus* ($n = 2$).

Centriolar apparatus

In all four zenarchopterid species the distal centriole extends into the implantation fossa and is capped by a dense mass (centriolar cap) which possibly contains remnants of the otherwise unrecognizable proximal centriole (Figs 2A–D; 3A–C; 4A–C). The distal centriole consists of nine elements, which only in *Hemirhamphodon* have been resolved into triplets of microtubules; it is probable that more favourable fixation would reveal triplets in the other species. The distal centriole is continuous with, and in the same longitudinal axis as, the doublets of the axoneme. A conspicuous basal plate at the base of the centriole in *Hemirhamphodon* has not been identified in the other species. In *Hemirhamphodon* each of the triplets gives rise, near its base, to a stout posterolaterally directed satellite ray tilted, in transverse section of the centriole, at about 30° to the radius, and in the same direction as the dynein arms of the axoneme. Satellites have also been identified in *Zenarchopterus* (Figs 4D)

Axoneme

The axoneme has the microtubular pattern usual for teleostean sperm of nine doublets, each with two dynein arms, and two inner singlets (Figs 2J–O; 3H–O; 4F, H). In *Hemirhamphodon*, from the anterior limit of the cytoplasmic canal, near the anterior end of the midpiece, to well behind the midpiece, the axonemal doublets are separated from the flagellar plasma membrane by a broad zone of cytoplasm. In cross section of the axoneme, this cytoplasmic zone is seen to contain 23 radial rodlets (decreasing slightly in number posteriad). These rodlets, which are negative for glycogen in the Thiéry test, are not seen in the other species. In *Dermogenys* and *Nomorhamphus*, a wide zone of cytoplasm is present around the doublets in the portion of the flagellum immediately behind the midpiece (Figs 2L; 3K). In both species, the submitochondrial dense layer curves out laterally in contact with the shoulder-shaped commencement of the cytoplasmic zone (Figs 2E; 3A–C). The opening of the cytoplasmic canal to the exterior is almost occluded by this approximation of the two structures. In both species the submitochondrial layer appears to consist of two parallel membranes. The plasma membrane of the axoneme, from the anterior limit of the cytoplasmic canal to the posterior limit of the canal is thickened and dense (Fig. 2E; 2A, C), its electron density being more pronounced that that of the submitochondrial layer. No particular widening of the cytoplasm around the axonemal doublets is apparent in the late spermatids of *Zenarchopterus* and it seems unlikely that this develops by maturity.

In *Zenarchopterus*, *Dermogenys* and *Nomorhamphus*, as in *Arrhamphus*, there is a pair of axonemal 'fins' in the plane of the central singlets. In *Dermogenys* and *Nomorhamphus*, the tips of the fins, as seen in transverse section of the flagellum, are filled with dense material and the overlying plasma membrane is also dense, giving an appearance in *Nomorhamphus* resembling a matchhead while in *Dermogenys* there is a circular swelling. Each density represents a continuous longitudinal rod-like thickening of the free edge of the fin. The length (radial extent) of a fin is variable along its longitudinal course but is maximally about 0.25 μm, a little wider than the 0.2 μm diameter of the doublet circlet in the axoneme, in both genera. In *Zenarchopterus* sperm, the fins are not so well defined, being wide-based and short, and the apical density is less developed, forming merely a dense cap of thickened membrane. It seems unlikely that at full maturity they reach the dimensions seen in *Dermogenys* and *Nomorhamphus*. Axonemal fins are absent in *Hemirhamphodon*.

Further posteriorly along the *Hemirhamphodon* flagellum the radial rods are absent from the axoneme and the plasma membrane is closely approximated to the doublets. In all species, at the posterior end of the axoneme the arrangement of the microtubules becomes progressively disrupted (Figs 2P, Q; 3P, Q; 4I–L). The B microtubules become open, C-shapes before ending.

Cladistic analysis

The following characters and character states were used in a cladistic analysis of trends in spermatozoal evolution in poeciliids (*Xiphophorus helleri* and *Gambusia affinis*, from Jamieson, 1991) and in exocoetoids. The plesiomorphic states are taken to be those which occur widely in the sperm of externally fertilizing teleosts (the basic teleostean aquasperm defined by Jamieson, 1991:59). This basic sperm is exemplified by the mullid *Upeneus prayensis* which Mattei (1970) chose to represent his type I spermatozoon. Some characters were treated as irreversible and others as unordered, as indicated (p = plesiomorphic, coded as zero; A1, A2 etc. = apomorphic states 1, 2 etc.). The Phylogenetic Analysis Using Parsimony program (PAUP) of Swofford (1990) was employed for the analysis.

1 Nucleus subspheroidal (p) flattened at right angles to plane of singlets (A1) Irreversible Up
2 Mitochondria in longitudinal series: 1, simple (p) > 1 (A1) 1 only, modified (mitochondrial derivative) (A2) Irreversible Up
3 Mitochondria circumferential (p) bilateral (A1) Unordered
4 Mitochondria spheroidal (p) slightly longer than wide (A1) much longer than wide (A2) wider than long (A3) Irreversible Up
5 Periaxonemal sheath absent (p) present, amorphous (A1) with rodlets (A2) Irreversible Up
6 Submitochondrial net absent (p) present (A1) Unordered
7 Axonemal fins well developed (p) reduced (A1) absent (A2) Irreversible Up
8 Nuclear spur absent (p) dorsal (A1) C-shaped (A2) Unordered

The matrix for these eight characters was as follows:

	12345678
Teleostean aquasperm	00000000
Xiphophorus helleri	11030112
Gambusia affinis	11030112
Arrhamphus sclerolepis	01000000
Zenarchopterus dispar	11130011
Hemirhamphodon pogonognatus	11112020
Dermogenys pusillus	12121001
Nomorhamphus celebensis	12121001

A Branch-and-bound search was made, with the following settings: Initial upper bound: unknown (compute via stepwise). Addition sequence: simple. Initial MAXTREES setting = 100. Branches having maximum length zero collapsed to yield polytomies. Keeping trees that satisfy constraint definition monophyletic Exocoetoidei, with *Zenarchopterus* the sister-group of *Hemirhamphodon* + *Dermogenys* + *Nomorhamphus* (relationships suggested by Tibbetts, 1992). Trees are rooted.

A single tree was obtained with the following characteristics: Tree length = 22 steps. Consistency index (CI) = 0.636. Homoplasy index (HI) = 0.364. Retention index (RI) = 0.805. Rescaled consistency index (RC) = 0.512.

This tree is illustrated in Fig. 5. Character changes and the morphology of the species investigated are indicated in a schematic representation of the tree in Fig. 6.

Trends in spermatozoal ultrastructure evident from this analysis are treated in the Discussion.

Discussion

A trend occurring homoplasically in poeciliids and zenarchopterids is elongation but chiefly flattening of the nucleus (character 1). Flattening in both groups is at right angles to the plane of the central singlet microtubules of the axoneme and in both the tip of the nucleus appears rounded in the plane of flattening but pointed in the plane at right angles.

The sperm of poeciliids and of exocoetoids can confidently be deduced to have originated independently from the aquasperm widely occurring in externally fertilizing teleosts (for review see Jamieson, 1991). We have arbitrarily attributed a single tier of mitochondria to this basic aquasperm (character 2), though it is possible that more than one tier is basic. It is noteworthy that the aquasperm of the externally fertilizing exocoetid

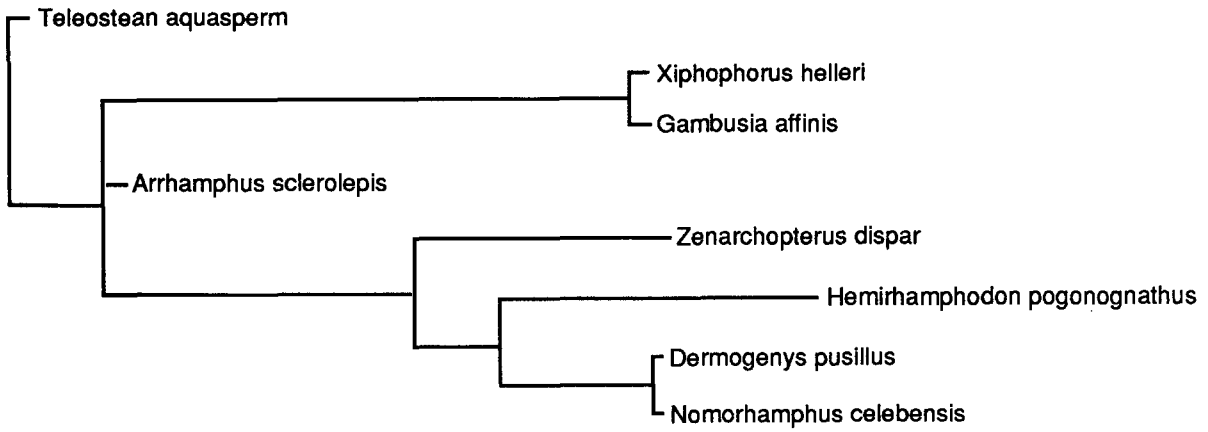


Fig. 5. Tree derived by branch and bound parsimony analysis of 8 spermatozoal characters (some multistate) for 7 studied atherinomorphs plus a basic teleostean aquasperm. Branch lengths reflect numbers of apomorphic changes in spermatozoal ultrastructure which are listed in Fig. 6. The novel sister-group relationship of *Dermogenys* and *Nomorhamphus* and unity of the Poeciliidae are well supported by the spermatozoal data.

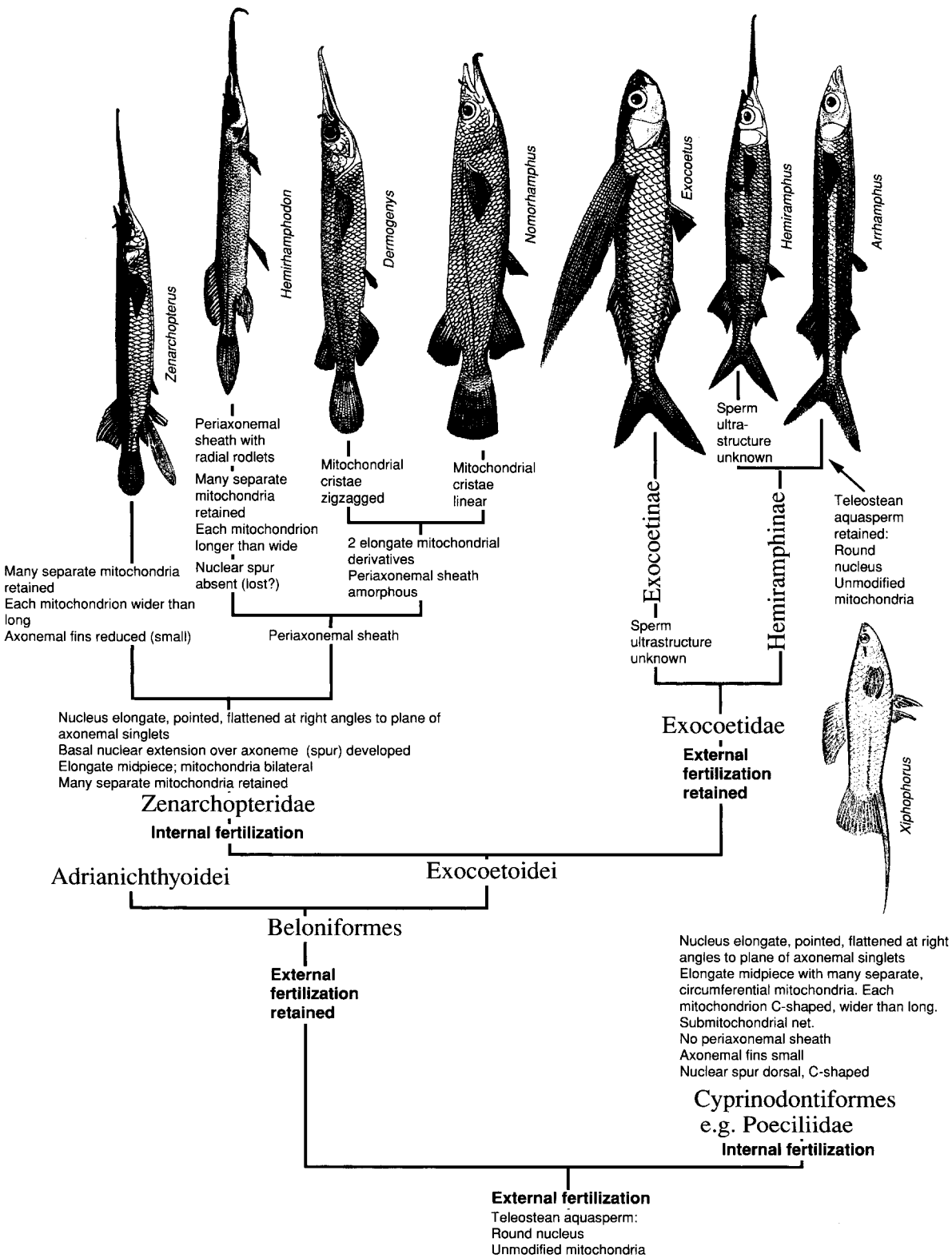
studied, *Arrhamphus sclerolepis*, already possesses more than one tier of mitochondria, as is characteristic of the internally fertilizing exocetoids, the Zenarchopteridae, though modified to a pair of longitudinal mitochondrial derivatives in *Dermogenys* and *Nomorhamphus*. The possession and form of the mitochondrial derivatives in the latter two genera appears to be an important synapomorphy between them. In contrast, Tibbetts (1992) from general morphology found *Zenarchopterus* to be the sister-group of *Nomorhamphus* + (*Hemirhamphodon* + *Dermogenys*).

Although poeciliids and zenarchopterids have independently elongated the midpiece and multiplied the number of mitochondria in it, a noteworthy difference between the two families (character 3) is the circumferential distribution of mitochondria in poeciliids whereas in zenarchopterids the mitochondria or mitochondrial derivatives are bilateral. Even where most nearly circumferential, in *Zenarchopterus dispar*, they are interrupted 'dorsally' by the posterior extension of the nucleus.

In the plesiomorphic condition for teleosts, as in *Arrhamphus*, the mitochondria are subspheroidal (character 4). They have departed radically from the subspheroidal form in the internally fertilizing fish and have multiplied along the lengthened midpiece. Although in longitudinal section of poeciliid sperm the individual mitochondria appear to be elongate, they are seen in cross section of the midpiece to be C-shaped and considerably more extensive circumferentially than longitudinally. In *Zenarchopterus* they have independently become more extensive circumferentially than longitudinally. In *Hemirhamphodon* they are moderately elongate, being two or three times as long as wide. In *Dermogenys* and *Nomorhamphus*, as noted above, they have been modified as a pair of mitochondrial derivatives, an apparently monophyletic event.

A wide cytoplasmic zone around the anterior

Fig. 6. Trends in the evolution of zenarchopterid, exocoetid and poeciliid spermatozoa. The interrelationships of poeciliids to the exocoetid *Arrhamphus* and to zenarchopterids are drawn from the parsimony analysis of spermatozoal ultrastructure (see also Fig. 5) but interrelationships of exocoetids are otherwise based on parsimony analysis of somatic morphology by Tibbetts (1992) as spermatozoal characters are plesiomorphic or unknown. Illustrations of fish, representative of their genera, are *Xiphophorus helleri* from Jordan (1907) and *Arrhamphus brevis*, *Zenarchopterus dispar*, *Hemirhamphodon pogonognathus*, *Dermogenys orientalis*, *Nomorhamphus celebensis*, *Hemiramphus far* and *Exocoetus volitans* from Weber & DeBeaufort (1922).


Zenarchopterus
Hemirhamphodon
Dermogenys
Nomorhamphus
Exocoetus
Hemiramphus
Arrhamphus
Xiphophorus
Periaxonemal sheath with radial rodlets
Many separate mitochondria retained
Each mitochondrion longer than wide
Nuclear spur absent (lost?)
Mitochondrial cristae zigzagged
Mitochondrial cristae linear
2 elongate mitochondrial derivatives
Periaxonemal sheath amorphous
Sperm ultra-structure unknown
Teleostean aquasperm retained:
Round nucleus
Unmodified mitochondria
Many separate mitochondria retained
Each mitochondrion wider than long
Axonemal fins reduced (small)
Periaxonemal sheath
Exocoetinae
Hemiramphinae
Sperm ultrastructure unknown
Nucleus elongate, pointed, flattened at right angles to plane of axonemal singlets
Basal nuclear extension over axoneme (spur) developed
Elongate midpiece; mitochondria bilateral
Many separate mitochondria retained
Zenarchopteridae
Internal fertilization
Exocoetidae
External fertilization retained
Adrianichthyoidei
Exocoetoidei
Beloniformes
External fertilization retained
Nucleus elongate, pointed, flattened at right angles to plane of axonemal singlets
Elongate midpiece with many separate, circumferential mitochondria. Each mitochondrion C-shaped, wider than long.
Submitochondrial net.
No periaxonemal sheath
Axonemal fins small
Nuclear spur dorsal, C-shaped
Cyprinodontiformes
e.g. Poeciliidae
Internal fertilization
External fertilization
Teleostean aquasperm:
Round nucleus
Unmodified mitochondria

region of the axoneme, the periaxonemal sheath (character 5), appears to have developed monophyletically in the ancestry of *Hemirhamphodon*, *Dermogenys* and *Nomorhamphus*. *Hemirhamphodon* has taken this development further by the acquisition of radial rodlets, in a circlet of 23 with a longitudinal repeat. The periaxonemal sheath is a distinctive zenarchopterid development, though apparently plesiomorphically absent in *Zenarchopterus*, and is not seen in poeciliids.

A distinctive development in poeciliids is the submitochondrial net (character 6) though the submitochondrial dense layer of zenarchopterid sperm is possibly its homologue.

A pair of fin-like extensions of the flagellum (character 7), approximately in the plane of the central singlets of the axoneme, is a synapomorphy of, and simultaneously plesiomorphic for, the Actinopteri (see Jamieson, 1991). Poeciliids have greatly reduced the fins. Exocoetoids have retained well developed fins where fertilization is external, as in *Arrhamphus*. *Dermogenys* and *Nomorhamphus* have retained fairly well developed fins, with some modification of their tips, but reduction has occurred, apparently independently, in *Zenarchopterus*, in which the fins are small, and *Hemirhamphodon* in which they are absent.

In basic teleostean aquasperm, as also in *Arrhamphus*, the nucleus does not extend posteriorly over the base of the axoneme. Such an extension, which we have loosely termed a 'spur' (character 8), has developed in poeciliids and independently in zenarchopterids. Whereas in poeciliids the posterior extension of the nucleus is a C-shaped structure embracing almost the entire circumference of the axoneme, in zenarchopterids it is a 'dorsal' plate. Its absence in *Hemirhamphodon* computes as a loss but it is possible that this genus basically lacks this extension.

Bearing in mind that modifications from the basic teleostean aquasperm have occurred independently in poeciliids and zenarchopterids, the above discussion summarises similar and different responses to internal fertilization in the two families. Similar changes have been elongation and flattening of the nucleus in the same plane, elongation of the midpiece and multiplication of the number of mitochondria, though with further modification of these two a pair of elongate mitochondrial derivatives in two zenarchopterids; a tendency to reduction of the axonemal fins; and development of a posterior extension of the nucleus over the base of the axoneme. Different responses have been a circumferential distribution of mitochondria in poeciliids against a bilateral arrangement in zenarchopterids; development of a periaxonemal sheath of cytoplasm exclusively in zenarchopterids, above *Zenarchopterus*; and of a submitochondrial net only in poeciliids (also seen independently in *Jenynsia*, see Jamieson, 1991).

All of these modifications relative to the aquasperm condition are here regarded as having been occasioned by the adoption of internal fertilization. They are thus directly attributable to a change in fertilization biology but to what extent their details are constrained by features of the genome peculiar to poeciliids, zenarchopterids or atherinomorphs or are demanded by minute differences in fertilization biology, or by a combination of the two, seems indeterminable. The functional significance of modifications of the sperm is partly treated in Jamieson (1991). Some of the character states, particularly those pertaining to elongation of the nucleus, elongation of the midpiece, and development of lateral elements to the axoneme, are seen in the phylogenetically very distinct Chondrichthyes (see Jamieson, 1991) but there have different expressions. It would be tempting to relate the differences to phylogenetic constraints but they might equally or also be the product of subtle differences in fertilization biology.

This study nevertheless demonstrates many modifications of internally fertilizing sperm independently acquired in two (poeciliid and zenarchopterid) lineages and shows that even where internal fertilization has been secondarily acquired, it offers major spermatozoal synapomorphies unifying the Poeciliidae and giving hitherto unrecognized synapomorphies, for instance acquisition of mitochondrial derivatives in *Dermogenys* and *Nomorhamphus*.

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