台灣櫻花鉤吻鮭(*Oncorhyhchus masou formosanus*)

精子的微細構造

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台灣陸封型鮭(*Oncorhynchus masou formosanus*; 眞骨魚類、鮭目、鮭科)精子的微細構造

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摘要 本文以穿透式及掃描式電子顯微鏡研究台灣陸封型鮭(櫻花鉤吻鮭、台灣鮭、台灣鱒)精子的微細構造，並且和其他鮭目魚類的精子做比較。台灣陸封型鮭與虹鱒(*O. mykiss*)，兩者精子的微細構造非常類似，只在兩中心粒的排列組合、粒線體的形狀和鞭毛內微管之堵塞與否，有些些微的出入。台灣陸封型鮭精子的微細構造和三種太平洋鮭屬(Oncorhynchus)的魚類一樣，皆有橢圓形不均質的細胞核，纖維狀結構連接兩中心粒，單一粒線體及鞭毛有側鰭等特徵。這些特徵屬固有子孫形質(autapomorphy)或許更可以衍伸到整個鮭亞科(Salmoninae)。從研究台灣陸封型鮭精子的微細構造所得的結論與以生化、生態、生活史、骨骼分析方式所歸納出之結果很吻合。本研究首次報導台灣陸封型鮭精子的幾個特殊形質，同時也討論藉精子的微細構造來探討鮭目(Salmoniformes)魚種系統分類之可行性。

關鍵詞：台灣、鮭、 精子、微細構造、太平洋鮭屬

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THE ULTRASTRUCTURE OF FORMOSAN LANDLOCKED SALMON, *ONCORHYNCHUS MASOU FORMOSANUS*, SPERMATOZOON (TELEOSTEI; SALMONIFORMES; SALMONIDAE)

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ABSTRACT Spermatozoa of Formosan landlocked salmon (*Oncorhynchus masou formosanus*), examined using transmission (TEM) and scanning (SEM) electron microscopy, are compared with spermatozoa of other Salmoniformes. Important features such as the alar sheet and necklace are described for the first time. Ultrastructurally there are only minor differences between the mature spermatozoa of *O. masou* and rainbow trout (*O. mykiss*), in particular the organization of the centriolar complex, the shape of the mitochondria, and the occurrence of ITD (intratubular differentiation) in the flagella. Our spermatozoan ultrastructural study of *O. masou* and available known spermatozoan ultrastructural reports from subfamily Salmoninae suggest that *O. masou* and three species of *Oncorhynchus* spermatozoa may represent an autapomorphy of the genus *Oncorhynchus* which could extend to the whole subfamily Salmoninae. Evidence is as follows: the presence of an elongated nucleus with highly heterogenous granular chromatin, a fibrous body connecting the two centrioles, a midpiece consisting of a single mitochondrion, and a flagellum with sidefin. Data from spermatozoan ultrastructure are in concordance with that from the biochemical, ecological, life history and osteological diagnostic characters. The use of spermatozoan morphology in the examination of taxonomic relationships of Salmoniformes is discussed.

Introduction

Masu salmon (*Oncorhynchus masou*) is distributed exclusively in the Far East, ranging from southwestern Kamchatka Peninsula to Taiwan (Nakajima and Fujio, 1993; Okazaki, 1986;88;90). This species, including four subspecies, has three life forms (Kiso, 1990; Kubo, 1980). One is a lacstrine form which spawns in rivers and grows in lakes. Another is a fluviatile form which lives in rivers throughout its life. The third is a sea-run form which spawns in rivers and grows up in the sea after one or two years of river life. The Formosan landlocked salmon (*Oncorhynchus masou formosanus*), a fluviatile form, is only found in the upper streams of Tachia River, Taiwan. This is the southern limit in the distribution range of salmonids (Hosoya *et al.*, 1992; Jan *et al.*, 1990; Numachi *et al.*, 1990; Oshima, 1957; Okazaki, 1988).

The scientific name and phylogenetic (systematic) position of the Formosan landlocked salmon have been very controversial. The phylogeny of Formosan landlocked salmon has been constructed on the basis of morphology and meristics (Behnke *et al.*, 1962; Hosoya *et al.*, 1992; Jan *et al.*, 1990), distribution (Lin *et al.*, 1990), growth and development (Yu *et al.*, 1985,86,87), and restriction analysis of mitochondrial DNA (Numachi *et al.*, 1990). Many of these studies have suggested that Formosan landlocked salmon are evolutionarily close to *O. masou* and other species of *Oncorhynchus*. Watanabe and Lin (1985) concluded that only a single salmonid, *Oncorhynchus masou formosanus*, exists in Taiwan. Numachi *et al.* (1990) speculated that Formosan landlocked salmon originated from the masu salmon distributed in the Sea of Japan and reached Taiwan through the Tsushima channel 100-800 thousand years ago when a cold current prevailed in the Sea of Japan. Hosoya *et al.* (1992) suggested that the Formosan landlocked salmon are similar enough to *O. masou* to be regarded as a subspecies of *masou*.

The use of spermatozoan ultrastructure to analyze relatedness among groups of organisms has become widespread in many animal groups, including fish (Baccetti, 1985; Billard, 1970; Jamieson, 1981;1991; Justine, 1991; Mattei, 1991). In addition, spermatozoan morphology has also provided interesting insights into phylogenetic relationships of a number of animal taxa (Franzen, 1970; Jamieson, 1991; Mattei, 1991; Mattei and Mattei, 1974; Gwo, 1995; Gwo and Arnold 1992; Gwo and Gwo, 1993; Gwo *et al.*, 1992;1995a,b). Therefore by comparing spermatozoan ultrastructures we may be able

to derive evolutionary relationships and levels of variability within and among groups of fishes. Though a great deal of work has been done on the morphology of spermatozoa in the Salmoniformes (Billard, 1983a,b; Drozdov *et al.*, 1981; Fribourgh, 1978; Fribourgh and Soloff, 1976; Jaana and Yamamoto, 1984; Lahnsteiner *et al.*, 1991; Lowman, 1953; Nicander, 1970; Radziun and Tomasik, 1985; Stein, 1981; Zirkin, 1975), only a few papers describe the details of the ultrastructure of mature spermatozoa (Billard, 1983b; Lahnsteiner *et al.*, 1991). In this paper, we investigated the ultrastructure of mature spermatozoa of Formosan landlocked salmon (*O. masou formosanus)* with emphasis on several organelles that have not been described in other species of Salmoniformes, to help clarify the taxonomy of this species. The ultrastructure of spermatozoa in the Salmoniformes is also reviewed.

Materials and Methods

Mature male *O. masou formosanus* (100--300 g body weight) were collected from Chichiawan stream near Wu-lin Farm, Taiwan, during the breeding season (October--December). Gentle pressure was applied on the abdomen of each fish to extrude the semen. Caution was exercised to prevent contamination of the semen with urine, blood, mucus or water.

Semen samples were fixed for 1-2 h in 2% glutaraldehyde in 0.12 M phosphate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in the same buffer for 1 h. The samples were dehydrated in a graded ethyl alcohol series. Dehydrated samples were either embedded in low viscosity Spurr resin or prepared for scanning electron microscopy as described below. Sections were stained on drops of 2% uranyl acetate followed by lead citrate, and observed in a JEM-1200 EX II transmission electron microscope at 80-kV accelerating voltage. For scanning electron microscopy, samples were fixed in glutaraldehyde and postfixed in osmium tetroxide as described above. Following dehydration in a graded ethyl alcohol series, samples were critical-point dried, coated with gold, and observed with the JSM-6300 SEM.

Results

The nucleus of spermatozoa of *O. masou formosanus* is ovoid-shaped, contains heterogeneously granular, strongly electron-dense chromatin with irregular small clear

lacunae, and is covered by a typical double nuclear envelope (Figs. A--C). The head has a length of 2.01 ± 0.04 μm (mean ± SD) and a diameter of 1.71 ± 0.03 μm (mean ± SD). The undulating nuclear envelope and plasma membrane are applied tightly to the anterior of the nucleus (Fig. B), but no acrosome is present anterior to the nucleus. The base of the nucleus is indented by a nuclear fossa, the length of which is about one-fourth of the length of the nucleus (Figs. B--E). The nuclear fossa is bell shaped in longitudinal section and circular in transverse sections (Figs. B--K).

The centriolar complex is present in the nuclear fossa and the proximal and distal centrioles which comprise it are located at a right angle to each other (Figs. B--E). Both centrioles display a characteristic nine-triplet pattern. The proximal centriole is oriented with its long axis perpendicular to the longitudinal axis of the head (Figs. B--E). A fibrous body consisting of osmiophilic disks alternating with lighter material adheres to the two centrioles laterally (Figs. C--E). Three dense bodies circle around the proximal centriole (Fig. C). Two additional dense bodies form two large mushroom-shaped projections which locate within the hollows in both sides of the upper nuclear fossa (Figs. C,D,F). These two dense bodies give rise to short electron-dense projections (fibers) which connect the dense body to the proximal centriole and anchor the proximal centriole to the nucleus as well. The distal centriole, which forms the basal body of the axoneme, extends from the level of the anterior end of the cytoplasmic canal to the basal nuclear fossa (Figs. B--E). In the transverse sections, the osmiophilic ring, embedded in an electron-dense material, surrounds the anterior end of the distal centriole (Fig. H). All nine of the outermost subtubules (C) of the triplets of the distal centriole disappear proceeding posteriorly, and the remaining two (A and B) innermost subtubules of each triplet, continuously connected to each other by an inner ring, elongate to form nine peripheral doublets (Fig. I). In the posterior region, nine radial alar sheets connect the basal body to the plasma membrane invagination (Figs C and J). An electron-dense structure is fixed at the junction between the midpiece and the beginning of the flagellum (Figs. C--E). In a cross-section, the basal body resembles a cartwheel with the alar sheets extending from the triplets to the cell surface (Fig. J).

Considerable amounts of cytoplasm are present adjacent to the mitochondrial ring (Figs. C,L,M). The cytoplasmic canal, bounded by a unusual fold of the plasma membrane, separates the midpiece from the flagellum (Figs. L,M). The mitochondria are fused to form a helical mitochondrion, which surrounds the cytoplasmic canal with one and a half

windings (Figs. A,L,M).

Three electron-dense particles, termed the necklace, were located in the invaginated part of the plasma membrane surrounding the neck of the flagellum (Fig. B). At this level, a flagellum that is transversely sectioned shows at this level Y-shaped bridges which link the doublets to the flagellar plasma membrane (Fig. K). Distal to this region, the axoneme has the classic 9 + 2 microtubular doublet construction (the central pair of microtubules is surrounded by nine peripheral doublets; Figs. L--Q), and Y-shaped bridges are absent. The plasma membrane, around the axoneme along the main part of the flagella, starts closely apposed to the axoneme; first one and then a second lateral extensions (lateral ridges; sidefins) appear behind the midpiece (Figs. L--R). The membranous extensions are filled with fine-granular material and do not contain any skeletal structures. No ITD (intratubular differentiation) was observed in the flagellum. The central singlet tubules, connected by a cross-bridge, are surrounded by an inner sheath (Figs. L--Q). Each of the nine doublets consists of subfibers A and B. Two dynein arms arise from subfiber A of each doublet and extend toward the next fiber. Structures similar to radial spokes and nexin are barely visible.

Discussion

Electron microscopy of a wide spectrum of teleost spermatozoa has demonstrated that important morphological characters can be identified among different species (Franzen, 1970, Mattei, 1988;1991, Gwo, 1995; Gwo *et al.* 1993; Gwo *et al.*, 1995a,b) and can be used for taxonomic purposes (Mattei and Mattei, 1974, Jamieson, 1991, Mattei, 1991, Gwo *et al.* 1993;1995a,b). Spermatozoan morphology often serves as an independent arbiter, capable in many cases of resolving contentious taxonomic and phylogenetic problems. It has, for example, proved useful in the demonstration of Anguilliformes-Elopidae relationships (Mattei and Mattei, 1974). The spermatozoan ultrastructure of *O. masou formosanus* closely resembles that of rainbow trout (*O. mykiss*) spermatozoa (Billard, 1970;1983a,b). However, these two species of the subfamily Salmoninae do exhibit some minor morphological differences, including the organization of the centriolar complex, the shape of the mitochondria and the appearance of ITD in the flagella. The Salmoninae spermatozoa examined to date show a certain structural homogeneity and provide support for the concept that ultrastructural features of spermatozoa can be useful in taxonomic studies (Baccetti *et*

al., 1984; Mattei, 1988;1991; Mattei and Mattei, 1974; Grier, 1973;1975;1976; Lahnsteiner *et al.*, 1991; Jamieson, 1991; Gwo *et al.*, 1993;1994;1995a,b). The present study also confirmed that there exist interspecific differences within the family of Salmonidae between species of the subfamilies Thymallinae and Salmoninae in the organization of the midpiece of the spermatozoa (Lahnsteiner *et al.*, 1991). The mitochondria of grayling spermatozoa have a helical form (Lahnsteiner *et al.*, 1991), while in most investigated salmoninae spermatozoa the mitochondria are cylindrical.

In *O. masou formosanus*, the fibrous body, consisting of osmiophilic disks alternating with lighter material, adheres to the two centrioles laterally. This can also be observed during spermiogenesis in rainbow trout (Billard, 1983b). A flagellar rootlet connects the centriolar complex to the nucleus in the early spermatid stage and is also involved in the migration of the centriolar complex toward the nucleus (Billard, 1983b). The rootlet disappears when the rainbow trout spermatozoan head is fully formed (Billard, 1983b). The fibrous body found in *O. masou formosanus* spermatozoa is similar in size, location, and appearance to that of the flagellar rootlet found in rainbow trout. We suspect that it may be the remnant of the flagellar rootlet. This idea remains to be tested. Clearly, an examination of spermiogenesis in this species may well shed more light on taxonomic and phylogenetic links in Salmoniformes. The dense bodies, located within the hollows in both sides of the upper nuclear fossa, resemble those of the centriolar complex appendage in the spermatozoa of flounder (*Platichthys flesus*; Jones and Butler, 1988) and *Oryzias latipes* (Grier, 1976). The alar sheet (called anchoring fibre apparatus, satellite projection, or centriolar satellite complex by other authors Afzelius, 1979; Jamieson 1981, Summers 1972) forms a nine-pointed star which apparently keeps the distal centriole anchored to the plasma membrane (Afzelius, 1979). The nine primary fibers have secondary processes in several animal groups (Afzelius, 1979). The fibrous body, the dense body, and the alar sheet appear to fasten the centrioles to the plasma membrane and are also responsible for the stabilization of the spatial relationship between the nucleus and the centriolar complex in fish spermatozoa, which is important during sperm tail movement. Summers (1972) proposed that the alar sheets may contain a centractile protein and they possibly also have a function in transporting ATP from the mitochondria to the flagellum.

Necklace are always found on the external surface of the flagellar neck-membrane, at the junction of the flagellum and plasma membrane, in several teleost spermatozoa (Gwo *et*

al., 1994; Gwo, 1995b). It has been proposed that the necklaces are involved in the control of localized membrane permeability, that is, the ionic permeability sites which control Ca^{+2} ion entry into the flagellum, thus triggering flagellar motility (Dentler, 1990). Initiation of spermatozoan motility in salmonid fishes is at first regulated at the plasma membrane of the flagellum by the transport of K^+ and Ca^{+2} through ion channels (Morisawa and Morisawa, 1990). Simultaneously with K^+ efflux, Ca^{+2} influx occurs and the rise of intracellular calcium level affects the capacity of the internal flagellar system to generate cAMP (cyclic adenosine monophosphate; Morisawa and Morisawa, 1990). Beating of the rainbow trout spermatozoan flagellum has been observed only when cAMP is applied to the basal region of a de-membranated flagellum, whereas it did not occur when cAMP was applied to other regions (Morisawa, 1987). The number of necklace has been reported to be species-specific (Gilula and Satir, 1972).

The family Salmonidae comprises three subfamilies: Salmoninae, Thymallinae and Coregoninae (Sanford, 1990). The Salmoninae have both freshwater and anadromous representatives, while the Thymallinae are restricted to freshwater alone. Most Coregoninae are also freshwater inhabitants (Sanford, 1990). Smith and Stearley (1989) mapped life history, ecological, biochemical and morphological traits onto the Salmonidae phylogeny and found the overall phylogenetic history of the Salmoninae supports a stepwise transition from freshwater forms to levels of intermediate anadromy to increasing loss of dependence on freshwater stages in *Oncorhynchus*. According to the dependence on freshwater in early life stage, six of the eight species of the genus *Oncorhynchus* can be divided into two groups: the freshwater dependent group and the sea water dependent group (Numachi, 1984; Okazaki, 1990). Chum salmon (*O. keta*) and pink salmon (*O. gorbuscha*), both belong to the sea water dependent group and migrate to sea shortly after hatching. The period of time they spend in freshwater is considerably shorter than the freshwater dependent species. They have reduced the freshwater phase of their life cycle to a minimum, emerging from the gravel ready to migrate. The freshwater dependent group consists of masu, coho (*O. kisutch*), chinook (*O. tshawytscha*), and sockeye *(O. nerka*). Sea-run forms of masu salmon (*O. masou*) usually spend one or two years in freshwater before migrating to sea in the spring as smolts (Kubo, 1980). All the females migrate to sea, while some males spend their life in freshwater (Kubo, 1980). The sea-run forms die after spawning, while landlocked forms are repeat spawners (Kubo, 1980). In terms of life history, the masu

salmon is intermediate between trout and salmon.

In recent years, numerous investigations based on conventional morphological methods and biochemical genetic methods have been applied to gain a better understanding of the phylogeny of salmoniformes. Masu salmon is considered the most primitive species among the genus *Oncorhynchus* (Neave, 1958; Numachi, 1984; Okazaki, 1988;1990). This species is morphologically and genetically more closely related to the genus *Salmo* than all the existing species of *Oncorhynchus* (Behnke *et al.*, 1962; Numachi, 1984; Okazaki, 1990). Neave (1958) suggested that *Oncorhynchus* diverged from *Salmo* at or near the beginning of the pleistocene, that is, about 2 million years ago, and that *O. masou* is most closely related to *Salmo* of all existing *Oncorhynchus* because of its unspecialized or primitive features in anatomical, ecological and physiological traits. This was also strongly supported by isozyme analysis and protein electrophoresis of salmonid species (Numachi, 1984; Utter *et al.*, 1973). In the present paper, the conclusions from the spermatozoan ultrastructure approach are consistent with those from numerical analyses of the general (non-spermatozoal) anatomy as well as the biochemical and mitochondrial DNA analysis criteria of this family (Behnke *et al.*, 1962; Numachi, 1984;1990; Okazaki, 1990). The most surprising discovery of the present study was the great similarity of spermatozoan ultrastructure between Formosan landlocked salmon and rainbow trout; a close relationship is suggested. Therefore, *O. masou*, the most primitive salmon, is trout-like and occupies an intermediate position between trout and salmon in the evolutionary scheme. Data from spermatozoan ultrastructure are also in concordance with that from the life history study conducted by Smith and Stearley (1989).

In *O. masou formosanus* and three species of *Oncorhynchus*, spermatozoa have an elongated nucleus with highly heterogenously granular chromatin, a special centriolar complex, and a single mitochondria (Billard, 1983a,b; Drozdov *et al.*, 1981; Jaana and Yamamoto, 1984). This may represent an autapomorphy of the genus *Oncorhynchus* which could extend to the whole subfamily Salmoninae. This subfamily comprises one species of *Hucho*, Radziun and Tomasik, 1985; one species of *Salvelinus,* Fribourgh, 1978; and two species of *Salmo*, Billard, 1983b; Nicander, 1970. In order to reevaluate the reliability of the phylogenetic relationship within the subfamily Salmoninae, ultrastructural studies of spermatozoa and spermiogenesis in this and other species of the family would be of considerable value.

The superorder Protacanthopterygii was originally erected to contain primitive teleostean fishes such as salmon, trout, and pike, together with certain obscure deep-sea groups (Nelson, 1984). Research in the last few years has shown that these groups were false friends, largely united on primitive characters (soft fin-rays, adipose fin, oviducts absent or incomplete and three upturned caudal vertebrae) which do not indicate relationships. Fink and Weitzmann (1982) found no indication that the esocoids (Family Esocidae and Umbridae) were closely related to other members of the Salmoniformes order; they suggested instead that esocoids are the most primitive euteleosts, with both ostariophysans and other salmoniforms being derived groups.

The fine structure of spermatozoon of the Northern pike, *Esox lucius*, has been briefly described (Billard, 1970; Stein, 1981). Both authors considered that the spermatozoa of Northern pike show no similarity to those of salmoniformes, whereas they have a strong resemblance to those of Cyprinidae spermatozoa (Billard, 1970). Baccetti *et al.* (1984) stated each species of the Cyprinidae is characterized by a particular organization of the spermatozoan organelles, in a uniform general pattern common to the whole family. The nucleus of Northern pike spermatozoa is round. The flagellum is inserted laterally to the nucleus. The mitochondria are not united in a single spherical or annular structure. These facts of spermatozoan ultrastructure do not support the proposition that esocoids are Salmoniformes. On the contrary, they suggest that there is a large difference between the esocoids and the Salmoniformes.

The observations reported here contribute to existing knowledge of comparative spermatology and may provide an additional clue to Protacanthopterygii or Salmoniformes phylogeny. While the results on spermatozoan fine structure obtained in the present study cannot resolve the phylogeny of Salmoniformes, our observations add further support to the opinions of Fink and Weitzman (1982) who showed that the esocoids and the Salmoniformes form clearly distinct groups.

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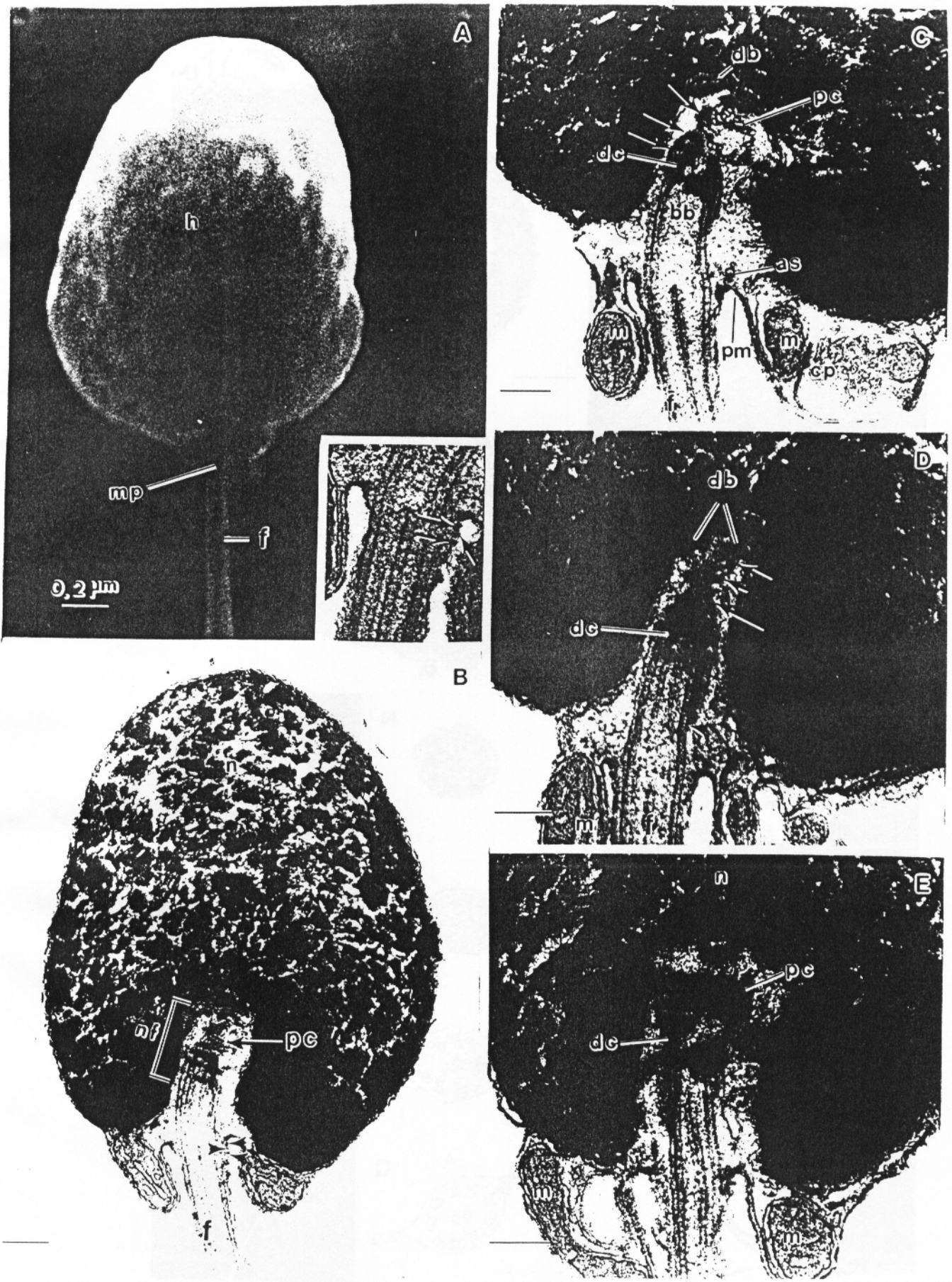
Figure A--Q Formosan landlocked salmon, *Oncorhynchus masou formosanus*, Spermatozoon.

A. Scanning electron micrograph of a spermatozoon showing the ovoid-shape head (h), midpiece (mp), and flagellum (f). X 29,400. Inset: Enlarged portion from Fig. B showing necklaces, the electron-dense particles (arrows), located in the invaginated part of the plasma membrane surrounding the neck of the flagellum. **B.** Sagittal longitudinal section of a spermatozoon showing the ovoid-shape nucleus (n) with the axial nuclear fossa (nf) which contains the centriolar complex. The chromatin is heterogeneously granular. No acrosome is present anterior to the nucleus. At the neck of the flagellum (f) the necklace is composed of three electron-dense particles (arrowheads). The flagellum is transversely sectioned at this level and shows Y-shaped bridges, which link the doublets to the flagellar membrane (see Fig. K). m: mitochondrion; pc: proximal centriole. X 37,600.

Figures C--E. Longitudinal sections (three different rotations of the spermatozoon) of the basal diplosome ultrastructure. **C.** The proximal centriole (pc) is perpendicular to the distal centriole (dc), the latter forming the basal body (bb) in the same longitudinal axis as the axoneme which arises from it. The pericentriolar matrix, embedded in an electron-dense material, surrounds the anterior end of the distal centriole. A fibrous body (arrows) consisting of osmiophilic disks alternating with lighter material adheres to the two centrioles laterally.Fibers connect the proximal and distal centrioles and also link the proximal centriole with the dense bodies (db) in the the central nuclear fossa. The alar sheets (as) originate at the distal centriole and fuse with the plasma membrane (pm). In a cross-section the basal body resembles a cartwheel with nine alar sheets extending from the triplets to the cell surface (see Fig. J). Considerable amounts of cytoplasm (cp) are present adjacent to the mitochondrial ring. f:flagellum. X54,500. **D.** Fibers (arrows) link diplosome and the dense bodies (db) at the base of the central nuclear fossa. The distal centriole (dc), which forms the basal body of the axoneme, consists of nine triplets of microtubules and the two outermost microtubules (arrowhead) elongated to form the doublets of the axoneme in flagellum (f). m: mitochondrion; f:flagellum. X 54,500. **E.** Microtubules present near the distal centriole (dc). m: mitochondrion; n: nucleus; pc: proximal centriole. X 59,000.

Figures F--Q. Successive, anteroposterior, transverse sections of spermatozoon of *Oncorhynchus masou formosanus* **F.** Cross section at the level of the proximal

centriole (pc). Along the centriole there is a dense body (db). X 40,000. **G.** A more posterior transverse section through the region of proximal centriole (pc). X 50,000. **H.** The distal centriole (dc) at the level of the osmiophilic ring showing the classic 9 + 0 microtubular triplet construction. The triplets are often obscured by the osmiophilic ring. X 72,500. **I.** The middle region of the distal centriole (dc). All nine of the outermost microtubules of the triplets of the distal centriole disappear proceeding posteriorly. A fine fibre connects A-subtubule of the neighboring triplets (arrow) and forms an inner ring. X 72,500. **J.** The basal region of the distal centriole (dc). The alar sheets (as) radiate from the distal centriole. X 70,000. **K.** The transitional region of the flagellum (f) showing the 9 + 0 microtubular doublet construction. Each doublet is connected to the plasma membrane by a Y-shaped link (Y). cc: cytoplasmic canal. X 43,500. **L.** The mitochondria (m) are fused to to form a helical mitochondrion, which surrounds the cytoplasmic canal (cc). X 58,700. **M.** The mitochondrion (m), bounded by an outer and inner membrane, exhibits transverse cristae (c). Arrowhead indicates one membrane in vicinity to the plasma membrane. Cytoplasma (cp) is visible adjacent to the mitochondrial ring. cc: cytoplasmic canal. X 68,500. **N--Q.** The flagellum has an axoneme with the conventional 9 + 2 microtubular pattern: the central pair of microtubules is surrounded by nine peripheral doublets. The central tubules are surrounded by a sheath and appear to be connected (arrow). Structures similar to radial spokes are just visible. No intratubular differentiation was observed in the flagellum. The plasma membrane, around the axoneme along the main part of the spermatozoan flagellum, starts closely apposed to the axoneme (Figs. L and M); first one and then a second lateral extensions (l) appear behind the midpiece (Figs. N--R). The membranous lateral extensions are filled with fine-granular material and do not contain any skeletal structures. A: subfiber A of the doublet. **N.** X 51,400, **O.** X 51,400, **P.** X 64,000. **Q.** X66,500. **R.** Scanning electron microscopy view of a spermatozoon. The lateral extension (sidefin; arrows) is evident. h: head; f:flagellum. X 84,000. Scales = 0.2 μm in Figures A--R.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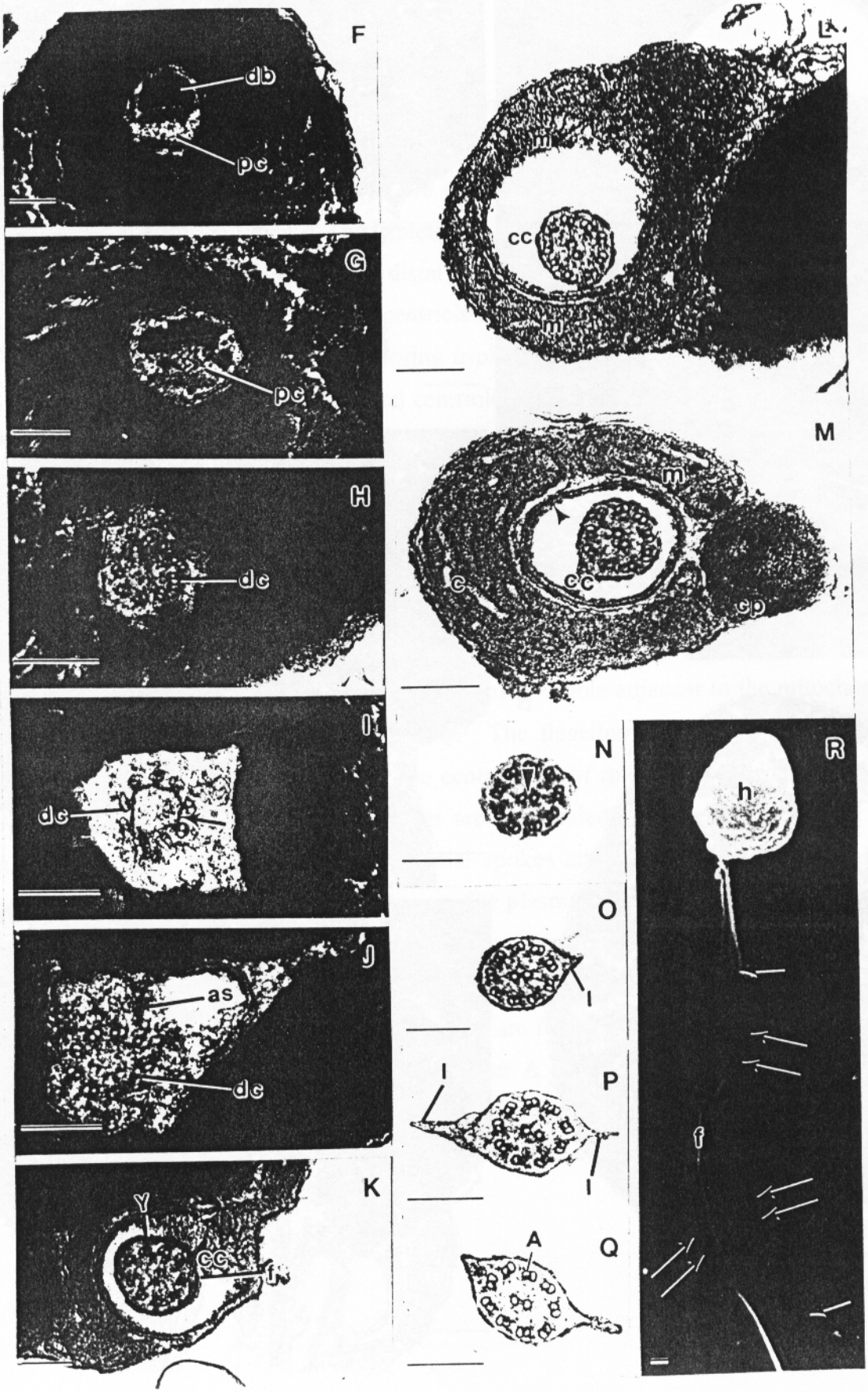
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