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Spermatozoal Ultrastructure and Phylogenetic Relationships of the Subfamily Gobioninae (Cyprinidae, Teleostei)

1. Ultrastructure of the Spermatozoa of the Korean Slender Gudgeon *Squalidus gracilis majimae*

Young Hwan Lee and Kgu Hwan Kim
Department of Biology Education, Taegu University, Kyungsan 712-714, Korea

한국산 모샘치아과(잉어과, 경골어강) 어류 정자의 미세구조와 계통학적 연구

1. 긴몰개 *Squalidus gracilis majimae* 정자의 미세구조

이 영 환 · 김 구 환
대구대학교 사범대학 생물교육과
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요 약

모샘치아과의 한국고유종인 긴몰개(Squalidus gracilis majimae)의 정자는 구형의 두부와 짧은 중편 및 긴 편모를 가지고 있으며 그 길이는 36.6 μm이었다. 두부에는 다른 경골어류에서와 같이 첨체를 가지고 있지 않으며 핵은 1.9 μm의 직경을 가지고 핵막 주변부에는 전자밀도가 낮은 염색질 부위를 포함하고 있었다. 핵와는 다른 잉어류에서와 같이 핵막이 얕게 함입되어 기부중심립만을 포함하고 있어서, 메기류의 기부 및 말단부 중심립을 포함하는 긴 핵와와는 큰 차이를 보여주었다. 두 중심립은 서로 약 140°의 각도로 배열되어 있었다. 미토콘드리아는 중편세포질에 3층으로 배열되어 있으나 축사를 둘러싸고 있지 않으며 축사와는 세포질관에 의해 분리되어 있었다. 정자의 꼬리에 대한 핵의 위치와 미토콘드리아의 비대칭적 배열은 잉어과 정자의 공통된 특징으로 나타나며, 미토콘드리아가 분포된 중편세포질은 다른 잉어류에서보다 훨씬 풍부하였다. 긴몰개의 정자는 현재까지 밝혀진 잉어과의 정자중에서 세포질관의 길이가 가장 길고, 미토콘드리아의 수도 가장 많이 나타났다. 편모에는 lateral fins가 관찰되지 않았다.

Key words : Ultrastructure, Anacrosomal aquasperm, Mitochondria, Asymmetry, *Squalidus gracilis majimae*

INTRODUCTION

Fishes present great spermatic diversity and different evolutionary tendencies within the fishes. Sperms of the Neopterygii, although greatly diversified, possess a common character which distinguishes them from the other fishes and even from the Vertebrates as a whole; they

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lack an acrosome (Mattei, 1988, 1991).

The ultrastructure of the spermatozoa has been extensively investigated in about 300 species of teleost fishes (Billard, 1970; Mattei and Mattei, 1976; Jamieson, 1991; Mattei, 1991) and has recently served as a criterion for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991). However, only several species of Cyprinidae have been examined for spermatozoal ultrastructure (Billard, 1970; Fribourgh *et al.*, 1970; Kudo, 1980; Stein, 1981; Kessel *et al.*, 1983; Ohta and Iwamatsu, 1983; Baccetti *et al.*, 1984; Guan, 1990; Guan and Afzelius, 1991; Ohta, 1991).

Cypriniformes have simple anacrosomal aquasperm and are characterized by a spherical head eccentrically placed on the tail, two variously oriented centrioles, and the tail of moderlate length with no lateral fins (Franzén, 1956; Baccetti and Afzelius, 1976; Baccetti *et al.*, 1984; Jamieson, 1991; Gwo *et al.*, 1995). All genera of Gobioninae except for *Gobio*, are restricted to eastern Asia including Korea (Nelson, 1994). They belong to Cyprinidae and contain the most Korean traditional species among freshwater teleost fishes (Kang and Kim, 1993). However, nothing has been reported on the fine structure of spermatozoa in Korean traditional cyprinids.

In the present study we examined the fine structure of the spermatozoa of the Korean slender gudgeon, *Squalidus gracilis majimae* and compared it with those of cyprinids. Further elucidation on the phylogeny and ultrastructural details of the speramtozoa in Gobioninae will be presented in the near future.

MATERIALS AND METHODS

Adult *Squalidus gracilis majimae* were collected during the breeding season in Naktong river, Korea, and kept in a controlled environment. For the experiment, mature spermatozoa were obtained by pressing both sides of the abdomen and kept in physiological saline in a small petri dish. Part of the material was examined and photographed with a phase contrast microscope. For transmission electron microscopy (TEM), semen and pieces of testis were dissected and fixed in 2.5~5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in the same buffer. They were then dehydrated in a graded ethanol series and embedded in Epon 812. The samples were sectioned on a LKB ultramicrotome, stained with 4% aqueous uranyl acetate, post-stained with lead citrate and examined with a Hitachi H-600 electron microscope.

For scanning electron microscopy (SEM), testes were fixed and dehydrated using the same procedures as for TEM. They were followed by isoamylacetate and subjected to critical point dryer. They were coated with gold by ion-sputter and examined and photographed with a scanning electron microscope.

RESULTS

The spermatozoon of *Squalidus gracilis majimae* is approximately 36.6 μm long and composed of a spherical head, a short midpiece and a tail region. There is thus no acrosome. The spermatozoon has the lateral implantation of the flagellum and its sperm tail attached obliquely to head (Fig. 1).

1. Head

The spherical nucleus of 1.9 μm in diameter is eccentrically positioned on the tail axis (Figs. 1, 2). The chromatin is highly electron-dense and

homogeneous, and contains vacuoles of various sizes (Fig. 3). The nucleus is characterized by electron lucent areas of the chromatin material, which are usually in the periphery of the nucleus (Fig. 4) and sometimes in the vicinity of the nuclear fossa (Fig. 5). This electron lucent chromatin is still in the mature spermatozoon. No acrosomal complex or vesicles are present anteriorly to the nucleus, where the undulating nuclear envelope is always in close contact with plasma membrane (Fig. 3). Mediolaterally the nucleus has an invagination, the nuclear fossa, where the proximal centriole is located (Fig. 5).

2. Centriolar complex

The proximal centriole has orientation of 140° with respect to the distal centriole appearing tangential to the nucleus. The proximal centriole lies in the shallow nuclear fossa. The distal centriole is parallel to the main axis of the tail and posteriorly extends to the level of the anterior end of the cytoplasmic canal (Fig. 5). Both centrioles have a conventional 9+0 microtubular triplet construction (Fig. 6). A few satellite fibers project from the centriole to the nuclear membrane.

3. Midpiece

The midpiece has the shape of an asymmetrical truncated cone and is pervaded sagittally by the cytoplasmic canal, an invagination of the plasma membrane. Mitochondria of 10 or more are arranged in three layers (Fig. 7) and are asymmetrically located in the postnuclear cytoplasm (Figs. 7-9). The postnuclear cytoplasm is abundant and of moderate extension, so that the cytoplasmic canal is approximately 2.0 μm deep. The mitochondria have loose matrix, and several simple cristae of irregular layers which are sometimes difficult to distinguish (Fig. 7). The mitochondria do not encircle the axoneme, most of which are in fact located in the abundant side of the postnuclear cytoplasm (Figs. 8, 9).

4. Tail

The sperm tail is approximately 34.6 μm long. The flagellum projects from the distal centriole and has the classic 9+2 microtubular doublet construction of axoneme (Fig. 10). The two central microtubules are surrounded by the central sheet which is interconnected with the peripheral microtubules by the centrifugal extending radiations. There are clear vesicles in cytoplasm under the plasma membrane of the anterior part of the tail (Figs. 3, 10). However, they are not observed in the posterior part of the tail, where the undulated plasma membrane is close to the axoneme (Fig. 5). The lateral fins are not present in the flagellum.

DISCUSSION

The ultrastructure of spermatozoa of *S. gracilis majimae* reveals some characteristics, typical of cyprinid spermatozoa. Cyprinid spermatozoa are characterized by a spherical nucleus with the shallow nuclear fossa, a midpiece containing mitochondria, a flagellum positioned tangentially to the nucleus, and no acrosome (Gwo *et al.*, 1995). However, the spermatozoa of *S. gracilis majimae* show significant differences of nuclear chromatin, the position of the proximal and the distal centrioles, the number of mitochondria, and the depth of the cytoplasmic canal, from those of the other cyprinids.

The nucleus of *S. gracilis majimae* is spherical and its diameter is about 2 μm as in most of the cyprinid species (Baccetti *et al.*, 1984). The nucleus is eccentrically positioned on the sperm tail

like most cyprinids. Electron-lucent areas of the chromatin appear in the periphery of the nucleus and are distinguished from the electron-dense chromatin occupying most of the nucleus. This light chromatin still remains in the nucleus of the mature spermatozoa. It is difficult to discern any evidence of the condensation processes of the nucleus.

The nuclear fossa is not deep in Cypriniformes, unlike Siluriformes with deep nuclear fossa containing the centriolar complex (Mattei, 1970; Jaspers *et al.*, 1976; Poirier and Nicholson, 1982; Emel'yanova and Makeyeva, 1991a, b; Lee, 1997). The shallow nuclear fossa of *S. gracilis majimae* containing only the proximal centriole is also found in other cyprinids. Jamieson (1991) suggested that the deep nuclear fossa is apomorphic as compared with the shallow nuclear fossa.

Two centrioles vary considerably in their relative position in teleost spermatozoa. In cyprinids the orientation of the centrioles is variable from 40° to 140°. The proximal centriole is perpendicular to the distal centriole only in *Alburnus* and *Barbus* of cyprinids. Jamieson (1991) reported that the proximal centriole perpendicular to distal centriole is a plesiomorphic feature in fish spermatozoa. Baccetti *et al.* (1984) reported in seven cyprinid species that the position of the nucleus with respect to the axis of the tail is correlated to the arrangement of centrioles. In *Liocassius ussuriensis* and *Clarias senegalensis* of siluroids two centrioles form an obtuse angle (Mattei, 1970) as in most cyprinids but the nucleus is parallel to the sperm tail unlike cyprinids having the sperm tail positioned tangentially to the nucleus. The correlation of the nuclear position and the centriolar arrangement in cyprinids is not shown in siluroids.

The number of mitochondria in *S. gracilis majimae* spermatozoon is 10 or more and located in the postnuclear cytoplasm. In cyprinid species they never fuse to form a mitochondrial derivative and their number varies from 1 in *Rhodeus ocellatus ocellatus* (Ohta and Iwamatsu, 1983; Ohta, 1991) and *Rhodeus sericeus sinesis* (Guan and Afzelius, 1991) to 10 in *Carassius auratus* (Baccetti *et al.*, 1984), with a higher frequency of 3 to 4. The mitochondrial number in the spermatozoon is the only character closely linked with phylogeny (Baccetti *et al.*, 1984). *S. gracilis majimae* spermatozoa have the most mitochondrial number and the deepest postnuclear canal among cyprinid species examined to date. Baccetti *et al.* (1984) suggested in cyprinid spermatozoa that the number and size of mitochondria determine the depth of the cytoplasmic canal.

The mitochondria of *S. gracilis majimae* are asymmetrically distributed in the area adjacent to the nucleus and do not surround the axoneme. This asymmetrical distribution of the mitochondria is the general pattern of cyprinid spermatozoa.

All cyprinid sperm examined to date lack the one or more lateral fins seen in the flagellum of most teleost sperm. The absence of the lateral fins in cyprinids is an apomorphic loss and an ostariophysian synapomorphy (Jamieson, 1991).

ABSTRACT

The spermatozoon of *Squalidus gracilis majimae* is approximately 36.6 μm in length and is characterized by a spherical nucleus with the clear chromatin, a short midpiece containing the mitochondria, and a flagellum positioned tangentially to the nucleus. An acrosome is absent as in all teleost fishes. The nucleus is about 1.9 μm in diameter and in its periphery contains the

electron-lucent chromatin distinguished from the electron-dense chromatin occupying most of the nucleus. The shallow nuclear fossa contains the proximal centriole, instead of two centrioles in deep nuclear fossa in siluroids. The proximal and distal centrioles are oriented approximately 140° to each other. The mitochondria of 10 or more in number are arranged in three layers and do not surround the axoneme. The asymmetrical distribution of the mitochondria and the eccentrical position of the nucleus with regard to the tail are the general pattern of the cyprinid spermatozoa. *S. gracilis majimae* spermatozoa have the most mitochondria and the deepest cytoplasmic canal among cyprinid species. The flagellum lacks the lateral fins.

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FIGURE LEGENDS

Fig. 1. Scanning electron micrograph of two spermatozoa showing the lateral implantation of the flagellum with respect to the nucleus.

Fig. 2. Scanning electron micrograph of a spermatozoon showing a spherical head (H), a short midpiece (Mi) and a tail (T).

Fig. 3. Longitudinal section through a spermatozoon showing the nucleus (N) containing nuclear vacuoles, the deep cytoplasmic canal (CC) and clear vesicles (V) in cytoplasm under the plasma membrane of the anterior part of the tail. Note the absence of acrosome and that the undulating nuclear envelope (arrows) is in close contact with plasma membrane at the anterior part of the nucleus.

Fig. 4. Transverse section through the nucleus showing an electron-lucent area (arrow) of the chromatin in its periphery.

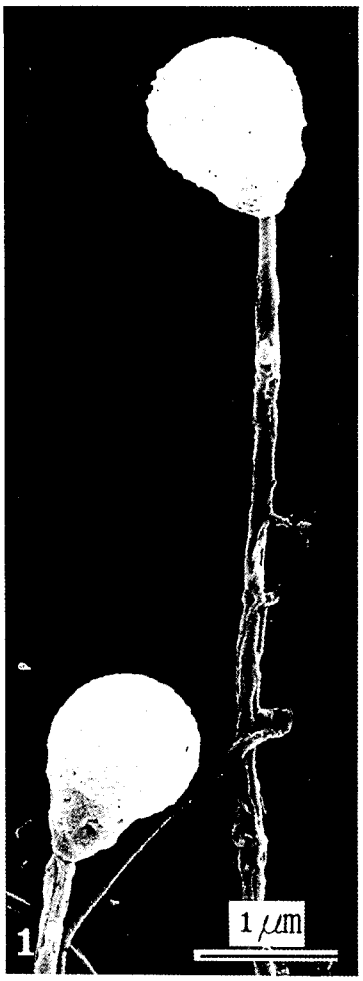
Fig. 5. Longitudinal section through the spermatozoon showing the shallow nuclear fossa containing the proximal centriole (PC) and two centrioles having the orientation of 140°. Note an electron-lucent chromatin (arrow) near the nuclear fossa and the undulating plasma membrane (arrowheads) surrounding the axoneme (Ax) in the posterior part of the sperm tail.

Fig. 6. Transverse section of the proximal centriole (PC) showing nine triplets. Note a few satellite fibers (arrows) between centrioles and nuclear membrane.

Figs. 7, 8. Longitudinal sections through the spermatozoa showing that the mitochondria (M) of two or three layers around the axoneme (Ax) are asymmetrically located in the postnuclear cytoplasm (PNC). Note 10 or 11 mitochondria which are distributed asymmetrically and having have loose matrix and simple cristae which are difficult to distinguish.

Fig. 9. Transverse section through the midpiece showing that the mitochondria are also asymmetrically distributed and do not surround the axoneme (Ax).

Fig. 10. Transverse section of the anterior part of the sperm tail containing vesicles (V) in cytoplasm surrounding the axoneme. Note the absence of lateral fins in the flagellum.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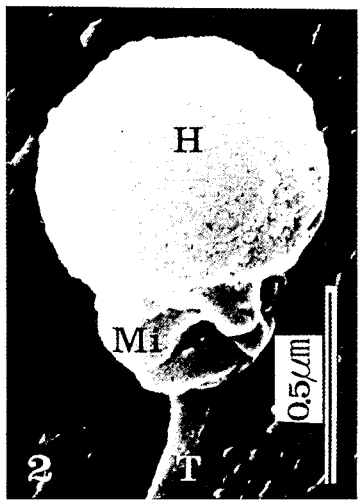
H
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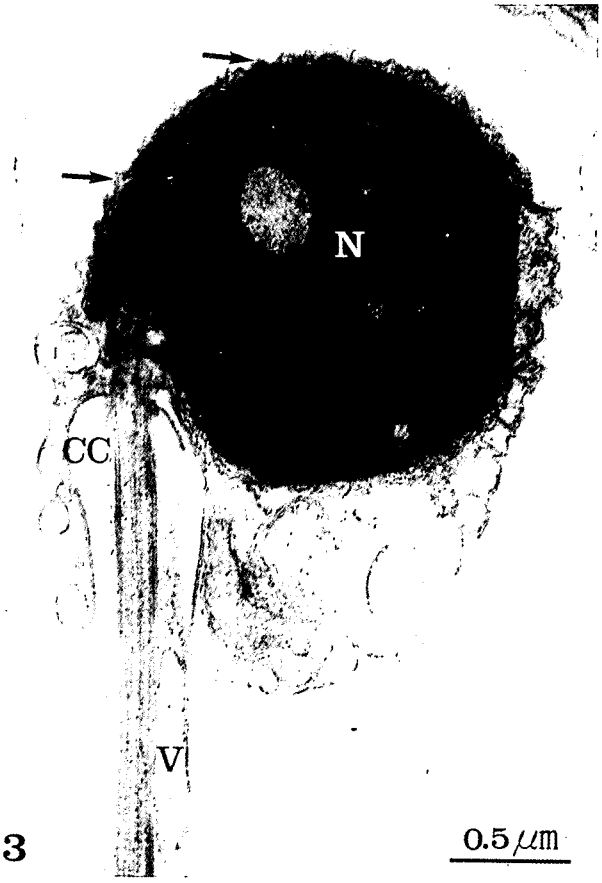
N
CC
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3
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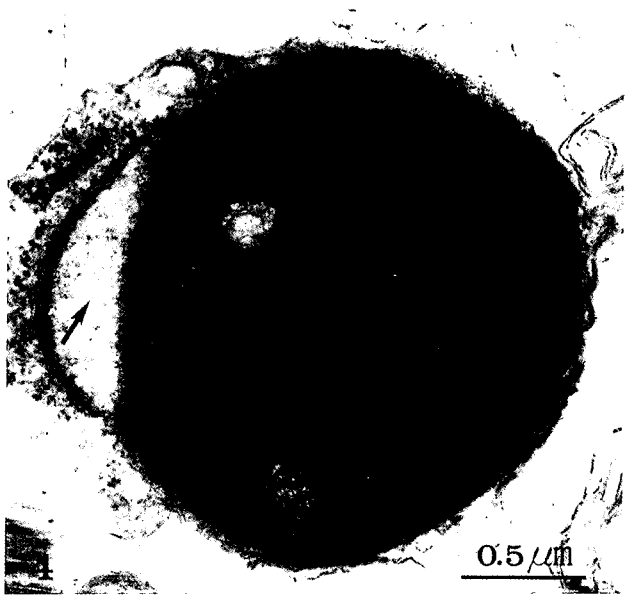
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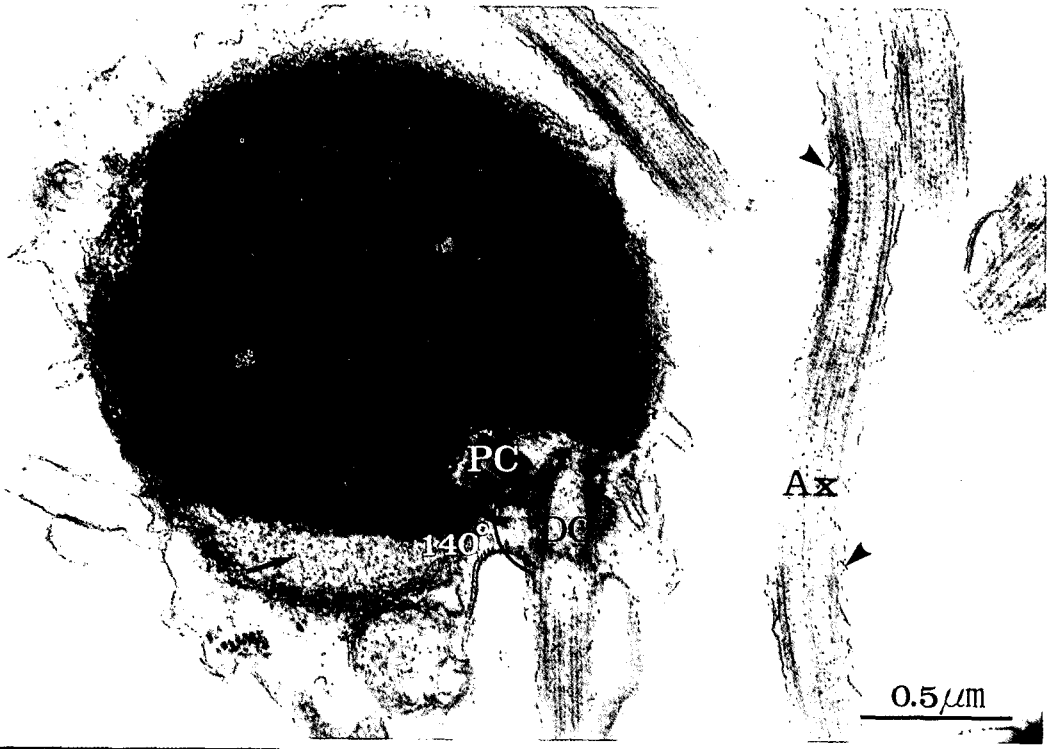

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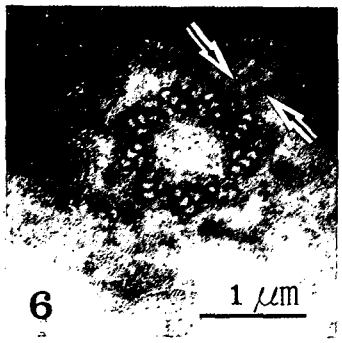

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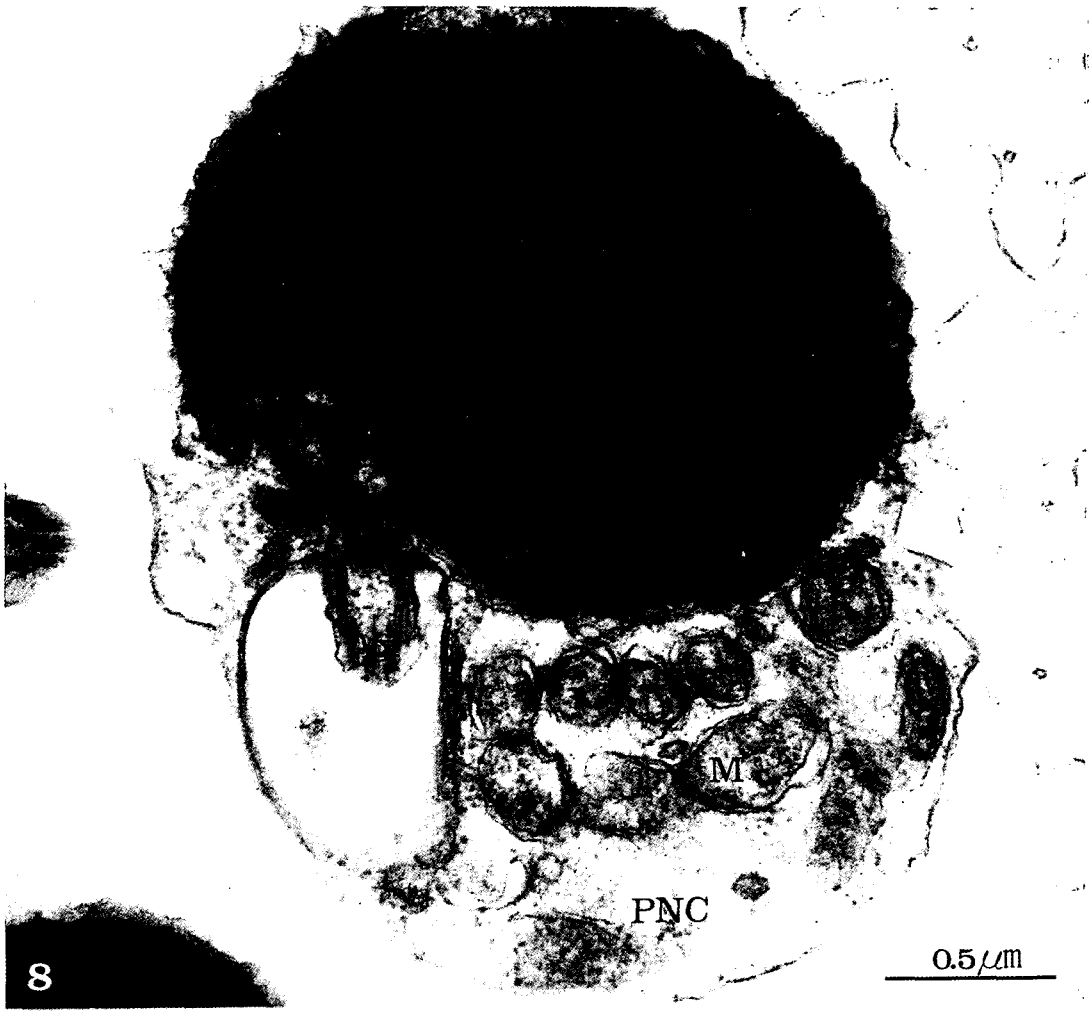

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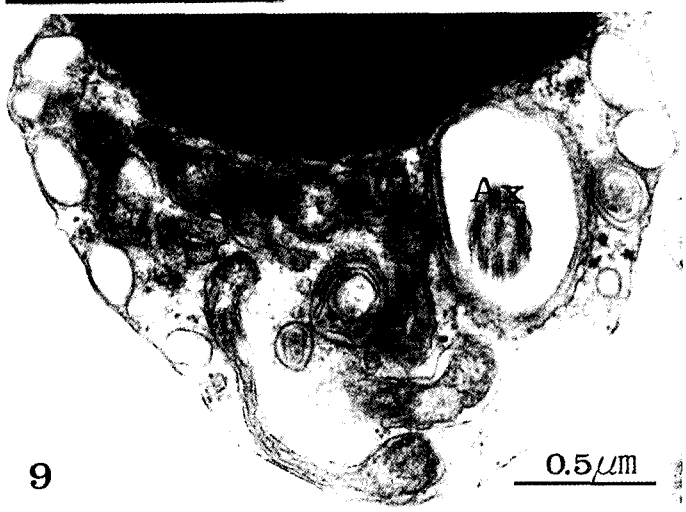

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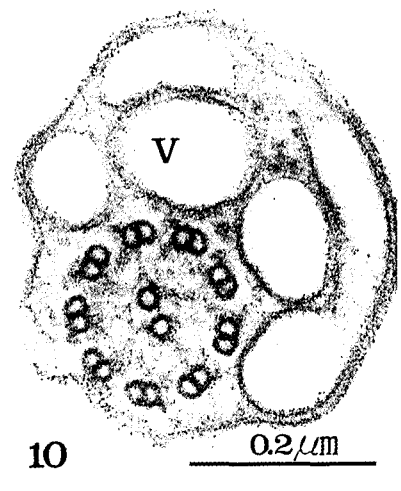

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