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ULTRASTRUCTURE OF SPERMATOZOA IN TWO MONOPISTHOCOTYLEAN MONOGENEANS: *ENCOTYLLABE* SP. (CAPSALIDAE) AND *TETRAONCHOIDES* SP. (TETRAONCHOIDIDAE)

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SUMMARY

Spermatozoa of *Encotyllabe* sp. (a capsalid monogenean parasitic in *Plectorhinchus mediterraneus* in Senegal) have two 9 + « 1 » axonemes and no cortical microtubules. Thus, they belong to sperm pattern 2 as defined by Justine, Lambert and Mattei (1985). The two synapomorphies for the capsalids and dionchids defined on the basis of spermiogenesis ultrastructure (a progressive loss of cortical microtubules in the zone of differentiation, and a bead-like mitochondrion through which the nucleus passes) are present in *Encotyllabe.* The mitochondrion is first pear-shaped and then bead-like. A single cortical microtubule is found in some rare sections of spermatids. Spermatozoa of *Tetraonchoides* sp. (a tetraonchoidid monogenean parasitic in *Uranoscopus* sp. in Senegal) are described here from preliminary observations. They possess one single axoneme and no cortical microtubule, and thus belong to sperm pattern 4 of Justine *et al.* (1985). The axoneme is terminated by isolated doublets without a central element. The presence of sperm pattern 4 in the Tetraonchoididae indicates close relationships with the other families having this pattern; this is consistent with classifications based on classical morphology.

RÉSUMÉ : **Ultrastructure des spermatozoïdes de deux Monogènes Monopisthocotylea : *Encotyllabe* sp. (Capsalidae) et *Tetraonchoides* sp. (Tetraonchoididae).**

Le spermatozoïde mûr de *Encotyllabe* sp. (Monogène Capsalidae, parasite de *Plectorhinchus mediterraneus* au Sénégal) possède deux axonèmes et aucun microtubule cortical. Il appartient donc au type 2 défini par Justine, Lambert et Mattei (1985). Les deux synapomorphies de la spermiogenèse définies auparavant pour l'ensemble Capsalidae + Dionchidae (disparition progressive des microtubules de la zone de différenciation et présence d'une grosse mitochondrie en forme de perle au travers de laquelle passe le noyau) sont retrouvées chez *Encotyllabe.* La mitochondrie adopte une forme en poire puis en perle au cours de la spermiogenèse. Un microtubule unique est trouvé dans quelques rares coupes de spermatides. Le spermatozoïde de *Tetraonchoides* sp. (Monogène Tetraonchoididae, parasite de *Uranoscopus* sp. au Sénégal), décrit ici à partir d'observations préliminaires, possède un seul axonème et aucun microtubule cortical, et appartient donc au type 4 de Justine *et al.* (1985). L'axonème se termine par des doublets isolés sans élément central. La présence du type 4 de spermatozoïdes chez les Tetraonchoididae indique des relations phylétiques proches avec les autres familles possédant ce type; ceci est cohérent avec les classifications basées sur la morphologie classique.

INTRODUCTION

In the parasitic Platyhelminthes or Cercomeridea (= Trematoda + Monogenea + Gyrocotylidea + Amphilinidea + Eucestoda), spermatozoon ultrastructure is relatively homogeneous and spermiogenesis is characteristic (Justine, 1991*a*). However, in the Monogenea, much variation exists, and this was used for phylogenetic studies. The monopisthocotylean monogenea show great variations, while the polyopisthocotylean are more homogeneous (Justine, Lambert and Mattei, 1985). A review (Justine, 1991*b*) and several studies (Malmberg and Afzelius, 1990; Tappenden and Kearn, 1990, 1991*a, b*; Schmahl and Obiekezie, 1991) have recently been published on monogenean spermatozoa.

In the family Capsalidae, spermatozoa were described in *Trochopus pini* (Tuzet and Ktari, 1971), *Megalocotyle hexacantha* (Justine *et al.*, 1985; Justine and Mattei, 1987), *Entobdella hipoglossi* and *E. soleae* (Tappenden and Kearn, 1991*b*) *M. grandiloba* (Justine and Mattei, 1983; Justine, 1983), and *Caballerocotyla* sp. (Justine and Mattei, 1987). Spermiogenesis was studied in the last three species. The present paper is the first spermatozoon description for *Encotyllabe* sp., but data on this material were previously used in a cladistic analysis (Justine, 1991*b*).

In the family Tetraonchoididae, the spermatozoon was

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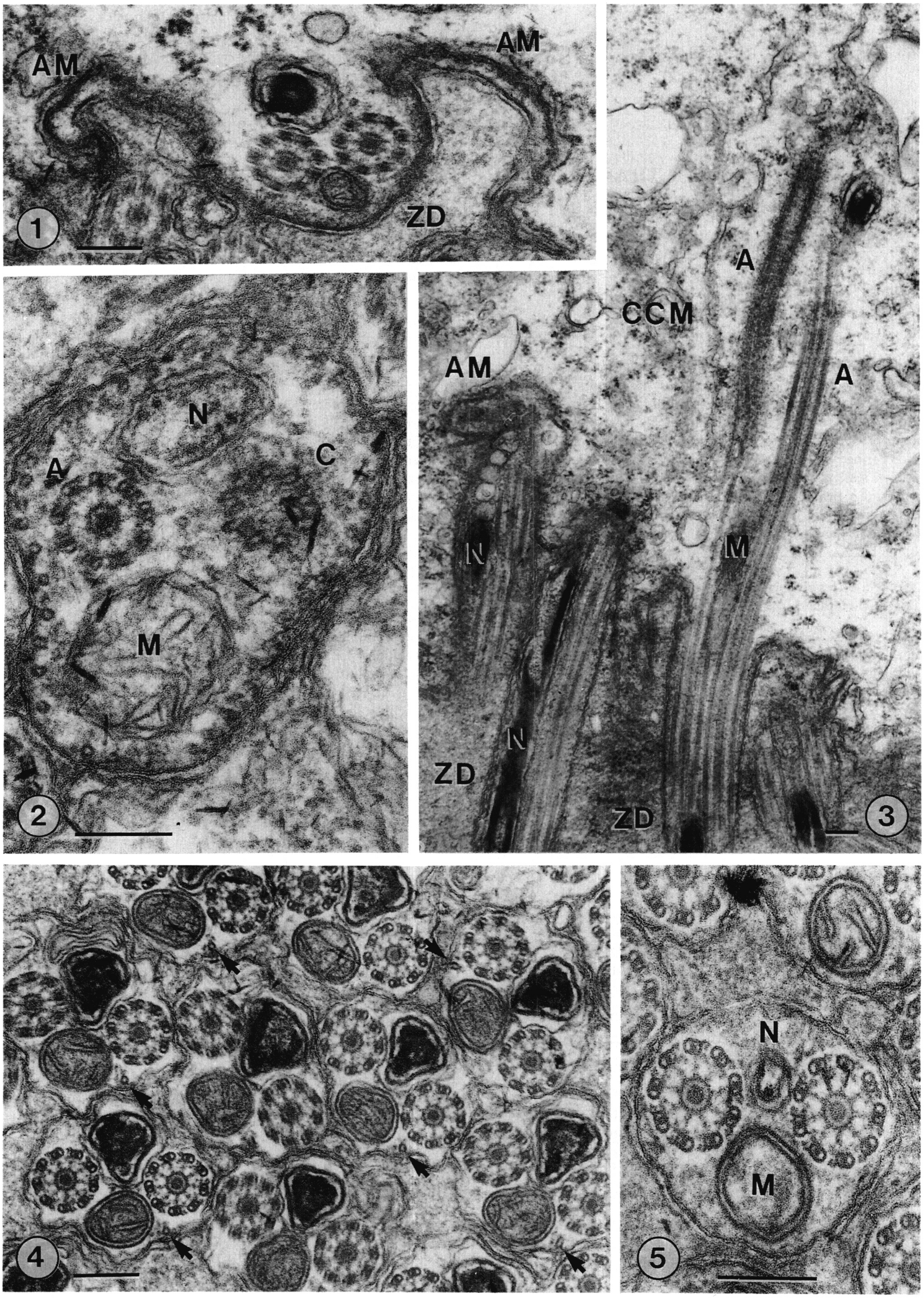

PLANCHE 1.

not described in any species and thus we found it useful to publish some preliminary observations on *Tetraonchoides* sp.

MATERIAL AND METHODS

The monogeneans were collected from fishes caught near Dakar, Senegal. Two individuals of *Encotyllabe* sp. were collected in a *Plectorhinchus mediterraneus;* one was used for ultrastructural study, and one was used for taxonomy but unfortunately was lost, thus prohibiting a specific identification. Several individuals of *Tetraonchoides* sp. were collected from *Uranoscopus* sp; their species identification is under study. The worms were processed for electron microscopy according to a routine glutaraldehyde-osmium tetroxide method (Justine and Mattei, 1983).

OBSERVATIONS

Spermiogenesis of *Encotyllabe* sp.

The spermatids are fused into a common cytoplasmic mass, and the zones of differentiation protrude at the periphery *(figs. 1, 3)*. At the onset of spermiogenesis, the zones of differentiation show a cortical row of longitudinal microtubules *(fig. 2)*. The nucleus and mitochondrion migrate into the zone of differentiation *(figs. 1-3)*. The cortical microtubule row later disappears and these microtubules are no longer found in the spermatids *(fig. 5)*. However, some rare transverse sections of spermatids in the testis *(fig. 4)* show a single cytoplasmic microtubule, located near the mitochondrion on the ventral face of the spermatozoon (according to the convention of Sato, Oh and Sakoda, 1967), on the left or right side of the mitochondrion.

During spermiogenesis a large mitochondrion, located in the common cytoplasmic mass, corresponds to each zone of differentiation and a long streamer comes from this mitochondrion and migrates into the zone of differentiation. The shape of this mitochondrion was described as bead-like in other species of the family Capsalidae (Justine and Mattei, 1983, 1987). The present observations in *Encotyllabe* allow a preciser description. Longitudinal sections of the mitochondrion (i.e. oriented along the axis of migration of the nucleus and mitochondrial streamer) show a pear-like shape, with the point directed toward the zone of differentiation *(fig. 6)*. At this stage *(fig. 7)* transverse sections of the mitochondrion are ring-shaped, with ribosome-rich cytoplasm in the middle of the ring. The nucleus migrates through the mitochondrion via a canal, and then continues its migration toward the zone of differentiation. This nuclear canal does not coincide with the cytoplasm encircled by the mitochondrion *(figs. 8, 9)*. Later in spermiogenesis *(figs. 10-11)* , the volume of the mitochondrion lessens since a lot of it was incorporated into the zone of differentiation during migration of the mitochondrial streamer. At this stage, most transverse sections of the mitochondrion are ring-shaped with a smaller diameter than before and show a section of the nucleus in the middle *(fig. 11)*; some sections still encircle some cytoplasm *(fig. 10)*.

Spermatozoa of *Tetraonchoides* sp.

Preliminary observations on this species deal only with mature spermatozoa. Transverse sections of spermatozoa in the seminal vesicle show one single axoneme, a mitochondrion and a nucleus *(fig. 12)*. The axoneme shows the 9 + « 1 » structure of the Trepaxonemata which is universal in the Monogenea, but the central element seems less electron-dense than usual for other species. Transverse sections of the axoneme extremity show peripheral doublets and no central element. These eight or nine peripheral doublets, located along the nucleus, are not organized into a circle. In more distal sections some of the doublets are simplified into singlets. This extremity of the spermatozoon, with an axoneme made up of randomly arranged doublets and singlets without a central element, is interpreted as posterior.

DISCUSSION

Encotyllabe sp.

Spermiogenesis in this species follows the scheme described in the capsalids (Justine and Mattei, 1983, 1987; Justine, 1983; Tappenden and Kearn, 1991*b*) and which is also found in the dionchid *Dionchus remorae* (Justine *et al.*, 1985; Justine and Mattei, 1987), in which the process of fertilization is also known (Justine and Mattei, 1986).

Figs. 1-5. — *Encotyllabe* sp. : spermatids in testis. A, axoneme; AM, arching membranes; CCM, common cytoplasmic mass; M, mitochondrion; N, nucleus; ZD, zones of differentiation. Bars = 200 nm.

Fig. 1. — Transverse section of a zone of differentiation, laterally protruding from the common cytoplasmic mass of the fused spermatids.

Fig. 2. — Transverse section of an early zone of differentiation, showing a continuous row of cortical longitudinal microtubules. C, centriole.

Fig. 3. — Longitudinal section of zones of differentiation protruding from the common cytoplasmic mass.

Fig. 4. — Transverse section of spermatids showing a single cortical longitudinal microtubule (arrow).

Fig. 5. — Transverse section of spermatid without cortical microtubule.

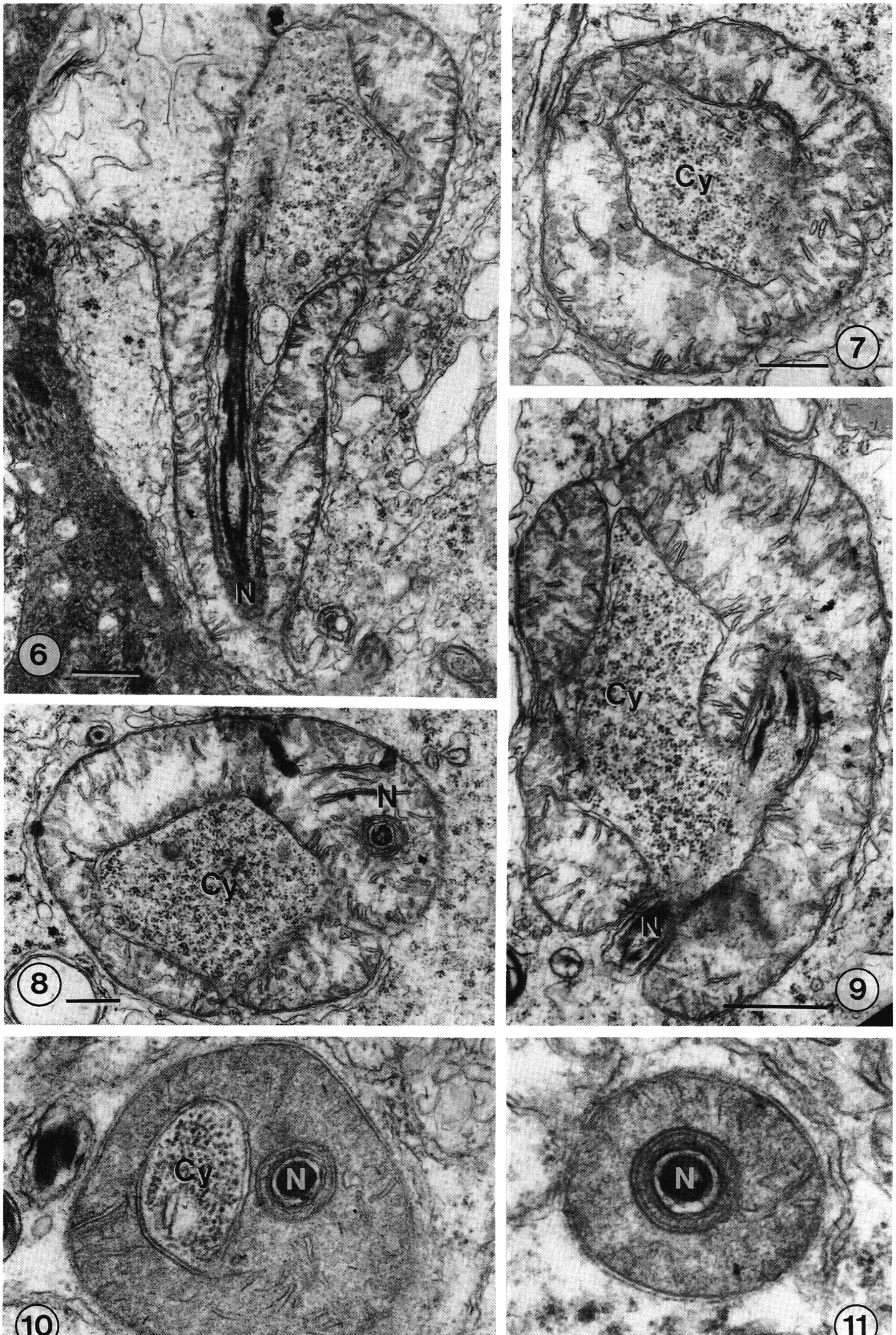

PLANCHE 2.

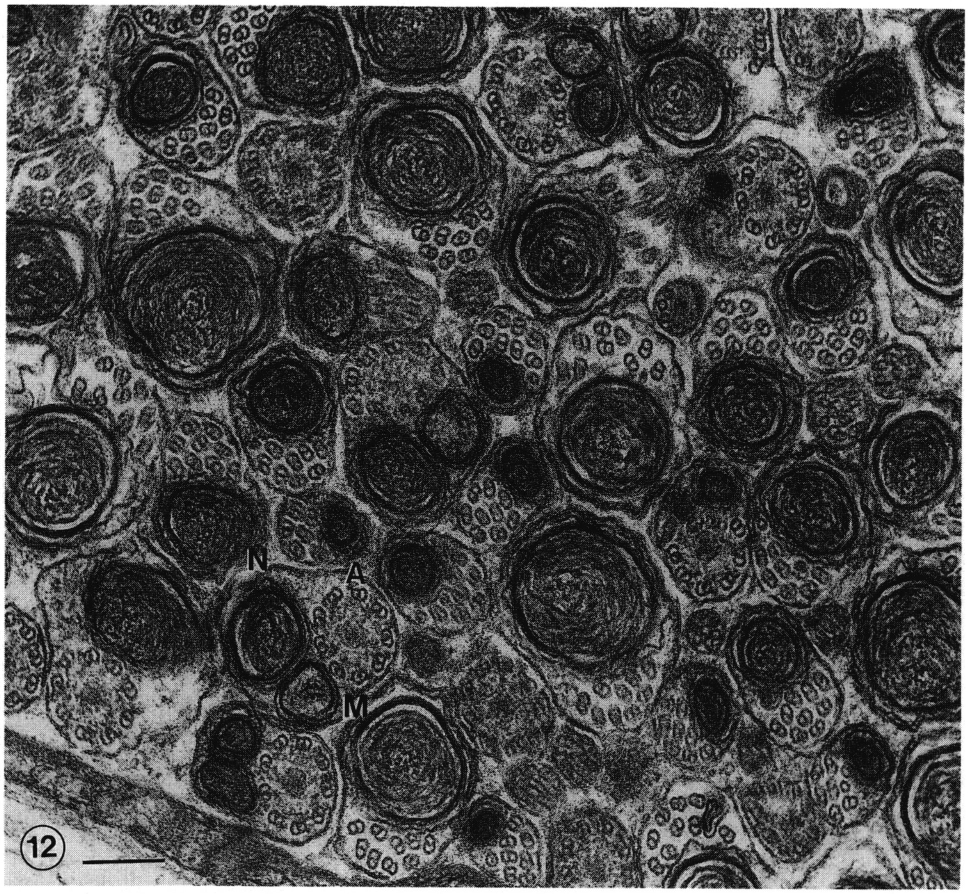

FIG. 12. — *Tetraonchoides* sp. Spermatozoa in the seminal vesicle. Note the presence of a single 9 + « 1 » axoneme (A), mitochondrion (M) and nucleus (N). Extremities show a disorganized axoneme made up of randomly arranged doublets and singlets without a central element. Bar = 200 nm.

Diagrams of spermiogenesis in the capsalids are found in previous publications (Justine and Mattei, 1983; Justine, 1983) and thus are not repeated here. From these descriptions, Justine and Mattei (1987) proposed two synapomorphies for the Capsalidae + Dionchidae defined on the basis of spermiogenesis: the bead-like shape of the mitochondrion, and the progressive loss of cortical microtubules in the zone of differentiation. These two synapomorphies are also found in *Encotyllabe.* A classification of monogenean spermatozoa into four patterns, based on the number of axonemes and the presence of cortical microtubules, was proposed by Justine *et al.* (1985). In this classification the spermatozoa of capsalids and dionchids, which have two axonemes and no cortical microtubules, belong to sperm pattern 2. Later (Justine, 1991*b*), sperm pattern 2 was divided into several subtypes. Subtype 2*a* includes spermatozoa with a spermiogenesis showing the two synapomorphies cited above, and hence *Encotyllabe* belongs to subtype 2*a*.

Ultrastructural details described here for the mitochondrion of *Encotyllabe* do not modify this classification, since a pear-shaped mitochondrion is simply considered as a minor variation from the bead-like model.

The presence of a single cortical microtubule in some sections of spermatids is here reported for the first time. A re-examination of unpublished photographs from the cap-

FIGS. 6-11. — *Encotyllabe* sp.: spermiogenesis, modifications in the mitochondrion. Cy, cytoplasm encircled by the mitochondrion; N, nucleus migrating via intramitochondrial canal. Bars = 400 nm.

Fig. 6. — Longitudinal section (oriented along the axis of nuclear migration) of a pear-shaped mitochondrion.

Fig. 7. — Transverse section (perpendicular to the axis of nuclear migration) of a mitochondrion showing ring shape and encircled cytoplasm.

Fig. 8. — Transverse section of mitochondrion showing encircled cytoplasm and a canal containing the migrating nucleus.

Fig. 9. — Oblique section of mitochondrion.

Figs. 10-11. — Transverse sections of mitochondrion in late spermatids.

salid *Megalocotyle grandiloba* spermiogenesis (described by Justine and Mattei, 1983) showed that some rare sections of spermatids also show this characteristic; *Encotyllabe* is thus not exceptional for this. Also, Malmerg and Afzelius (1990) described in the spermatids of *Myxinidocotyle californica* (Acanthocotylidae) the presence of one cortical microtubule, but in another acanthocotylid, *Acanthocotyle lobianchi,* Malmberg and Afzelius (1990) and Tappenden and Kearn (1990) did not mention the presence of a cortical microtubule. Unpublished observations on *A. lobianchi* and *My. californica* (Justine, Afzelius, Malmberg and Mattei, in preparation) show that a single microtubule may be found in some sections of spermatids. This single cortical microtubule in capsalid and acanthocotylid spermatids is probably very short (present in some rare sections only) and its presence in fully mature spermatozoa is uncertain. Thus, the definition of sperm pattern 2 (« mature spermatozoa with two axonemes and no cortical microtubules ») is provisionaly kept since the presence of the single microtubule in mature spermatozoa is not proved.

Tetraonchoides SP.

Preliminary observations reported here show that spermatozoa of *Tetraonchoides* have only a single axoneme. Spermatozoa of the Cercomeridea classically have two axonemes incorporated in the cytoplasm; some exceptions are known in the cestodes (Euzet, Swiderski and Mokhtar-Maamouri, 1981), the Digenea and the Monogenea (lists in Justine, 1991*a*). In the monopisthocotylean monogeneans, spermatozoa with a single axoneme were found in the pseudodactylogyrids, diplectanids, ancyrocephalids, calceostomatids, tetraonchids and amphibdellatids (list of species in Justine, 1991*b*). These spermatozoa belong to sperm pattern 4 (one axoneme, no cortical microtubules) defined by Justine *et al.* (1985). The family Tetraonchoididae, to which *Tetraonchoides* belongs, is thus the seventh in which this structure is found. This structure is interpreted as derived compared to the structure with two axonemes. In a cladistic analysis of the Monogenea, based on spermatozoon and spermiogenesis characteristics, Justine (1991*b*) proposed a new taxon Monoaxonematoidea in which all families with sperm pattern 4 are included. Inclusion of the family Tetraonchoididae in this taxon is consistent with Lebedev's classification (1988) in which the tetraonchoidids are included in the order Tetraonchidea with the amphibdellatids, tetraonchids and bothitrematids. However, sperm structure is still unknown in this last family.

A study of spermiogenesis in *Tetraonchoides* is necessary for phylogenetic analysis. The very atypical axoneme extremity with randomly arranged doublets could be proposed as an autapomorphy for the family Tetraonchoididae.

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REFERENCES

Euzet L., Swiderski Z., Mokhtar-Maamouri F. : Ultrastructure comparée du spermatozoïde des Cestodes. Relations avec la phylogénèse. *Ann. Parasitol. Hum. Comp.,* 1981, *56,* 247-259.

Justine J.-L. : A new look at Monogenea and Digenea spermatozoa. *In:* The Sperm Cell (André J., ed.) *Martinus Niijhoff,* The Hague, 1983, 454-457.

Justine J.-L., Mattei X. : Étude ultrastructurale comparée de la spermiogenèse des Monogènes. 1. *Megalocotyle* (Monopisthocotylea Capsalidae). *J. Ultrastruct. Res.,* 1983, *82,* 296-308.

Justine J.-L., Lambert A., Mattei X. : Spermatozoon ultrastructure and phylogenetic relationships in the monogeneans (Platyhelminthes). *Int. J. Parasitol.,* 1985, *15,* 601-608.

Justine J.-L., Mattei X. : Ultrastructural observations on fertilization in *Dionchus remorae* (Platyhelminthes, Monogenea, Dionchidae). *Acta Zool. (Stockholm),* 1986, *67,* 97-101.

Justine J.-L., Mattei X. : Phylogenetic relationships between the families Capsalidae and Dionchidae (Platyhelminthes, Monogenea, Monopisthocotylea) indicated by the comparative ultrastructural study of spermiogenesis. *Zool. Scripta.,* 1987, *16,* 111-116.

Justine J.-L. : Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Can. J. Zool.,* 1991*a, 69,* 1421-1440.

Justine J.-L. : Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. *Int. J. Parasitol.,* 1991*b,* sous presse.

Lebedev B. I. : Monogenea in the light of new evidence and their position among platyhelminths. *Ang. Parasitol.,* 1988, *29,* 149-167.

Malmberg G., Afzelius B. A. : Sperm ultrastructure in *Myxinidocotyle* and *Acanthocotyle* (Platyhelminthes, Monogenea, Acanthocotylidae). *Zool. Scripta.,* 1990, *19,* 129-132.

Sato M., Oh M., Sakoda K. : Electron microscopic study of spermatogenesis in the lung fluke *(Paragonimus miyazakii). Z. Zellforsch.,* 1967, *77,* 232-243.

Schmahl G., Obiekezie A. : Fine structure of spermatogenesis in polyopisthocotylid monogeneans *(Protomicrocotyle ivoriensis, Gastrocotyle* sp.*). Parasitol. Res.,* 1991, *77,* 115-122.

Tappenden T., Kearn G. C. : Spermiogenesis and sperm ultrastructure in the monogenean parasite *Acanthocotyle lobianchi. Int. J. Parasitol.,* 1990, *20,* 747-753.

Tappenden T., Kearn G. C. : Spermiogenesis and sperm ultrastructure in the monocotylid monogenean *Calicotyle kroyeri. Int. J. Parasitol.,* 1991*a, 21,* 57-63.

Tappenden T., Kearn G. C. : Crystalline bodies associated with spermatozoa in the vas deferens of the monogeneans *Entobdella soleae* and *E. hipoglossi. Parasitol. Res.,* 1991*b, 77,* 421-424.

Tuzet O., Ktari M.-H. : Recherches sur l'ultrastructure du spermatozoïde de quelques Monogènes. *Bull. Soc. Zool. Fr.,* 1971, *96,* 535-540.