ULTRASTRUCTURE OF SPERMIOGENESIS AND SPERMATOZOA IN *EUZETREMA KNOEPFFLERI* (PLATYHELMINTHES, MONOGENEA)

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Summary :
Euzetrema knoepffleri is a monogenean parasite in the relict urodele amphibian *Euproctus montanus* in the island of Corsica. Spermiogenesis was studied by electron microscopy. The spermatid has a zone of differentiation containing two parallel centrioles, an elongating nucleus and mitochondrion, and no peripheral microtubule. The spermatozoon is formed by a lengthening of the zone of differentiation. The mature spermatozoon contains two axonemes of the platyhelminth 9+"1" pattern, a mitochondrion, a nucleus, and no peripheral microtubule. The spermatozoon corresponds to type 2 in the classification of Justine, Lambert & Mattei (1985). This type 2 is characteristic of certain monopisthocotylean Monogenea. In the absence of supplementary apomorphies, it is not possible to specify the phylogenetic position of *Euzetrema* in the Monopisthocotylea from spermatozoal ultrastructure.

KEY WORDS : *Euzetrema knoepffleri*, ultrastructure, spermatozoon, phylogeny, Monogenea.
MOTS CLES **:** Euzetrema knoepffleri, *ultrastructure, spermatozoïde, phylogénie, Monogènes.*

Résumé : ULTRASTRUCTURE DE LA SPERMIOGENÈSE ET DU SPERMATOZOÏDE DE *EUZETREMA KNOEPFFLERI* (PLATHELMINTHES, MONOGENEA)

Euzetrema knoepffleri *est un monogène parasite de l'amphibien urodèle relicte* Euproctus montanus *en Corse. La spermiogenèse a été étudiée en microscopie électronique à transmission. La spermatide montre une zone de différenciation contenant deux centrioles parallèles, le noyau et la mitochondrie en élongation, et aucun microtubule périphérique. Le spermatozoïde se forme par allongement de la zone de différenciation. Le spermatozoïde mûr contient deux axonèmes du type 9+"1" de Plathelminthes, une mitochondrie, le noyau, et aucun microtubule périphérique. Le spermatozoïde correspond au type 2 de la classification des spermatozoïdes de Justine, Lambert & Mattei (1985). Ce type 2 est caractéristique de certains Monogènes Monopisthocotylea. En l'absence d'apomorphies supplémentaires, la structure du spermatozoïde ne permet pas de préciser la position phylogénétique de* Euzetrema *à l'intérieur des Monogènes Monopisthocotylea.*

INTRODUCTION

Spermatozoal ultrastructure is one of the available tools for understanding the phylogeny of the Platyhelminthes (Justine, 1991a; Justine, 1991b; Justine, 1993; Justine, Lambert and Mattei, 1985a). We present here observations on spermiogenesis and spermatozoa of a monogenean, namely *Euzetrema knoepffleri* Combes, 1965. *Euzetrema* belongs to the lagotrematidae, a family comprising only three species with a very discontinuous geographical distribution (Fournier, 1980). Moreover, the host of *Euzetrema knoepffleri*, the urodele amphibian *Euproctus montanus*, is a relict species found in the island of Corsica (Combes, 1965; Combes, Jourdane and Knoepffler, 1974).

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MATERIAL AND METHODS

The host *Euproctus montanus* was collected in Corsica. Adult monogeneans were fixed in 3% glutaraldehyde in a pH 7.3, 0.1 M cacodylate buffer at 4°C for 3 hrs, then rinsed and post-fixed in 1% osmium tetroxide in the same buffer. Dehydration was performed in an acetone- propylene oxide series, blocks were embedded in Epon, and grids were contrasted with uranyl acetate and lead citrate. Glycogen demonstration was performed after Thiéry (1967).

OBSERVATIONS

In contrast to many parasitic Platyhelminthes, the testis of *Euzetrema* contains only few stages of spermiogenesis and spermatozoa; this could be related to the annual cycle of this species.

Early spermatids are attached to a cytophore. Nurse cells send extensions between the germinal cells (Fig. a). The early spermatid is round, with a round nucleus and mitochondria grouped at one pole of the cell (Fig. a). Then the nucleus becomes pyriform with the mitochondria grouped at its base, and two axo-

nemes growing near its pointed extremity (Figs. b, c). The axonemes, at this stage, show no central core and thus have a 9+0 structure (Fig. c). Two centrioles are present (Fig. d) and thus two free axonemes are probably present in spermatids, but sections showing two elements were not found.

Later, the spermatid lengthens and free flagella are no longer seen. A zone of differentiation with two free axonemes and a middle cytoplasmic process, such as seen in the Polyopisthocotylea and Digenea, was never observed. Transverse sections of the lengthening spermatid exhibit two axonemes of the trepaxonematan 9+"1" type, the elongating nucleus and the elongating mitochondrion (Fig. e). In the proximal part of the spermatid, transverse sections show two centrioles made up of nine triplets (Fig. f). No peripheral microtubule was seen in any stage of spermiogenesis. During the elongation process, dynein arms of the immature axonemes are indistinct (Fig. g). In some spermatids, the elongating extremity shows more than two sections of axonemes (Fig. h), thus indicating that the extremity makes a loop on itself, and axonemal tips have their central core on the outside of the circle of nine doublets (Fig. h).

The mature spermatozoon, observed in the testis (Figs. i, j) and vas deferens (Fig. k) shows two regions: a) a nuclear region (Fig. i), with nucleus, mitochondrion, two axonemes and abundant beta glycogen demonstrated with Thiéry's reaction (Fig. j); and b) a region without nucleus (Fig. k), containing only two axonemes with a section of mitochondrion in some sections. Exceptionally, a section showing one single peripheral microtubule (Fig. k) was found among many observed sections.

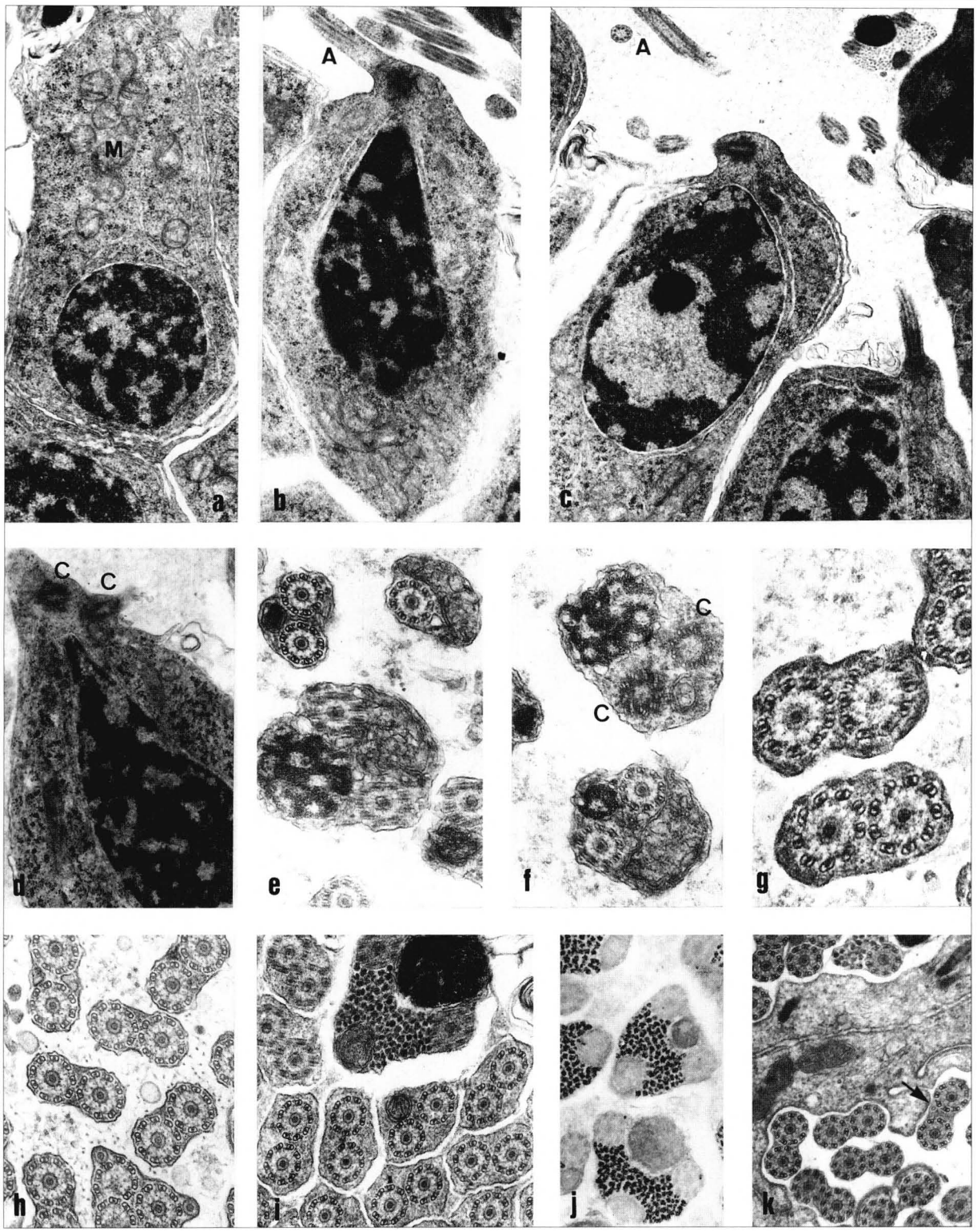
DISCUSSION

The phylogenetic value of monogenean spermatozoon ultrastructure is currently well accepted and the number of species described is growing rapidly. A recent review (Justine, 1993) listed 68 species, and since, nine studies have been published, on *Octomacrum lanceatum* (Hathaway, Hathaway and Kritsky, 1993a), *Lagarocotyle salamandrae* (Hathaway, Hathaway and Kritsky, 1993b), *Isancistrum subulatae* (Malmberg and Lilliemark, 1993), *Pseudodiplorchis americanus* (Cable and Tinsley, 1993), *Entobdella soleae* (Tappenden, Kearn and Evans-Gowing, 1993), *Monocotyle helicophallus* and *Calicotyle australiensis* (Watson and Rohde, 1994a), *Gonoplasius* sp. (Rohde and Watson, 1994a), *Polylabroides australis* (Rohde and Watson, 1994b) and *Neopolystoma* sp. (Watson and Rohde, 1994b). The present observations were included in an unpublished thesis (Fournier, 1980) and because of the current interest in monogenean spermiogenesis we thought it was useful to allow other researchers access to the observations on *Euzetrema knoepffleri* by publishing them.

The spermatozoa of monogeneans have been classified into four patterns (Justine *et al.*, 1985a) and a fifth pattern was later added (Justine, Le Brun and Mattei, 1985b). In this classification, *E. knoepffleri* corresponds to type 2 (two axonemes, peripheral microtubules absent). Type 2 was separated into several subtypes, and *E. knoepffleri* corresponds to subtype 2c (Justine, 1991a). Recently, a single peripheral microtubule was found in some cases (Justine, Mattei and Euzet, 1991) and the definition of type 2 has been amended (Justine, Afzelius, Malmberg and Mattei, 1993) as "spermatozoa with two axonemes and no cortical microtubules, except a single microtubule much shorter than the spermatozoon". The pattern of *E. knoepffleri* corresponds well to the definition of type 2; the single microtubule exceptionally found is probably much shorter than the spermatozoon. Cladistic analyses of the Monogenea based on sperm ultrastructure (Justine, 1991a; Justine, 1993) use many characters, and not only the classification of sperm patterns. In these analyses, the pattern found in *E. knoepffleri* corresponds to the most plesiomorphic pattern found in the Monopisthocotylea, and lack of other apomorphies does not allow its use for understanding the phylogenetic position of *Euzetrema* within the Monopisthocotylea.

Watson and Rohde (1994a) recently claimed that the limits between patterns 2 and 3 were not clear and thus proposed a type 2/3. It is true that *Calicotyle australiensis* and *C. kroyeri* have spermatozoa showing two axonemes and several cortical microtubules (Tappenden and Kearn, 1991; Watson and Rohde, 1994a) and, thus, this should lead to a re-examination of the definition of type 3. However, microtubules of mature spermatozoa in type 3 clearly originate from the cortical microtubules, with associated ornamentation, located in the spermatid's early zone of differentiation, while the origin of the single (sometimes absent) microtubule in type 2 is less clear. In other words, the single microtubule of type 2 might be not homologous with the cortical microtubules with ornamentation of type 3. The combination of types 2 and 3 into a single type would require a demonstration of homology.

A study of spermiogenesis in *Iagotrema*, a parasitic monogenean found in a south American turtle and supposedly related to *Euzetrema*, would be useful to clarify the phylogenetic position of the family Iagotrematidae.



Figs. a-k. – Spermiogenesis in *Euzetrema knoepffleri*

a. Early spermatid with round nucleus. Mitochondria (M) are grouped at one pole of the cell. Note processes of nurse cells. x 20 000. b. Early spermatid, with pyriform nucleus and axoneme (A). x 15 000. c. Two early spermatids, same stage as b. The axoneme (A) has no central core. x 15 000. d. Early spermatid, section showing two centrioles (C). x 15 000. e. Elongating late spermatid, transverse section showing two axonemes and elongating nucleus and mitochondrion. x 36 000. f. Late spermatid, transverse section at the base of the zone of differentiation, showing two centrioles (C). x 36 000. g. Late spermatid, elongating extremity. Dynein arms are indistinct. x 36 000. h. Transverse section of distal extremities of spermatids. x 25 000. i. Transverse section of mature spermatozoa in testis, showing nuclear region with nucleus, and a region without nucleus. x 40 000. j. Beta-glycogen in mature spermatozoon, evidenced by Thiéry's reaction. x 30 000. k. Transverse section of mature spermatozoa in vas deferens. Note single microtubule in one section (arrow). x 30 000.

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