Sperm ultrastructure of *Isancistrum subulatae* (Platyhelminthes: Monogenea: Gyrodactylidae)

G. Malmberg and **Y. Lilliemarck**

Swedish Museum of Natural History and Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden; Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden

Key words: spermatozoa, *Isancistrum*, Monogenea, ultrastructure

Abstract. Spermatozoa of *Isancistrum subulatae* have an ultrastructure with two axonemes of the 9 + 1 type, in parallel with the nucleus and one mitochondrion. In the released *Isancistrum* spermatozoa the nucleus and mitochondrion may have a rather circular cross-section. No cortical microtubules were established. The ultrastructure of the *Isancistrum* spermatozoa thus corresponds to sperm pattern 2, according to Justine et al. (1985).

The absence of anchors in the ontogeny of monogeneans may indicate a primitive condition, and often primitive monogeneans parasitise primitive hosts (Bychowsky 1957, Malmberg and Fernholm 1989, Malmberg 1990). This applies to the family Acanthocotylidae, parasitising myxinids and rajids, and also to the genus *Isancistrum* (Gyrodactylidae) parasitising squids. In most gyrodactylid genera (generally parasitising fish), however, peduncular anchors (Malmberg 1990) develop. This raises the question, whether *Isancistrum* represents a primitive or secondary monogenean condition.

Justine et al. (1985) used the ultrastructure of spermatozoa in various families for arranging the Monogenea phylogenetically in four groups, each characterized by a sperm pattern. They considered "pattern 1" to be the most primitive, from which the remaining three were derived by simplification. In the subgroup of sperm pattern 2, they included the families Udonellidae, Gyrodactylidae and Iagotrematidae. The sperm pattern of the Gyrodactylidae was based on Kritsky's (1976) description of the ultrastructure of the spermatozoa and spermiogenesis of *Gyrodactylus eucaliae* Ikezaki et Hoffman, 1957. In this paper, the sperm pattern of a second gyrodactylid, *Isancistrum subulatae* Llewellyn, 1984, is presented.

MATERIALS AND METHODS

The material emanates from specimens of *Isancistrum subulatae* Llewellyn, 1984, infesting the squid *Alloteuthis subulata*. The squid specimens were trawled off the shore of Whitesand Bay, Plymouth on July 26, 1974 and then kept in an aquarium for two days, when a number of squid arms with live specimens of *I. subulatae* were fixed in glutaraldehyde. Until preparation for TEM (April 1991), the material was stored in the fixative at room temperature.

Specimens of *I. subulatae* were detached from the squid arms, washed in distilled water, post-fixed for 1 h in a cold 1% solution of osmium tetroxide, dehydrated in ethanol, embedded in Polarbed (Polaron), sectioned, section-stained with uranyl acetate and lead citrate and examined with a JEOL 100 S electron microscope.

RESULTS

A cross-sectioned spermatozoon (or late spermatid) of *Isancistrum subulatae* can be seen to contain four parallel structures: an electron-dense nucleus, a moderately electron-dense mitochondrion and two axonemes (Fig. 1). The nucleus, at least at this stage, is round in cross-section. In certain cross-sections a nuclear membrane can be discerned and likewise the mitochondrial membranes. The mitochondrion has an elliptic cross-section. The axone-

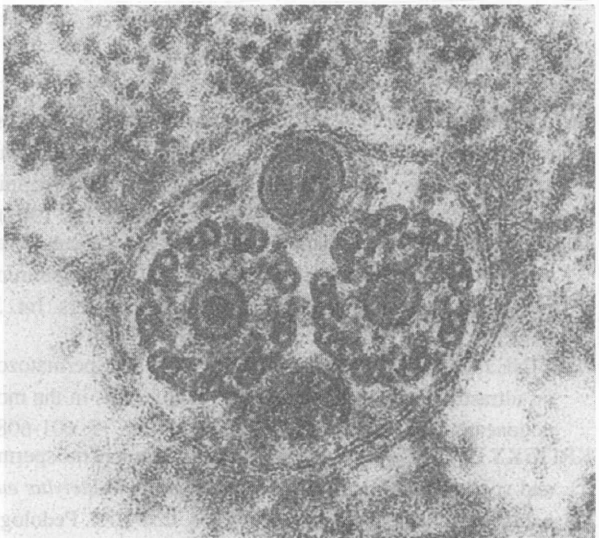

Fig. 1. *Isancistrum subulatae* Llewellyn, 1984. Electron micrograph of a transverse section of a spermatozoon (or late spermatid). (x 112 000)

mes are of the kind that is commonly seen in flatworm spermatozoa: nine microtubular doublets surrounding a rather prominent central hub. Some fuzzy material is seen around the four structural cell components.

DISCUSSION

Our investigation shows that the spermatozoa of *Isancistrum subulatae* Llewellyn, 1984 have two axonemes and principally the same ultrastructure as *Gyrodactylus eucaliae* Ikezaki et Hoffman, 1957 (see Kritsky 1976). Thus, according to Justine et al. (1985), the spermatozoal ultrastructure of *Isancistrum* conforms to sperm pattern 2. This pattern, however, is found in families as separate as the Acanthocotylidae (see Malmberg and Afzelius 1990, Tappenden and Kearn 1990), the Gyrodactylidae, the Udonellidae, the Iagotrematidae, the Capsalidae and the Dionchidae (see Justine et al. 1985). The families Acanthocotylidae and Gyrodactylidae are certainly more closely related to each other than to any of the remaining four families. The early lyrval development of the haptor is very similar until the haptor of acanthocotylids stops developing (Malmberg and Fernholm 1989, Malmberg 1990) and their larval haptoral attachment structures (hinged marginal hooks) are the same. The further development, however, indicates a distinct evolutionary gap: in the Gyrodactylidae (with the exception of the genus *Isancistrum*), peduncular anchors develop, which move into the growing haptor (opisthaptor), while in the Acanthocotylidae (in front of the small haptor), a large pseudohaptor develops (Malmberg and Fernholm 1989). The opisthaptor of *Isancistrum* may represent an intermediate evolutionary capacity level (Malmberg 1986).

Investigating genera of the Acanthocotylidae, Malmberg and Fernholm (1989) found that the pseudohaptor of *Myxinidocotyle* represents a more original condition than that of *Acanthocotyle*. Malmberg and Afzelius (1990) investigating spermatozoa found cortical microtubules in *Myxinidocotyle*, but not in *Acanthocotyle* and presumed that the spermatozoa of *Myxinidocotyle* represent a primitive form of sperm pattern 2. Because sperm pattern 2 is present in different monogenean main lines, they concluded that this pattern, in the Monogenea, represents an evolutionary capacity level (Malmberg 1986) and does not necessarily indicate a close relationship between the families. These ideas correspond with the division of pattern 2 into the subtypes 2a, 2b, 2c (Justine 1991), made on a basis of results concerning presence or absence of microtubules in the spermiogenesis. Thus the presence of sperm pattern 2 in the genera *Isancistrum* and *Gyrodactylus* may imply that all members of the family Gyrodactylidae are characterized by this pattern. Further studies on the spermiogenesis to establish the sperm pattern 2 subtype of *Isancistrum* and other gyrodactylid genera may reveal different evolutionary steps and contribute to the discussion on primitiveness or reduction of the *Isancistrum* opisthaptor.

Acknowledgements. We thank Profesor Björn Afzelius for valuable help and advice concerning transmission electron microscopy and for providing the electron micrograph. We also acknowledge valuable help when collecting the material given by Professor Jack Llewellyn, Birmingham, U.K. and the Laboratory of the Marine Biological Association, Plymouth, U. K. Financial support was provided by the Swedish Natural Science Research Council.

REFERENCES

BYCHOWSKY B. E. 1957: Monogenetic trematodes, their systematics and phylogeny. Zool. Inst. Leningrad, Akad. Nauk USSR, Moscow. (In Russian.) (English translation: Virginia Inst. Mar. Sci. Transl. Ser. 1, AIBS, Washington DC 1961).

JUSTINE J. L. 1991: Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. Int. J. Parasitol. 21: 821-838.

JUSTINE J. L., LAMBERT A., MATTEI X. 1985: Spermatozoon ultrastructure and phylogenetic relationships in the monogeneans (Platyhelminthes). Int. J. Parasitol. 15: 601-608.

KRITSKY D. C. 1976: A study of the ultrastructure of the sperms and spermatogenesis in the monogenean *Gyrodactylus eucaliae* Ikezaki et Hoffman, 1957. Proc. Inst. Biol. Pedology (Vladivostok) 34: 70-76. (In Russian.)

LLEWELLYN J. 1984: The biology of *Isancistrum subulatae* n. sp., a monogenean parasitic on the squid, *Alloteuthis subulata*, at Plymouth. J. Mar. Biol. Ass. U.K. 64: 285-302.

MALMBERG G. 1986: The major parasitic platyhelminth classes - progressive or regressive evolution? Hydrobiologia 132: 23-29.

MALMBERG G. 1990: On the ontogeny of the haptor and the evolution of the Monogenea. Syst. Parasitol. 17: 1-65.

MALMBERG G., AFZELIUS B. 1990: Sperm ultrastructure in *Myxinidocotyle* and *Acanthocotyle* (Platyhelminthes, Monogenea, Acanthocotylidae). Zool. Scr. 19: 129-132.

MALMBERG G., FERNHOLM B. 1989: *Myxinidocotyle* gen. n. and *Lophocotyle* Braun (Platyhelminthes, Monogenea, Acanthocotylidae) with description of three new species from hagfishes (Chordata, Myxinidae). Zool. Scr. 18: 187-204.

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

Received 21 May 1992

Accepted 9 December 1992