FOLIA PARASITOLOGICA 45: 75-76, 1998.

SPERMATOZOON ULTRASTRUCTURE IN TWO MONOPISTHOCOTYLEAN MONOGENEANS FROM MALAYSIA: *PSEUDODACTYLOGYROIDES MARMORATAE* AND *SUNDANONCHUS MICROPELTIS*

Isabelle Mollaret[1], **L.H. Susan Lim**[2], **Göran Malmberg**[3], **Björn Afzelius**[4] and **Jean-Lou Justine**[1]

[1]Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, ERS 156 CNRS, Muséum National d'Histoire Naturelle, 61 rue Buffon, F-75231 Paris cedex 05, France;

[2]Institute of Advanced Studies, University of Malaya, 591 00 Kuala Lumpur, Malaysia;

[3]Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden;

[4]Department of Ultrastructure Research, Wenner-Gren Institute, Biology E4, Stockholm University, S-106 91 Stockholm, Sweden

Sperm ultrastructure has been shown to be a valuable tool for phylogenetic studies of Platyhelminthes (Justine J.-L. 1991: Can. J. Zool. 69: 1421-1440). In the Monogenea, sperm ultrastructure is relatively homogeneous in the Polyopisthocotylea, but various structures are found in the Monopisthocotylea and can be used as characters for phylogeny (Justine J.-L., Lambert A., Mattei X. 1985: Int. J. Parasitol. 15: 601-608; Justine J.-L. 1991: Int. J. Parasitol. 21: 821-838; Justine J.-L. 1993: Bull. Fr. Pêche Piscic. 328: 137-155). Sperm ultrastructure has been described for more than 40 species and the addition of any new data, even fragmentary, increases the validity of the database of sperm characters. We have examined the spermatozoa of two species of monopisthocotylean monogeneans from Malaysian teleosts. Although our observations did not provide detailed information on spermiogenesis, the information obtained on spermatozoa has phylogenetic significance.

Specimens of monogeneans were collected from fishes of peninsular Malaysia: *Pseudodactylogyroides marmoratae* Lim, 1995 (Ancyrocephalidae) from *Oxyeleotris marmorata* Bleeker, 1852 (Eleotrididae), and *Sundanonchus micropeltis* Lim et Furtado, 1985 (Sundanonchidae) from *Channa micropeltes* (Cuvier) (Channidae). Living specimens were fixed in 2.5% glutaraldehyde in a buffer solution of 0.1 M sodium cacodylate, rinsed and postfixed for 1 h in 1% osmium tetroxide in the same buffer, dehydrated in ethanol, and embedded in Polarbed (Polaron). Ultrathin sections were contrasted with lead citrate and uranyl acetate and examined with a Hitachi H-600 microscope.

In *Pseudodactylogyroides marmoratae* (Fig. 1a-n), transverse sections of the filiform spermatozoon, observed in the seminal vesicle, show three elements: an axoneme with the 9 + "1" trepaxonematan structure, a mitochondrion and the nucleus (Fig. 1f). No cortical microtubules are present. The anterior extremity shows a centriole adjunct (Fig. 1a,b). The filiform nucleus and mitochondrion appear to lie very close to the centriole adjunct, as demonstrated by serial sections (Fig. 1c-e). The distal extremity contains only the axoneme and mitochondrion (Fig. 1f), indicating that the mitochondrion is longer than the nucleus. The distal extremity of the axoneme shows in an anterio-posterior sequence: 9 doublets and the central core (i.e. a normal 9 + "1" structure) (Fig. 1j), doublets and singlets, and the central core (Fig. 1g, upper section), 9 singlets and the central core (Fig. 1h, k), and eventually 9 singlets without the central core (Fig. 1i, 1,m). Spermiogenesis was not observed. Sections of spermatozoa were found within a sclerified structure (Fig. 1n). This structure presented two tubular sections enclosed in a common muscular sheath, and corresponds to the curved sclerified copulatory organ described in *P. marmoratae* by Lim (Lim L.H.S. 1995: Syst. Parasitol. 31: 25-32).

In *Sundanonchus micropeltis* (Fig. 1o,p), the number of sections of germ cells obtained was low and observations are fragmentary. Transverse sections of the anterior part of the spermatozoon show a 9 + "1" axoneme and a mitochondrion (Fig. 1o), and sections of the posterior extremity show the nucleus and several singlet microtubules (Fig. 1p). These microtubules are interpreted as originating from the disorganisation of the distal extremity of the axoneme.

The spermatozoa of *Pseudodactylogyroides marmoratae* and *S. micropeltis* both have a single axoneme and no cortical microtubules, and thus conform to Type 4 in the classification of Justine et al. (Justine J.-L., Lambert A., Mattei X. 1985: Int. J. Parasitol. 15: 601-608).

The spermatozoon of *P. marmoratae* is similar in its ultrastructure to that of *Pseudodactylogyrus bini* (Schmahl G., El-wasila M. 1992: Folia Parasitol. 39: 19-31) and *P. anguillae* (Le Brun N., Lambert A., Justine J.-L. 1986: Ann. Parasitol. Hum. Comp. 61: 273-284). A centriole adjunct is present in the two genera. Recent studies (Mollaret I., Justine J.-L. 1997: Tissue & Cell, 29: 699-706) have shown that, in *Pseudodactylogyrus* sp., the nucleus is located in the central region of the filiform spermatozoon; comparative studies with the same method (nuclear labelling with fluorescent dyes) should perhaps be attempted to detect differences between the different species. Thus, sperm ultrastructure is very similar in Pseudodactylogyridae and Ancyrocephalidae.

To our knowledge, it is the first time that spermatozoa have been observed by transmission electron microscopy within the sclerified copulatory apparatus of a monogenean. This confirms that this organ conducts sperm during copulation.

The distal extremity of the sperm axoneme varies in the monopisthocotylean monogeneans; it made up of: i) disorganized doublets and singlets close to the nucleus in *Tetraonchoides* sp. (Justine J.-L., Mattei X., Euzet L. 1991: Ann. Parasitol. Hum. Comp. 66: 173-178); ii) 8 long singlets associated with the mitochondrion in *Diplectanum* sp. (Justine J.-L., Mattei X. 1984: J. Ultrastruct. Res. 88: 77-91); and iii) singlets circling the nucleus in *S. micropeltis*. This character could be of phylogenetic interest when more data are available. From a morphological study of the adults and

---

Address for correspondence: J.-L. Justine, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Muséum National d'Histoire Naturelle, 61 Rue Buffon, F-75231 Paris cedex 05, France. Phone: ++33 1 40793503; Fax: ++33 1 40793499; E-mail: justine@mnhn.fr

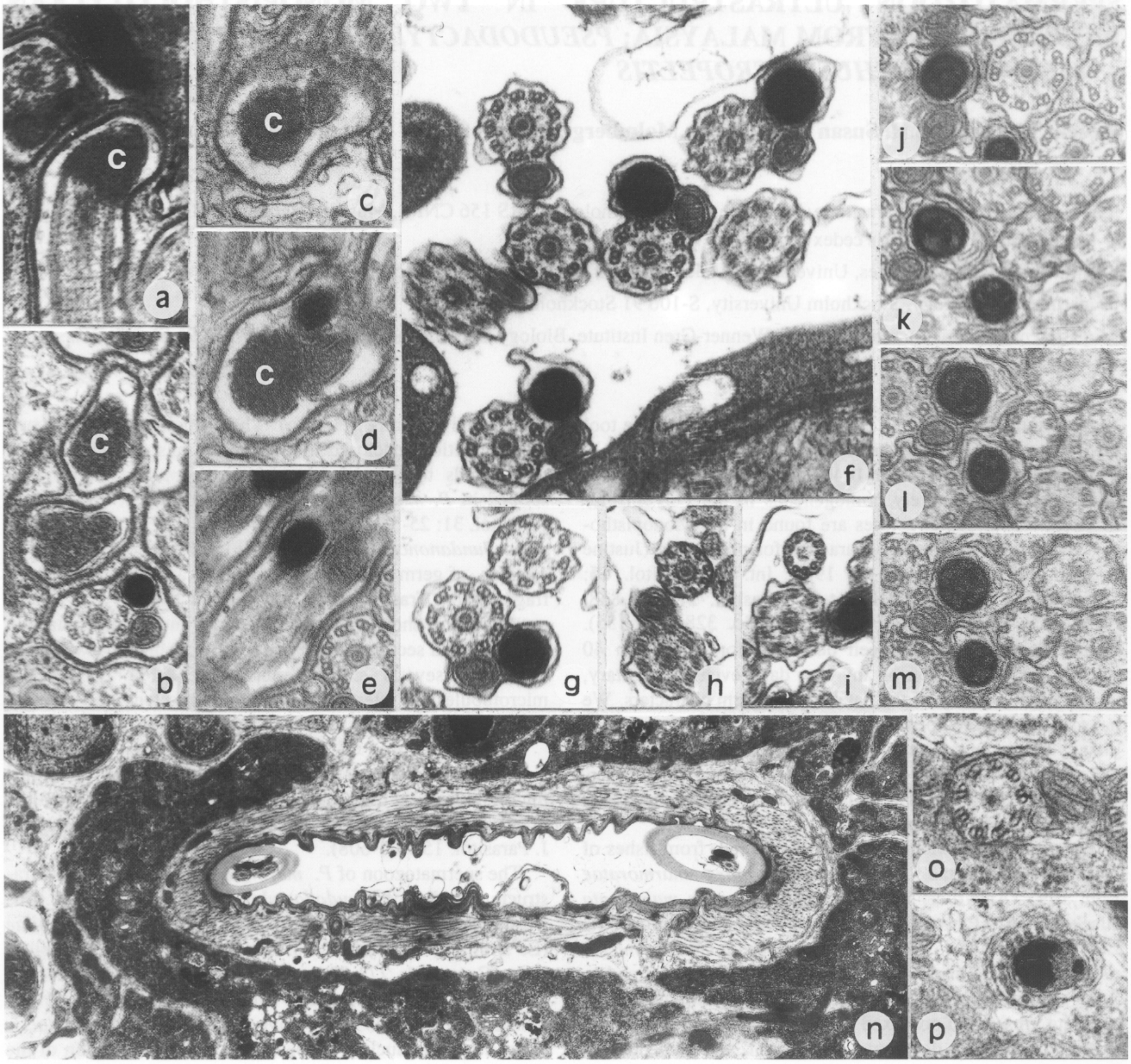

Fig. 1. a-n. Spermatozoa of *Pseudodactylogyroides marmoratae*. **a** – longitudinal section of centriole adjunct; **b** – centriole adjunct (upper two transverse sections); **c-e** – serial section of the anterior extremity of a spermatozoon, showing that the mitochondrion **(c)** and the nucleus **(d)** begin just posteriorly to the centriole adjunct; **f** – various sections of spermatozoa, showing the typical structure with 9 + "1" axoneme, mitochondrion and nucleus; **g-i** – transverse sections of distal extremity of the spermatozoon; **j-m** – serial sections of distal extremity; **n** – section in sclerified copulatory organ. Two transverse sections of the copulatory organ are seen within a common muscular sheath; note the presence of spermatozoa within the sclerified part; **Fig. 1. o,p.** Spermatozoa of *Sundanonchus micropeltis*. **o** – transverse section in anterior region, with mitochondrion and 9 + "1" axoneme; **p** – transverse section in posterior region with nucleus and 9 singlets originating from the distal extremity of the axoneme. × 50,000 (a, b, h, i); × 60,000 (c-g, j-m, o-p); × 8,000 (n). c – centriolar adjunct.

comparison with other families, Kritsky and Lim (Kritsky D.C., Lim S.L.H. 1995: Inv. Biol. 114: 285-295) predicted that sperm structure in the Sundanonchidae should belong to Type 4 of Justine et al. (1985), which has one axoneme without cortical microtubules. Type 4 has been found in the ancyrocephalids, pseudodactylogyrids, diplectanids, tetraonchids, tetraonchoidids, amphibdellatids, calceostomatids, and anoplodiscids (for a review, see Justine J.-L. 1995: Mém. Mus. Natn. Hist. Nat. 166: 55-86), and now in the sundanonchids. The present study confirms the prediction of Kritsky and Lim (op. cit.), and demonstrates that general morphology and sperm ultrastructure are two complementary tools for the study of phylogeny in the Monogenea.

This work was partly supported by a BQR from the Muséum, the INTAS grant No. 93-2176, a grant from the French Embassy in Malaysia, and a grant from the French Embassy in Australia. Prof. B.G.M. Jamieson kindly edited the English.

Received 28 June 1997 Accepted 8 September 1997