Zoological Studies **49**(2): 265-269 (2010)

Zoological Studies

Ultrastructure of Spermatozoa of *Prionospio japonica* (Annelida: Spionidae) from Taiwan

Vasily I. Radashevsky[1,*], Arkadiy A. Reunov[1], Olga V. Yurchenko[1], Yana N. Alexandrova[1], and Hwey-Lian Hsieh[2]

[1]*A.V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok 690041, Russia*
[2]*Biodiversity Research Center, Academia Sinica, Nankang, Taipei 115, Taiwan*

(Accepted June 25, 2009)

Vasily I. Radashevsky, Arkadiy A. Reunov, Olga V. Yurchenko, Yana N. Alexandrova, and Hwey-Lian Hsieh (2010) Ultrastructure of spermatozoa of *Prionospio japonica* (Annelida: Spionidae) from Taiwan. *Zoological Studies* **49**(2): 265-269. *Prionospio japonica*, a common inhabitant of estuaries in the northwestern Pacific Ocean, releases gametes into the water, where fertilization and planktotrophic larval development occur. Each spermatozoon has a biradially symmetrical acrosome 0.3 ± 0.1 μm long, a spherical nucleus 1.7 ± 0.2 μm in diameter, 4 spherical mitochondria 0.8 ± 0.1 μm in diameter, 2 centrioles situated perpendicular to each other, and a flagellum with a 9 × 2 + 2 organization of microtubules. The biradially symmetrical acrosome, which is also present in *P.* cf. *queenslandica*, is unique among polychaetes examined to date and possibly an apomorphy shared by a group of closely related *Prionospio* species. http://zoolstud.sinica.edu.tw/Journals/49.2/265.pdf

Key words: Sperm, Morphology, Polychaeta.

Gametes in the polychaete order Spionida show considerable variation in morphology. Thin- and thick-enveloped oocytes, as well as short- and long-headed spermatozoa were reported in various species (see Blake 2006). Although it is generally accepted that sperm morphology is correlated with the mode of fertilization and the morphology of oocyte envelopes (Franzén 1956), their relationships in spionids are not yet understood.

Gamete morphology, observed by light microscopy, was incorporated into hypotheses on phylogenetic relationships of families and subfamilies within the Spionida (Söderström 1920, Hannerz 1956, Blake and Arnofsky 1999). The fine morphology of gametes among members of the Spionida was previously examined only in some species of the family Spionidae. Ultrastructural features of oogenesis were described for *Streblospio benedicti*, *Spio setosa*, *Polydora cornuta* (Eckelbarger 1980 1988 1992 1994), and *Marenzelleria viridis* (Bochert 1996a). The sperm ultrastructure was studied in *Polydora ciliata* (Franzén 1974), *Dipolydora socialis*, *Pol. cornuta*, *Polydora websteri*, *Str. benedicti* (Rice 1981), *Prionospio fallax* (Franzén and Rice 1988), *Prionospio* cf. *queenslandica* (Rouse 1988), *Pseudopolydora* sp. (Rouse 1988, as *Tripolydora* sp.), *Boccardiella hamata*, *Pseudopolydora paucibranchiata* (Rice 1992), *Mar. viridis* (Bochert 1996b), *Polydora neocaeca* (Williams 2000), and *Scolelepis laonicola* (Vortsepneva et al. 2006, as *Asetocalamyzas laonicola*). Those investigations showed that the gamete ultrastructure might be relevant to developing hypotheses of the relationships between generic and less-inclusive taxa of the Spionidae. However, the small number of species for which these details are known still precludes the use of ultrastructural characters in

*To whom correspondence and reprint requests should be addressed. E-mail:radashevsky@mail.ru

phylogenetic hypotheses.

Prionospio japonica Okuda is a common inhabitant of estuaries in the northwestern Pacific Ocean. Females and males release their gametes into the water, where fertilization and planktotrophic larval development occur. The oocytes are 100-105 μm in diameter and have smooth membranes about 2 μm thick (Radashevsky and Hsieh in preparation). The purpose of the present study was to describe the ultrastructure of spermatozoa of specimens of *P. japonica* collected in northwestern Taiwan.

MATERIAL AND METHODS

Adults of *P. japonica* were collected in the Tanshui River estuary, Taipei City, northern Taiwan in Feb.-Apr. 2006. The gamete-bearing segments of mature males were cut off and fixed in a primary fixative for 2 h at 4°C. The fixative was composed of 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 21 mg/ml NaCl to provide tonicity equal to that of the water from near the bed of the estuary. Specimens were washed in several changes of buffered sodium cacodylate with NaCl added and then post-fixed in 2% buffered OsO_4 for 2 h. After dehydration in a graded ethanol series and acetone, they were embedded in Spurr resin (Spurr, EMS). Semi- and ultrathin sections were made using a Leica UC6 ultramicrotome. Semi-thin sections were stained with methylene-blue and examined with a light microscope (Leica DM4500 B). Images were taken with a digital camera (Leica DFC300 FX). Ultrathin sections from 5 males were stained with 2% alcoholic uranyl acetate and aqueous lead citrate and then viewed with a transmission electron microscope (JEM 100S at 80 kV) in the Institute of Marine Biology, Vladivostok (IMBV), Russia. The precision of measurements of 10 spermatozoa was maximally to the nearest 0.2 μm (0.1 μm for the acrosome and mitochondria). Representative adult specimens were fixed in a 10% formaldehyde solution, rinsed in fresh water, transferred to 70% ethanol, and deposited in the IMBV.

RESULTS

In fertile segments of mature males, the coelomic cavity was filled with spermatozoa (Fig. 1). Within the spermatozoa, the acrosome was an electron-dense biradially symmetrical structure, 0.3 μm long, which was penetrated almost to its anterior end by a narrow sub-acrosomal space (Figs. 2A-D). When sectioned vertically through the short axis, the acrosome appeared round to slightly oval, 0.5 μm wide, with a central invagination (Figs. 2A, B, D). When sectioned vertically through the long axis, the acrosome appeared elongate, 2.4 μm wide, with the base curving inwards at each end; thus the entire structure resembled a telephone receiver (Figs. 2B, C). In a vertical section through the long axis, the acrosome showed about 10 periodic indentations on the anterior and posterior edges (Figs. 2B, C). The sub-acrosomal space was filled with material of moderate electron density that extended around the base of the acrosome and also anteriorly to interspaces of the acrosome and plasma membrane (Figs. 2C, D).

The nucleus was spherical, 1.7 μm in diameter, and of uniform electron density (Figs. 2A, B). Posteriorly, it had slight indentations accommodating the mitochondria and an anchoring apparatus (Fig. 2E).

The midpiece had 4 spherical mitochondria, each 0.8 μm in diameter, and 2 centrioles (Fig. 2E). A lipid globule, located on the external side of the mitochondria (Fig. 2F), often appeared on sections. The mitochondria had numerous tabular cristae (Figs. 2E, F). The centrioles were situated perpendicular to each other, with the distal centriole functioning as the basal body of the

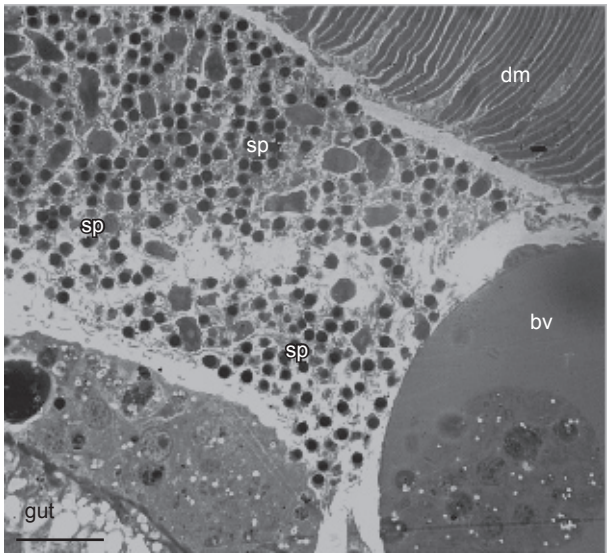

Fig. 1. *Prionospio japonica.* Semi-thin cross-section through a male fertile segment showing separate spermatozoa floating in the coelom. bv, blood vessel; dm, dorsal muscles; gut, midgut; sp, spermatozoa. Scale bar = 10 μm.

flagellum (Fig. 2E). The microtubule organization of flagella was 9 × 2 + 2 (Fig. 2G).

DISCUSSION

The sperm of *P. japonica* appeared to be quite similar to that of *P.* cf. *queenslandica* (Rouse 1988) and *P. fallax* (Franzén and Rice 1988). In all 3 species, the spermatozoa have spherical nuclei, 4 spherical mitochondria, and 2 centrioles situated perpendicular to each other. *Prionospio japonica* and *P.* cf. *queenslandica* are unique among polychaetes examined to date in possessing acrosomes with biradial symmetry. The only longitudinal section of spermatozoa from *P. fallax* provided by Franzén and Rice (1988: fig. 17A) appears identical to the longitudinal sections through the short axis of the acrosomes of *P.* cf. *queenslandica* and *P. japonica*. However, whether *P. fallax* has a radially or biradially symmetrical acrosome remains unknown. Neither indentations on the anterior and posterior edges of the acrosome nor a lipid globule on the mitochondria, as in *P. japonica*, were reported for *P.* cf. *queenslandica* or *P. fallax*.

Acrosomes, specific organelles responsible for penetration of oocyte envelopes, show great variation among polychaetes (Sawada 1984, Jamieson and Rouse 1989, Rice 1992). In most spionids, they are short (sub)spherical structures

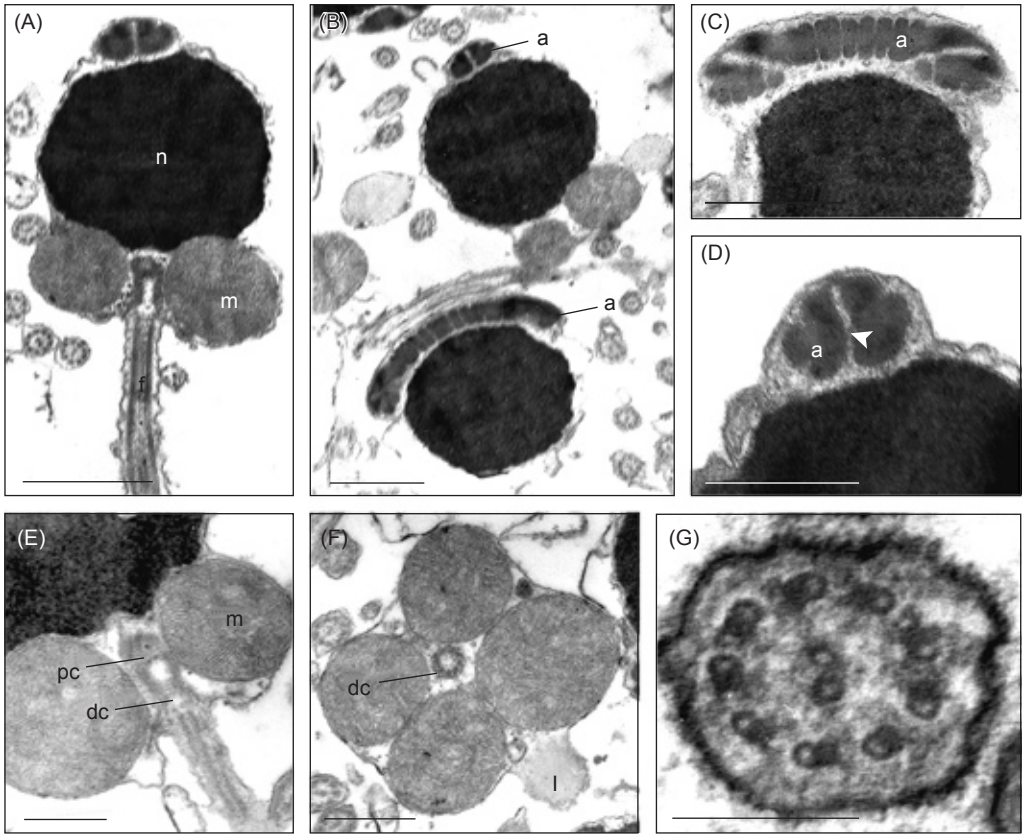

Fig. 2. *Prionospio japonica*. Spermatozoon ultrastructure. (A) Longitudinal section through a spermatozoon. (B) Longitudinal sections through 2 spermatozoa, the upper one with an acrosome sectioned through the short axis, and the lower one with an acrosome sectioned through the long axis. (C) Longitudinal section through the long axis of an acrosome, resembling a telephone receiver with periodic indentations on the anterior and posterior edges. (D) Longitudinal section through the short axis of an acrosome, showing a central invagination (arrowhead). (E) Longitudinal section through the midpiece, showing spherical mitochondria and proximal and distal centrioles situated perpendicular to each other. (F) Transverse section through the midpiece, showing 4 spherical mitochondria, a distal centriole, and a lipid drop. (G) Transverse section through the flagellum, showing a 9 × 2 + 2 arrangement of microtubules. a, acrosome; dc, distal centriole; l, lipid globule; m, mitochondria; n, nucleus; pc, proximal centriole. Scale bars: A-D = 1 μm; E, F = 0.5 μm; G = 0.2 μm.

about 1 μm long, but in some species, acrosomes are elongated and sharply pointed, e.g., about 7 μm long in *Streblospio benedicti* (Rice 1981), 25 μm long in *Boccardiella ligerica* (Rullier 1960: fig. 12, as *Polydora* (*Boccardia*) *redeki*), and 36 μm long in *Rhynchospio nhatrangi* (Radashevsky 2007: fig. 5A). Because very little is known about the fine structure of oocyte envelopes or fertilization biology in the Spionida, the reasons for acrosome diversity in this group remain unknown.

Acrosomes with similar longitudinal sections through the short axis as in *Prionospio* species are also present in various species from less closely related polychaete families, e.g., the Eunicidae (*Marphysa sanguinea*, Jamieson and Rouse 1989: fig. 1B), Maldanidae (*Clymenella* sp., Jamieson and Rouse 1989: fig. 7F), Nereidae (*Tylorrhynchus heterochaetus*, Jamieson and Rouse 1989: fig. 3I), Opheliidae (*Armandia* sp., Jamieson and Rouse 1989: fig. 2G), Orbiniidae (*Haploscoloplos elongatus*, Rice 1992: fig. 32), Oweniidae (*Owenia fusiformis*, Jamieson and Rouse 1989: fig. 2B), Polynoidae (*Alentia gelatinosa*, Franzén and Rice 1988: fig. 17B; *Lepidonotus* sp., Jamieson and Rouse 1989: fig. 3A), Serpulidae (*Pomatoleios kraussi*, Sawada 1984: fig. 3D; *Serpula* sp.; Jamieson and Rouse 1989: fig. 4D), and Terebellidae (*Streblosoma acymatum*, Jamieson and Rouse 1989: fig. 4A). Reviewing sperm ultrastructure in polychaetes, Sawada (1984: 102) classified this kind of acrosome as “beret-like”, while Jamieson and Rouse (1989: 100) called it “an inverted bowl with thickened rim”. This kind of radially symmetrical acrosome might have independently emerged in species from different families due to convergent or parallel evolution. In *Prionospio*, biradially symmetrical acrosomes may have evolved from the nearest common ancestor within the Spionidae clade, thus evidencing close relationships between species. Further studies of sperm ultrastructure may elucidate relationships among numerous species of the *Prionospio* complex whose generic classification has been changed several times in the last century and remains uncertain at present (see Sigvaldadóttir 1998).

Acknowledgments: Our sincere thanks go to T. Worsfold for careful editorial assistance, and 2 anonymous reviewers for editing and making comments on the manuscript after submission. Financial support was provided by the National Science Council of Taiwan (NSC94-2621-B-001-014 and NSC95-2923-B-001-001-MY2) and the Russian Foundation for Basic Research (RFBR project 05-04-90589) through the Taiwan-Russian Joint Research Cooperative Programme (contract RP05B11), the RFBR (projects 09-04-01235, 09-04-10029, and 09-04-98540), and the Far Eastern Branch of the Russian Academy of Sciences (FEB RAS project 09-III-A-06-209).

REFERENCES

Blake JA. 2006. Spionida. *In* G Rouse, F Pleijel, eds. Reproductive biology and phylogeny of Annelida. Vol. 4. Reproductive biology and phylogeny. Enfield, NH: Science Publisher, pp. 565-638.

Blake JA, PL Arnofsky. 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. Hydrobiologia **402:** 57-106.

Bochert R. 1996a. An electron microscopic study of oogenesis in *Marenzelleria viridis* (Verrill 1873) (Polychaeta: Spionidae) with special reference to large cortical alveoli. Invertebr. Reprod. Dev. **29:** 57-69.

Bochert R. 1996b. An electron microscopic study of spermatogenesis in *Marenzelleria viridis* (Verrill, 1873) (Polychaeta: Spionidae). Acta Zool.-Stockholm **77:** 191-199.

Eckelbarger KJ. 1980. An ultrastructural study of oogenesis in *Streblospio benedicti* (Spionidae), with remarks on diversity of vitellogenic mechanisms in Polychaeta. Zoomorphology **94:** 241-263.

Eckelbarger KJ. 1988. Oogenesis and female gametes. *In* W Westheide, CO Hermans, eds. The ultrastructure of Polychaeta. Microfauna Marina 4. Stuttgart, New York: Gustav Fischer Verlag, pp. 281-307.

Eckelbarger KJ. 1992. Polychaeta: oogenesis. *In* FW Harrison, SL Gardiner, eds. Microscopic anatomy of invertebrates. Vol. 7: Annelida. New York: Wiley-Liss, pp. 109-127.

Eckelbarger KJ. 1994. Diversity of metazoan ovaries and vitellogenic mechanisms: implications for life history theory. Proc. Biol. Soc. Wash. **107:** 193-218.

Franzén Å. 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. Zool. Bidrag Uppsala **31:** 355-482.

Franzén Å. 1974. Sperm ultrastructure in some Polychaeta. *In* BA Afzelius, ed. The functional anatomy of the spermatozoon. Proceedings of the Second International Symposium Wenner-Green Center, Stockholm, Aug. 1973. Oxford and New York: Pergamon Press, pp. 267-278.

Franzén Å, SA Rice. 1988. Spermatogenesis, male gametes and gamete interactions. *In* W Westheide, CO Hermans, eds. The ultrastructure of Polychaeta. Microfauna Marina 4. Stuttgart, New York: Gustav Fischer Verlag, pp. 309-333.

Hannerz L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil, and Poecilochaetidae n. fam. in the Gullmar Fjord (Sweden). Zool. Bidrag Uppsala **31:** 1-204.

Jamieson BGM, GW Rouse. 1989. The spermatozoa of the

Polychaeta (Annelida): an ultrastructural review. Biol. Rev. **64:** 93-157.

Radashevsky VI. 2007. Morphology and biology of a new *Rhynchospio* species (Polychaeta: Spionidae) from the South China Sea, Vietnam, with the review of *Rhynchospio* taxa. J. Nat. Hist. **41:** 985-997.

Rice SA. 1981. Spermatogenesis and sperm ultrastructure in three species of *Polydora* and in *Streblospio benedicti* (Polychaeta: Spionidae). Zoomorphology **97:** 1-16.

Rice SA. 1992. Polychaeta: spermatogenesis and spermiogenesis. *In* FW Harrison, SL Gardiner, eds. Microscopic anatomy of invertebrates. Vol. 7: Annelida. New York: Wiley-Liss, pp. 129-151.

Rouse GW. 1988. An ultrastructural study of the spermatozoa from *Prionospio* cf. *queenslandica* and *Tripolydora* sp.: two spionid polychaetes with different reproductive methods. Acta Zool.-Stockholm **69:** 205-216.

Rullier F. 1960. Morphologie et développement du Spionidae (Annélide Polychète) *Polydora* (*Boccardia*) *redeki* Horst. Cah. Biol. Mar. **1:** 231-244.

Sawada N. 1984. Electron microscopical studies of spermatogenesis in polychaetes. *In* A Fischer, HD Pfannenstiel, eds. Polychaete reproduction: progress in comparative reproductive biology. Fortschritte der Zoologie, Band 29. Stuttgart, New York: Gustav Fischer Verlag, pp. 99-114.

Sigvaldadóttir E. 1998. Cladistic analysis and classification of *Prionospio* and related genera (Polychaeta, Spionidae). Zool. Scr. **27:** 175-187.

Söderström A. 1920. Studien über die Polychätenfamilie Spionidae. Inaugural-Dissertation. Uppsala, Sweden: Almquist & Wicksells.

Vortsepneva EV, AE Zhadan, AB Tzetlin. 2006. Spermiogenesis and sperm ultrastructure of *Asetocalamyzas laonicola* Tzetlin, 1985 (Polychaeta), an ectoparasite of the large spionid *Scolelepis* cf. *matsugae* Sikorsfi, 1994, from the White Sea. Sci. Mar. **70:** 343-350.

Williams JD. 2000. Spermiogenesis and spermatozoon ultrastructure of *Polydora neocaeca* (Polychaeta: Spionidae) from Rhode Island. Invertebr. Reprod. Dev. **38:** 123-129.