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Spermiogenesis and spermatozoon ultrastructure of *Polydora neocaeca* (Polychaeta: Spionidae) from Rhode Island

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Summary

The ultrastructure of spermatozoa and spermiogenesis *in Polydora neocaeca* is described. Most morphological features and measurements of cellular components of the spermatozoa are similar to previously studied members of the genus *Polydora.* However, early spermatids contain finely granular chromatin which becomes finely tibrillar in late spermatids and uniformly electron-dense in mature spermatozoa. Fibrillar chromatin morphology has not been reported within the Spionidae and may represent a species-specific trait or a previously unrecognized transient developmental process. Spermatozoa of *Polydora neoceaca* exhibit an elongate, cylindrical nucleus and a bullet-shaped acrosome that contains channels of electron transparent material. No microtubules are associated with nuclear elongation. The basal body of the axoneme occupies a short implantation fossa in the nucleus. The middlepiece contains 6-10 mitochondria closely aligned with the axoneme. Platelets, presumably carbohydrate storage bodies, are found along the nucleus and middlepiece. Additional electron-dense granules occur in the cytoplasm of the middlepiece.

Key words: Annelida, polydorid, sperm, reproduction

Introduction

Sperrnatogenesis, sperrniogenesis, and spermatozoon ultrastructure of polychaetes have been reviewed in several papers (Sawada, 1984; Franzén and Rice, 1988; Jamieson and Rouse, 1989; Rice, 1992; Rouse, in press). Sperm morphology of 30 polychaete species belonging to the family Spionidae was recently reviewed (Blake and Arnofsky, 1999). Sperm ultrastructure has provided investigators data for phylogenetic analyses and characters to distinguish members of sibling species complexes. While Rasmussen (1973) found that *Polydora cornuta* Bosc and *P. ciliata* (Johnston) were morphologically indistinct, studies of sperm ultrastructure have indicated that they are distinct species (Rice, 1981). Similarly, Eckelbarger and Grassle (1987) found significant differences in sperm ultrastructure of five sibling species of *Capitella*, and Nordheim (1989) showed that sibling species of *Protodrilus* could also be distinguished based on sperm ultrastructure. Due to the extent of inter- and intra-familial variability of reproductive modes, the use of sperm ultrastructure in phylogenetic analysis of polychaetes at taxonomic levels higher than genera has been questioned (Jamieson and Rouse, 1989; Nordheim, 1989; Rice, 1992). However, recent research suggests that sperm ultrastructure, when used in combination with morphological data, can be used in systematic studies at higher taxonomic levels (Rouse, 1995; Rouse and Fauchald, 1995).

In a review of the Spionidae from California, Blake (1996) resurrected and redefined the genus *Dipolydora* Verrill, to which a number of species formerly placed

in the genus *Polydoru* have been referred. Thus, sperm ultrastructure of three species in the newly restricted genus *Polydoru* has been examined with transmission electron microscopy (TEM): *P. cornuta* (Rice, 1981), *P. ciliata* (Franzén, 1974; Franzén and Rice, 1988), and *P. websteri* Hartman (Rice, 1981). Sperm ultrastructure of four additional polydorids (spionid genera that contain a modified fifth segment) has been described (Rice, 1981; Rouse, 1988; Rice, 1992). The purpose of the present investigation is to document spermiogenesis and spermatozoa in *Polydoru neocuecu*, a recently described species from the east coast of North America (Williams and Radashevsky, 1999). *P. neocuecu* closely resembles *P. caeca* Webster, a permanently invalid secondary homonym for which Hartman (1943) proposed the replacement name *P. websteri*. This work led to the taxonomic revision of *P. websteri* (Radashevsky and Williams, 1998; Radashevsky, 1999), including the designation of a lectotype supported by subsequent authors (Read, 2000; Petersen, 2000). Previous reports of *P. websteri* along the east coast of America may include *P. neocuecu;* both species bore into mollusc shells and are potential pests of commercially important bivalve species. The morphology, ecology, reproduction, and larval development of *P. neocaeca* have been documented (Williams and Radashevsky, 1999). Sperm ultrastructure of *P. neocuecu* is compared to previously studied spionids.

Materials and Methods

Specimen collection

Live *Crepidula fornicutu* Linné and gastropod shells inhabited by *Pagurus longicarpus* Say were collected intertidally at a depth of O-2 m along the bank of the Pettasquamscutt River underneath the Sprague Bridge in Narragansett, Rhode Island (41°27′N, 71°27′W) and on the mudflat of Galilee, Rhode Island (41°23′N, 71°30′W). Specimens of *P. neocuecu* were removed from their burrows by cracking the gastropod shells with a hammer or pliers. After removal, the specimens were isolated and maintained at approximately 12.0°C in unfiltered seawater or artificial seawater (Instant Ocean; salinity=32.0‰) until prepared for TEM preparation.

TEM preparation

Specimens were fixed for 2 h in 3% glutaraldehyde in Na-cacodylate buffer (pH 7.2) with 3% NaCl, placed in three changes of Na-cacodylate buffer for 30 min, each at room temperature, postfixed for 2 h in 1% OsO_4 in 0.1 M cacodylate buffer at 4°C, and dehydrated in an ascending ethanol series at 4°C. After warming to room temperature in 95% ethanol, the specimens were placed in four changes of 100% ethanol. Following dehydration, the samples were cleared in propylene oxide at room temperature, infiltrated and embedded in ERL-4206 resin, transferred to BEEM capsules and cured in a 70°C oven overnight (Spurr's 16-h cure mixture; Spurr, 1969).

Thick and thin sections were cut with a Sorvall MT-2 Porter-Blum ultramicrotome. Semi-thin sections (1 μm) were stained with 1% toluidine blue/l % sodium borate in distilled water. Thin sections (70-90nm) were mounted on 300 copper mesh grids and stained for 5 min in a drop of 2% uranyl acetate in 50% methanol and rinsed with distilled water. The sections were additionally stained for 5 min with 0.3% lead citrate solution followed by two rinses in 0.2N NaOH for 1 min each and distilled water. Both the uranyl acetate and lead citrate stains were filtered (0.22 μm) before use.

Thick sections were viewed using a compound microscope (Olympus BH-2). Thin sections were viewed in TEM mode using a JEOL 1200EX electron microscope.

Results

Sperm develop in the middle 1/3 to 1/2 of the body segments. The mean percent of segments containing developinggametes was 38.1 ± 14.8 (mean&SD, n=11) in males. The nephridia of gametogenic segments are enlarged in the posterior to medial area of the coelomic space and become tightly packed with sperm.

Spermiogenesis

A common cytoplasmic bridge or cytophore (Fig. lb) joins eight spermatids, which persists to late spermiogenesis.

Nucleus

In early spermatids the nucleus is spherical; later a depression forms at the anterior end of the nucleus which is occupied by the acrosome (Fig. la, b). The chromatin is finely granular in early spermatids (Fig. lb) and becomes fibrillar during maturation (Fig. 2a-c) as the nucleus elongates and becomes cylindrical. The chromatin fibrils are oriented along the dorso-ventral axis of the developing spermatids and

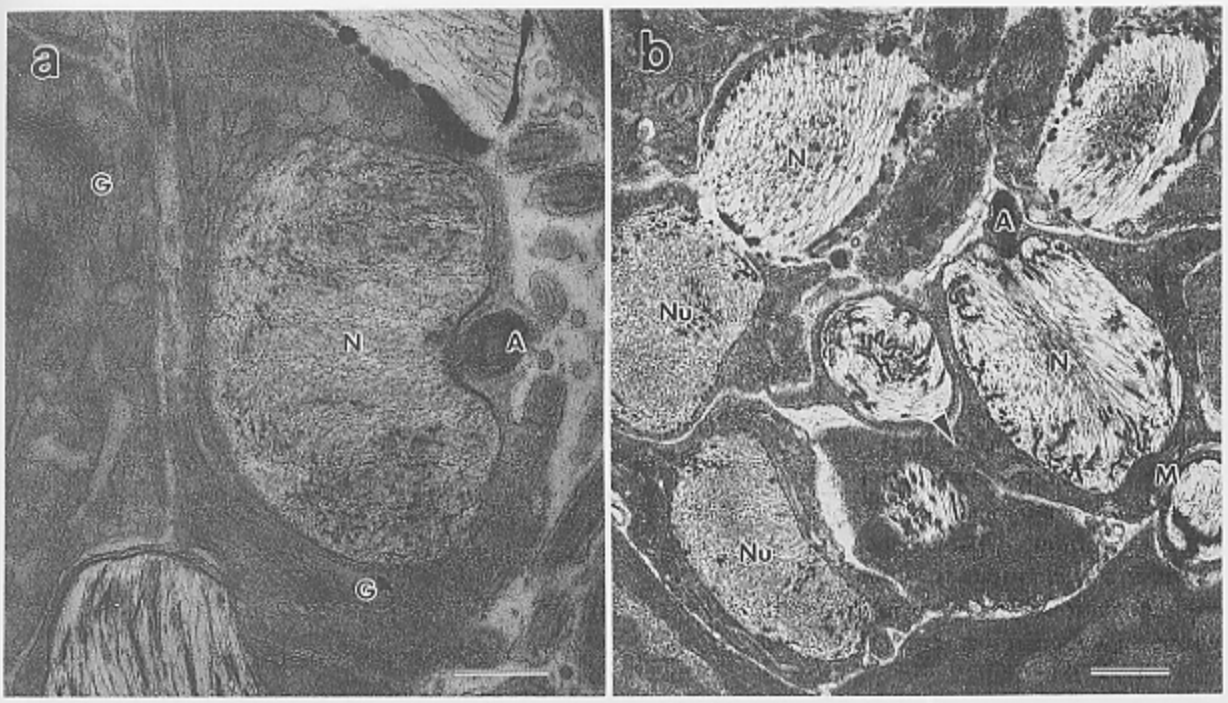

Fig. 1. Early spermatids of *Polydora neocaeca*. a. Cross section through spermatid nucleus of *Polydora neocaeca* showing (A) acrosome; (G) Golgi complex; (N) nucleus; scale bar = 500 nm. b. Cross section of early spermatids showing (A) acrosome; (Nu) nucleus with granular chromatin; (N) nucleus with fibrillar chromatin; (M) mitochondria; arrow indicates cytoplasmic bridge; scale bar = 1.0 µm.

globular electron-dense patches associated with the chromatin are found at the anterior end of the nucleus (Fig. 2c). At this stage the nuclei are 1.6±0.2 µm in diameter (n=11).

The chromatin remains fibrous until late spermiogenesis when it becomes lamellar (Fig. 2b,c). Condensation begins in the middle to posterior portion of the nucleus; chromatin in the anterior end of the nucleus condenses last. The nuclei of mature spermatozoa contain uniformly electron-dense chromatin and are 1.1±0.3 µm in diameter (Fig. 3a, c–e). The centriolar fossa (implantation fossa) is formed at the posterior end of the nucleus (Figs. 2a–c; 3a). No microtubules are associated with the process of nuclear elongation.

Acrosome

A proacrosomal granule is found near the Golgi complex in early spermatids. In later spermatids the cylindrical acrosome is situated in a depression at the anterior end of the nucleus (Fig. 1a, b). As the spermatid nucleus elongates during maturation, the acrosomal depression disappears. The posterior end of the acrosome contains channels of high electron density alternated with electron transparent areas (Figs. 2d; 3a, b).

Middlepiece

In early spermatids the mitochondria are spherical and situated near the base of the nucleus (Fig. 1b). Later in development up to ten mitochondria become packed around the base of the nucleus. As the middlepiece forms, the mitochondria elongate and migrate posteriorly, becoming closely aligned with the axoneme (Figs. 2a, b; 3a, f).

Flagellum

The basal body of the flagellum occupies a shallow centriolar fossa at the posterior end of the nucleus (Fig. 2a–c). The annulus (ring centriole) is positioned at the posterior end of the middlepiece and is composed of two sets of nine spokes radiating from the point of the junction of the plasma membrane with the axoneme (Fig. 3g). The flagella contain an axoneme with the typical 9+2 microtubule arrangement (Fig. 3h).

Cytoplasmic inclusions

Electron-dense platelets are found beneath the plasma membrane along the middlepiece to within approximately 1.5 µm of the anterior end of the nucleus

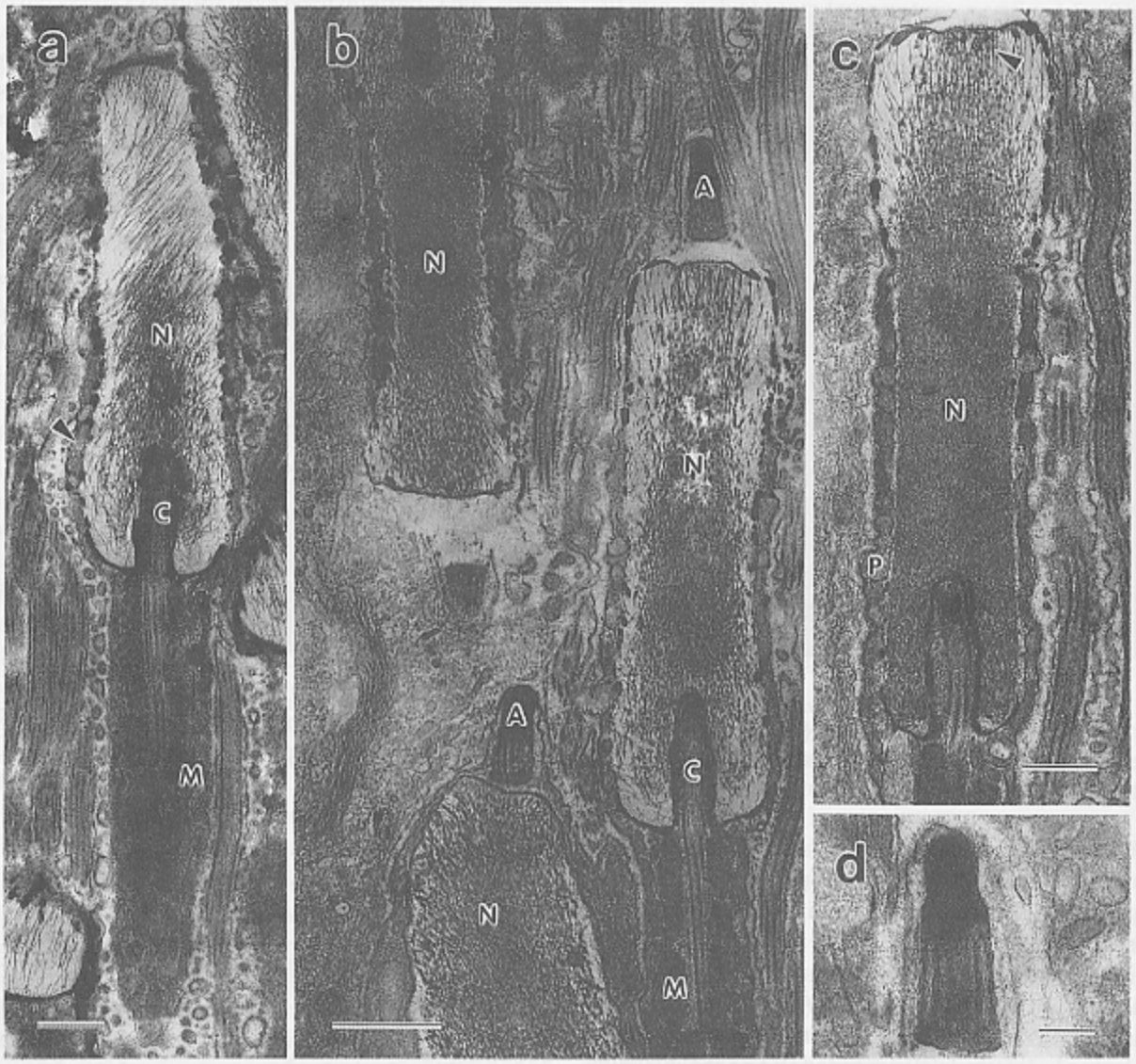

Fig. 2. Spermatids of *Polydora neocaeca* showing nuclear condensation. a. Longitudinal section of spermatid showing (N) nucleus; (M) mitochondria; (C) centriolar fossa; platelets indicated by arrowhead; scale bar = 500 nm. b. Longitudinal section of spermatids showing (A) acrosome; (N) nucleus; (C) centriolar fossa; (M) mitochondria; scale bar = 1.0 µm. c. Longitudinal section of late spermatid showing (N) nucleus; (P) platelets; arrowhead indicates globular electron-dense patches associated with chromatin fibrils; scale bar = 500 nm. d. Longitudinal section of acrosome showing channels of high electron density alternated with electron transparent areas; scale bar = 200 nm.

(Figs. 2a–c; 3a, d–f). The platelets are disc-shaped and are 0.3±0.1 µm in length (n=24). The middlepiece also contains electron-dense circular granules homogeneously dispersed between the mitochondria and platelets (Fig. 3a, f). The granules are approximately 15 nm in diameter.

Mature spermatozoa

The spermatozoa have elongate, cylindrical nuclei with uniformly electron-dense chromatin (Fig. 3a). The middlepiece contains 6–10 mitochondria which are moderately electron dense, with some cristae evident (Figs. 2a, b; 3f). The mitochondria extend along the axoneme to within 1.0 µm of the posterior end of the middlepiece. The acrosome is bullet shaped and positioned at the anterior end of the nucleus (Fig. 2d). The spermatozoa have the following measurements: acrosome 0.9±0.1 µm (n=10), nucleus 4.8±0.4 µm (n=11), middlepiece 4.2±0.4 µm (n=7), centriolar fossa 1.2 ± 0.1 (n=12).

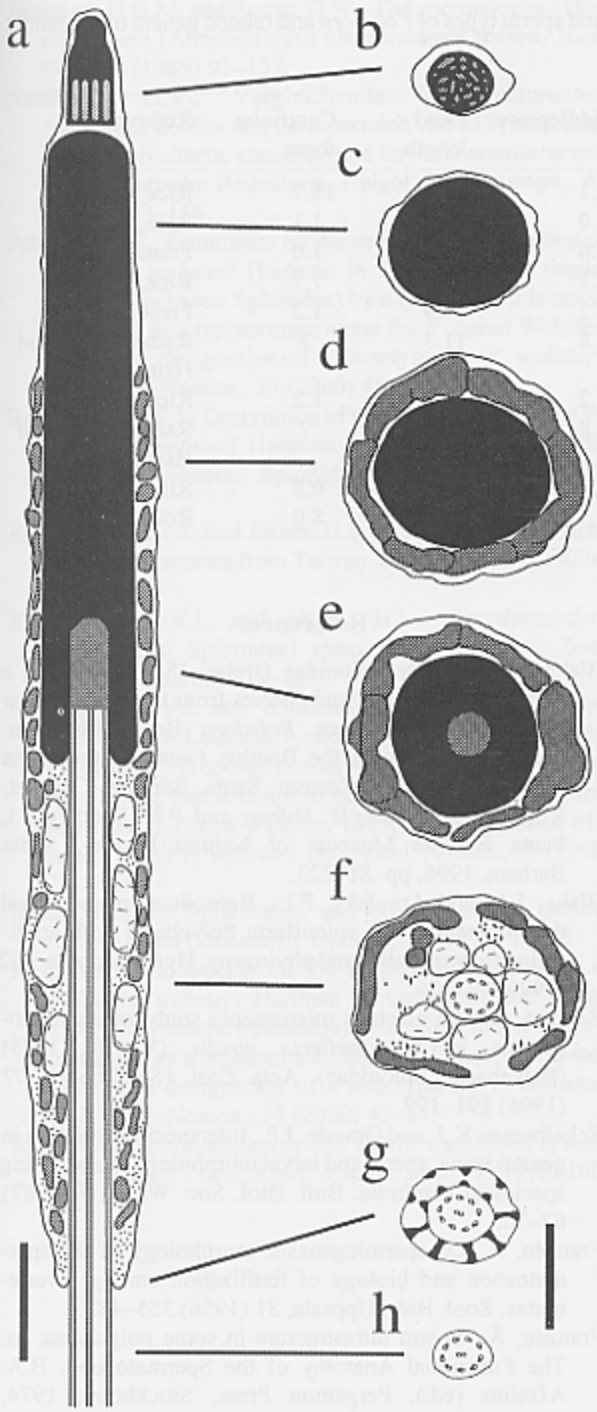

Fig. 3. Spermatozoa of *Polydora neocaeca*. a. Diagram of spermatozoon in longitudinal section. b. Cross section of posterior end of acrosome. c. Cross section of anterior end of nucleus. d. Cross section of middle portion of nucleus. e. Cross section of nucleus through the centriolar fossa. f. Cross section of middlepiece. g. Cross section through ring centriole. h. Cross section through the flagellum showing 9+2 axoneme. Scale bar: a = 1.0 µm; b–h = 500 nm.

Discussion

The family Spionidae contains species which produce ect-aquasperm (released into the water and fertilize externally) or introsperm (sperm transferred to females without exposure to water) (Blake and Arnofsky, 1999). Due to the lack of knowledge of fertilization biology, the sperm type has been inferred from spermatozoon morphology in many of these species. Members of the genus *Polydora* produce spermatophores which are released into the ambient water and collected by the palps of neighboring females. Upon contact with the palps, the spermatophores break apart and sperm travel to the seminal receptacles of females; fertilization is assumed to occur at the time of egg deposition (Rice, 1978). Several studies have shown that sperm morphology reflects the mode of sperm transfer (e.g., Franzén, 1956; Rice, 1981). The mode of fertilization in *P. neocaeca* has not been observed but based on sperm morphology and sperm found packed in the nephridia, the species presumably produces spermatophores.

P. neocaeca exhibits the characteristic conical acrosome, elongate nucleus, implantation fossa, and platelets found in other *Polydora*. The acrosome, nucleus and middlepiece lengths of *P. neocaeca* are similar to that of *P. ciliata* and *P. websteri*, but can be distinguished from *P. cornuta* based on centriolar fossa length (Table 1). The platelets surrounding the middlepiece and posterior portion of the nucleus in *P. neocaeca* have been found in all *Polydora* examined and are presumptive carbohydrate storage bodies derived from the Golgi complex, such as those found in other polychaetes (see Rice, 1992). Rice (1979) found in *P. cornuta* that the number of these carbohydrate bodies decreased while sperm are stored in the seminal receptacles. Acrosome and middlepiece morphology are similar to that of *P. cornuta, P. websteri, P. ciliata*, and *Dipolydora socialis* (Schmarda) (Table 1).

Late spermatids of *P. neocaeca* contain fibrous chromatin that condenses into a uniformly electron-dense nucleus, a feature which has not been found in previously studied spionids (Franzén, 1974; Rice, 1981; Franzén and Rice, 1988; Rouse, 1988; Rice, 1992; Bochert, 1996). A similar process of chromatin condensation has been documented in the holoplanktonic polychaetes, *Vanadis formosa* and *Krohnia lepidota* (Rice and Eckelbarger, 1989). In contrast, *P. cornuta, P. ciliata,* and *P. websteri* exhibit coarsely granular chromatin in early spermatids which develops into densely granular lamella in late spermatids and finally into uniformly electron-dense nuclei in mature

Table 1. Dimensions of the cellular components of spermatozoa and sperm types of *Polydora* and related genera of the family Spionidae. All measurements are reported in microns.

Species	Acrosome	Nucleus	Middlepiece	Head length	Centriolar fossa	Reference
Boccardiella hamata	*0.4*	15.7	18.1	24.4	15.7	Rice, 1992
Dipolydora socialis	1.1	5.4	4.0	10.5	1.1	Rice, 1981
Polydora ciliata	*0.9*	4.0	4.6	9.5	1.0	Franzén, 1974
Polydora cornuta	*0.8*	7.7	6.1	14.6	7.7	Rice, 1981
Polydora neocaeca	*0.9*	4.8	4.2	9.9	1.2	Present study
Polydora triglanda	1.9	5.6	3.8	11.3	?	Radashevsky and Hsieh, 2000a
Polydora websteri	1.0	5.9	3.2	10.1	1.2	Rice, 1981
Pseudopolydora diopatra	1.2	5.3	2.8	9.3	?	Radashevsky and Hsieh, 2000b
Pseudopolydora paucibranchiata	1.0	5.4	3.6	10.0	*0.8*	Rice, 1992
Tripolydora sp.	0.7	8.0	7.5	16.2	8.0	Rouse, 1988

spermatozoa (Franzén, 1974; Rice, 198 1). The spionid, *Streblospio benedicti* Webster, contains granular chromatin in early spermatids which develops into a patchy fibrous morphology in late spermatids and uniformly electron-dense in mature spermatozoa (Rice, 198 1). Chromatin condensation begins at the anterior end of the nucleus in *Streblospio benedicti.*

Rice (1992) suggested that closely related species (e.g., polydorids) might exhibit similar chromatin morphologies and patterns of condensation. The chromatin morphology of *P. neocaeca* spermatids may be unique to this species or may be exhibited only during a short period in the development of the spermatozoa not previously described in other members of the genus. Further investigations of sperm ultrastructure must be initiated in order to determine if the presence of fibrous chromatin is a species-specific trait or a transient developmental process in spermiogenesis. Additionally, identification of the method of sperm transfer and description of spermatophore morphology could help to distinguish this species from other closely related species in the genus.

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