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# Spermatogenesis in *Perotrochus quoyanus* (Fischer & Bernardi) (Gastropoda: Pleurotomariidae)

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## ABSTRACT

The male reproductive system, ultrastructure of spermatozoa and spermatogenesis are described for the pleurotomariid *Perotrochus quoyanus* (Fischer & Bernardi). Gross morphology of the male reproductive system of *P. quoyanus* agrees in all essential details with that of *Mikadotrochus beyrichii*. In all features, spermatozoa of *Perotrochus quoyanus* closely resemble those of *Perotrochus westralis* Whitehead, 1987, as well as spermatozoa of certain members of the Trochoidea (Trochidae, Liotiidae). Spermatozoa of *P. quoyanus* have a conical acrosomal vesicle with a finely ridged anterior layer, a short, rod-shaped nucleus with numerous lacunae, a midpiece consisting of five (rarely four) mitochondria surrounding a pair of centrioles, a rootlet connecting the centrioles and axoneme to the nucleus, and a flagellum (55–58 μm long) that is continuous with the distal centriole. Investigated species of Haliotidae and Scissurellidae (*Sinezona* sp.) differ from *Perotrochus* in acrosomal substructure, and, in the case of *Sinezona*, also in midpiece and nuclear morphology.

*Key Words*: Spermatozoa, Spermatogenesis, Mollusca, Gastropoda, Pleurotomariidae, *Perotrochus*, male reproductive tract.

## INTRODUCTION

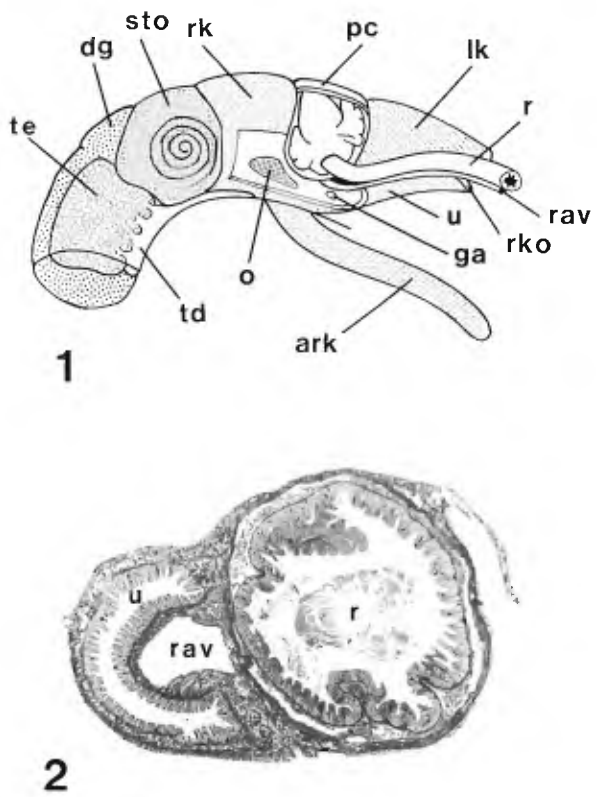
Living species of the Pleurotomariidae have a host of primitive gastropod features including a prominent labial shell slit as well as paired gills, auricles, osphradia, kidneys, and hypobranchial glands (Woodward, 1901; Bouvier & Fischer, 1902; Fretter, 1966; Hickman, 1984; Haszprunar, 1988). The Haliotidae and Scissurellidae, which share these features and classically have been assigned to the Pleurotomarioidea, are now considered sufficiently different from the Pleurotomariidae to warrant their placement into separate superfamilies, while the Pleurotomarioidea is considered most closely related to the Trochoidea based on the shared presence of a glandular urinogenital duct in females (for discussion see Haszprunar, 1988, 1989; McLean, 1989). Basic features of pleurotomariid anatomy, including radular morphology, have been known for more than a century (Dall, 1889; Bouvier & Fischer, 1899, 1902; Pelseneer, 1899; Woodward, 1901; Fretter, 1964, 1966), but it is only in recent years that the advent of deep-sea submersible craft has allowed the biology and habitat of living specimens to be studied in detail and *in situ* (Yonge, 1973; Harasewych *et al.*, 1988, 1992).

The field of comparative spermatology has, over the last twenty years, contributed greatly to the resolution of taxonomic and phylogenetic problems in numerous phyla (Baccetti & Afzelius, 1976; Wirth, 1984; Jamieson, 1987), including the Mollusca (Nishiwaki, 1964; Popham, 1979; Giusti, 1971; Kohnert & Storch, 1984a,b; Koike, 1985; Healy, 1983, 1986, 1988a; Hodgson *et al.*, 1988). Among the Gastropoda, studies of archaeogastropod (*s.l.*) spermatozoa and spermiogenesis (Kohnert & Storch, 1983; Azevedo *et al.*, 1985; Koike, 1985; Hodgson & Bernard, 1988; Healy, 1988b, 1989, 1990a,b) are becoming increasingly important since it is from this broad assemblage that origins for the caenogastropod and euthyneuran groups are sought (Cox, 1960; Ponder, 1973; Haszprunar, 1988). The recent discovery of pronounced sperm dimorphism in the trochoidean *Zalipais laseroni* Kershaw, 1955, including a multi-tailed, oligopyrene paraspermatozoon (Healy, 1990b), has drawn attention to the fact that comparatively little is known of the range of sperm morphologies existing in the Vetigastropoda. Healy (1988b) provided the first ultrastructural information on spermatozoa of the Pleurotomariidae [*Perotrochus westralis* Whitehead, 1987,[1] as *Pleurotomaria africana* (Tomlin, 1948)], but, because of limitations imposed by the state of preservation of the testes, was unable to trace events of spermatogenesis or give substructural detail of certain sperm features. Using glutaraldehyde-fixed testicular material of *Perotrochus quoyanus*, we present the first ultrastructural study of sperm development in a pleurotomariid gastropod.

[1] For a discussion of the nomenclature of this species, see Wagner and Coomans (1990).

## MATERIAL AND METHODS

Three male specimens of the pleurotomariid *Perotrochus quoyanus* (Fischer & Bernardi, 1856) were collected using the research submersible JOHNSON-SEA-LINK II, 1.03 nautical miles west of Ilets-á-Goyaves, off Basse Terre, Guadeloupe, West Indies (16°10′33″N, 61°49′00″W) at a depth of 350–360 m. Specimens were maintained in refrigerated aquaria for six days prior to cracking the shells and excising the testes. For scanning electron microscopy (SEM), samples were prepared by teasing apart sections of fresh testes in filtered seawater, transferring droplets of sperm suspension to coverslips, and fixing with glutaraldehyde vapor (25% glutaraldehyde in a covered petri dish). The coverslips were passed through a graded acetone series (20–100%), critical-point dried, and coated with gold-palladium. The sperm were examined using a Hitachi S-570 SEM at an accelerating voltage of 10 kv. Measurements are based on SEM photographs of sperm and calibration grids of standard size (2160 lines/mm at 15,000 X for acrosomes, nuclei, and mitochondria, 19.7 lines/mm at 1,500 X for tails). For transmission electron microscopy (TEM), 1–2mm$^3$ pieces of testicular tissue were fixed with 5% glutaraldehyde in 0.2 M cacodylate buffer and shipped to the senior author. Upon arrival, samples were further fixed in cold 3% 0.2M cacodylate-buffered glutaraldehyde and washed thoroughly in cacodylate buffer before being placed into a 1% solution of osmium tetroxide (prepared in 0.2M cacodylate buffer) for two hours. Tissues were again rinsed in buffer, then dehydrated using an ascending series of ethanols (20–100%). Spurr's epoxy resin was used to embed the tissues (Spurr, 1969). Ultrathin sections were cut with an LKB IV Ultratome, collected on uncoated 200-mesh copper grids, and stained using either the double lead stain of Daddow (1983) or a single lead procedure (20 minutes uranyl acetate, 10 minutes lead citrate). Sections were examined using a Hitachi 300 transmission electron microscope operated at 75 kV. Remaining soft tissues were fixed in 10% formaldehyde in seawater and transferred to 70% ethanol for dissection. Shell fragments retained as voucher specimens are housed in the National Museum of Natural History, Smithsonian Institution (USNM 878154).




**Figures 1–2.** Male reproductive tract of *Perotrochus quoyanus* (Fischer & Bernardi). **1.** Diagrammatic representation of male reproductive system, viewed from right side. Walls of pericardium and right kidney removed to reveal contents. **2.** Transverse section midway along pallial gonoduct, viewed from anterior. ark, anterior lobe of right kidney; dg, digestive gland; ga, genital aperture; lk, left kidney; o, opening of anterior lobe of right kidney; pc, pericardium; r, rectum; rav, right afferent branchial vessel; rk, right kidney; rko, right kidney opening; sto, stomach; td, testicular duct; te, testis; u, urinogenital duct.

## RESULTS

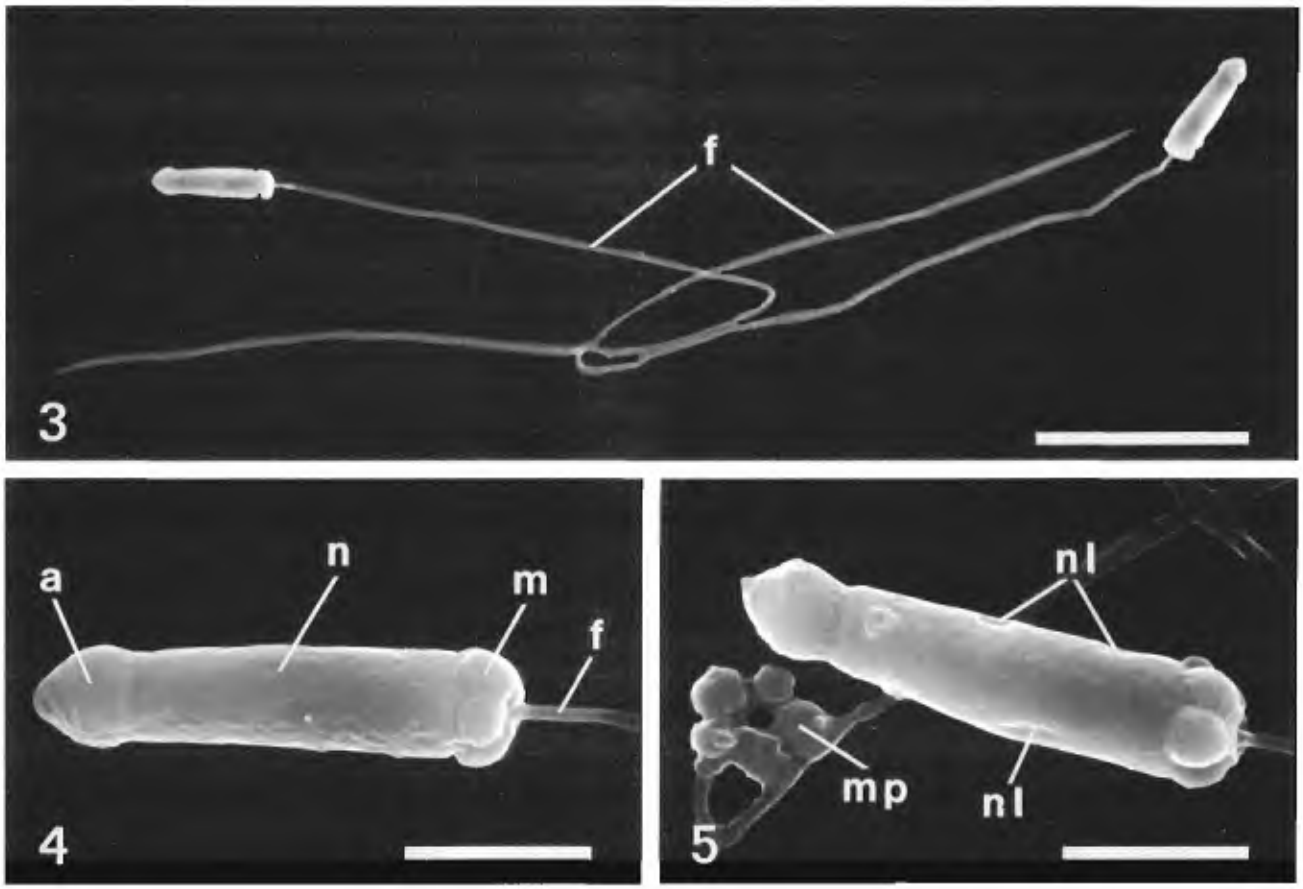
### Male Reproductive System

The mustard-colored testis (fig. 1, te) lines the right wall of the digestive gland (fig. 1, dg) , and empties into a thin-walled testicular duct (fig. 1, td) situated ventral to both these organs. This duct becomes tubular along the ventral surface of the stomach (fig. 1, sto) and continues anteriorly, emptying (fig. 1, ga) into the ureter portion of the right kidney (fig. 1, u) anterior and to the right of the opening (fig. 1, o) of the anterior lobe of the right kidney (fig. 1, ark), which is situated in the cephalic hemocoel. The ureter/urinogenital duct (figs. 1, 2, u) runs anteriorly along the roof of the mantle cavity to the right of the rectum (figs. 1, 2, r), envelops the right afferent branchial vessel (figs. 1,2, rav), and drains into the mantle cavity through a transversely oriented right kidney opening (fig. 1, rko), approximately 1/4 of the distance from the rear of the mantle cavity to the rear of the mantle slit. The urinogenital ducts of all three individuals lacked a glandular lining.

The testes of two animals were full of mature spermatozoa, while that of the third animal were almost entirely spent. Although only scattered groups of developing cells remained, we were able to identify basic features of spermatogonia, spermatocytes and spermatids.

### Mature Testicular Spermatozoa (SEM observations)

Spermatozoa of *Perotrochus quoyanus* consist of a conical acrosomal complex (fig. 4, a), a rod-shaped nucleus (fig. 4, n), a cluster of five, equal-sized, spherical mitochondria (fig. 4, m, fig. 5, mp) at the base of the nucleus, and a single 55–58 $\mu$m long flagellum (figs. 3–4, f, table




**Figures 3–5.** *Perotrochus quoyanus*. Mature testicular sperm, SEM. **3.** Two spermatozoa including entire flagella (f). **4,5.** Acrosome (a), nucleus (n) and mitochondria (m) of two spermatozoa. Nuclear lacuna (nl) and detached midpiece (mp) consisting of five mitochondria visible in figure 5.
Scale bars: **3** = 10 μm; **4,5** = 2 μm.

1). The acrosomal complex (externally, the acrosomal vesicle proper) is approximately 1.15 μm long, tapers slightly at contact with the nucleus, and has a maximum diameter of 1.18 μm (figs. 4,5, table I). The nucleus measures 3.7 μm in length, is broadest posteriorly, with a maximum diameter of 1.4 μm. Irregular indentations on the nuclear surface (fig. 5, nl) can be correlated by TEM with nuclear lacunae (figs. 6, 7, 11, nl) occurring beneath the nuclear and plasma membranes. These indentations are not, therefore, nuclear pores. Spherical mitochondria (diameter 0.8 μm) obscure the attachment site of the flagellum. The flagellum narrows markedly towards its insertion point within the midpiece (figs. 13,15).

### Mature Testicular Spermatozoa (TEM observations)

**Acrosome:** The acrosomal vesicle is broadly conical, with a rounded anterior surface and flattened basal surface (fig. 7, av). The vesicle has a length of 0.90–0.93 μm and the maximum diameter of 1.28 μm at its base is wider than the apex of the nucleus (figs. 7,8, n). A deep, narrow invagination extends anteriorly from the base of the vesicle and is filled with a diffuse, faintly fibrous material (figs. 8, 10, sm). Some sections clearly indicate an eccentric, slightly angular alignment for the invagination relative to the sperm longitudinal axis (figs. 8, 10). Beneath the anterior face of the acrosomal vesicle is an electron-lucent layer containing fine ridges with a periodicity of 12–14nm (figs. 7,9, rl). A similarly electron-lucent layer, lacking discernible ridged substructure, forms the basal rim of the acrosomal vesicle (fig. 7, br). A loose, fibrous deposit of subacrosomal material occupies the space between the base of the acrosomal vesicle and the nuclear apex (fig. 8, sm).

**Nucleus:** The mature nucleus (fig. 6, n) is short (3.7 μm) and almost cylindrical, with a shallow depression anteriorly (figs. 7, 8, n) and five (rarely four) shallow depressions surrounding a centriolar fossa posteriorly (figs. 14, 15, n). The anterior depression is associated with subacrosomal material (fig. 8, sm), while the posterior depressions act as sockets for the midpiece mitochondria (fig. 15, m). Dense material linking the proximal and

**Table 1.** Dimensions of mature spermatozoa from SEM observations. Linear measurements in μm. (n = 30, 10 sperm from each of three individuals.)

| | Mean | Range | Standard deviation ($\sigma$) |
|---|---|---|---|
| Acrosome | | | |
| Length | 1.10 | 1.01–1.19 | 0.07 |
| Width | 1.08 | 1.01–1.18 | 0.05 |
| Nucleus | | | |
| Length | 3.67 | 3.52–3.78 | 0.08 |
| Width (anterior) | 0.98 | 0.95–1.03 | 0.02 |
| Width (posterior) | 1.19 | 1.13–1.34 | 0.08 |
| Mitochondria | | | |
| Diameter | 0.80 | 0.68–0.93 | 0.09 |
| Flagellum | | | |
| Length | 56.5 | 52.7–61.1 | 3.26 |

distal centrioles is continuous with a hollow rootlet (figs. 14, 15, r), the bulbous end of which fills the centriolar fossa. Numerous irregularly shaped lacunae (figs. 6, 7, 11, nl) occur within the nucleus, some of which open underneath the nuclear membranes, though not to the plasma membrane or cell surface. Nuclear contents are highly electron dense and consist of tightly packed fibers (diameter 16 nm) set in a finely granular matrix.

**Midpiece:** Five (rarely four) spherical (diameter 0.6–0.8 μm) mitochondria (figs. 12, 15, m), each having curved, plate-like cristae, surround the proximal and distal centrioles to form the sperm midpiece (fig. 12). The centrioles (figs. 14, 15, pc, dc), arranged at a 90° angle to each other, are hollow, cylindrical structures composed of triplet microtubules and embedded in a pericentriolar matrix (triplets often obscured by matrix, see fig. 15 inset). Nine satellite fibers (figs. 15, 16, sf) connect the distal centriole to an annulus (figs. 15,16, an), a ring-shaped deposit of material lining the inner surface of the plasma membrane. The flagellar axoneme, therefore, is anchored to the midpiece and nucleus via the centrioles and rootlet as well as by the radial set of satellite fibers.

**Flagellum:** The flagellum measures approximately 55–58 μm in length and consists of a 9+2 axoneme enclosed by the plasma membrane (figs. 15, f; 17). Many spermatozoa were observed with an angularly offset flagellar-centriolar apparatus (fig. 13). This misalignment could be due to tight packing of sperm within the testis or even slight immaturity, since our SEM observations on free sperm show a normal, posteriorly projecting flagellum (figs. 3–5). Occasionally, a dense body is enclosed with the axoneme by the plasma membrane (fig. 17, db). Its position along the flagellum could not be determined. Further study is required to determine whether this structure is a true sperm feature of *P. quoyanus* or an artifact of fixation. In the distal region of the flagellum, the 9+2 substructure of the axoneme degenerates into singlet microtubules (fig. 17, arrow).

### Spermatogenesis

Spermatogenic cells present within the testis consisted principally of isolated clumps of spermatocytes and spermatids (fig. 18, spc, spt). Most of the testis space in ripe males was found to be almost totally occupied by tightly packed mature spermatozoa. To some extent the process of reconstructing events of spermatogenesis was hampered by the occurrence of many abnormally developing spermatocytes and spermatids. The morphology and possible significance of these cells is treated in the final section of these results.

**Spermatogonia:** Spermatogonia (fig. 19) were only rarely observed. They can be distinguished from spermatocytes and spermatids by their oblong, usually lobulate nucleus (fig. 19, n; length 6.0 μm, breadth 4.0 μm), prominent nucleolus (fig. 19, nc; diameter 0.7 μm), well-developed nuclear pores (fig. 19, arrows), numerous small mitochondria (fig. 19, m; diameter 0.3–0.4 μm), and more extensive cytoplasm. Endoplasmic reticular cisternae, where visible, are scattered and poorly developed. The presence of centrioles and Golgi complex could not be confirmed in the limited number of observed cells.

**Spermatocytes:** Spermatocytes (fig. 20) have a spherical to ovoid nucleus (fig. 20, n; diameter 4.0–4.5 μm) that appears to lack either a nucleolus or prominent nuclear pores. The small electron-dense patches visible in many cells (fig. 20, arrowheads) may be sites of synaptinemal complexes, although these structures are more easily discerned in moribund spermatocytes that have partially lost nuclear contents (fig. 38, arrows). Mitochondria (fig. 20, m; diameter 0.6–0.75 μm) markedly larger than those of spermatogonia are pressed slightly into the surface of the nucleus. Highly electron-dense proacrosomal vesicles (fig. 20, pav; diameter 0.1–0.2 μm) of Golgian origin are found throughout the cytoplasm. The axoneme (fig. 21, ax) develops intracellularly from one of a pair of orthogonally arranged centrioles (fig. 21, pc, dc) positioned close to the concave face of the Golgi complex (fig. 21, G). Even at this early stage in axoneme formation, satellite fibers (fig. 21, sf) are associated with the future

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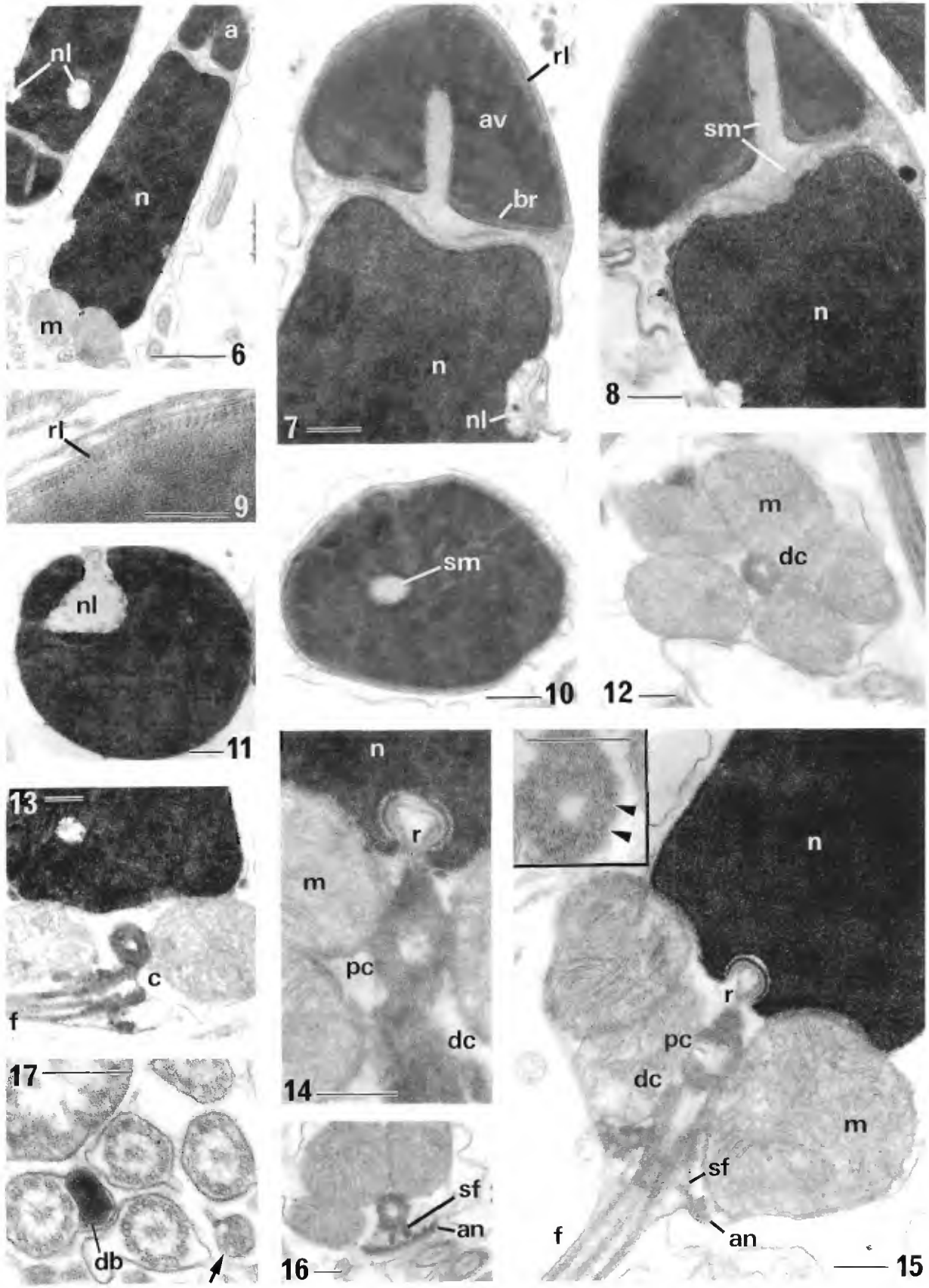
**Figures 6–17.** *Perotrochus quoyanus*. Mature testicular sperm, TEM. **6.** Acrosome (a), nucleus (n), nuclear lacunae (nl), and mitochondria (m) of two spermatozoa. **7,8.** Acrosomal vesicle (av) showing ridged layer (rl), basal rim (br), subacrosomal material (sm), and apex of nucleus (n) with nuclear lacuna (nl). **9.** Detail of ridged layer (rl) in acrosome. **10.** Transverse section through acrosomal vesicle showing subacrosomal material (sm). **11.** Transverse section through nucleus showing nuclear lacuna (nl). **12.** Transverse section through midpiece, five mitochondria (m) surround the distal centriole (dc). **13.** Angularly offset centriolar (c)—flagellum (f) apparatus of a spermatozoon. **14.** Detail of centriolar fossa and attached rootlet (r), proximal (pc) and distal centrioles




(dc), and mitochondria (m). **15.** Base of nucleus (n), rootlet (r), proximal (pc) and distal (dc) centrioles, satellite fibers (sf), annulus (an), flagellum (f), and mitochondria (m). Inset: triplet microtubules of proximal centriole (arrowheads). **16.** Oblique section showing distal centriole and three of nine satellite fibers (sf) attached to annulus (an). **17.** Transverse section through flagella. Note distal region (right) and dense body (db) (left). Arrow indicates singlet microtubules in distal region of flagellum.
Scale bars: **6** = 1 μm; **7,8,10–17** = 0.25 μm; **9** = 0.1 μm.

distal centriole. Endoplasmic reticular cisternae are poorly developed.

**Spermatids (Spermiogenesis):** Spermatids can be divided into three categories based on the condensed state of the nucleus: early cells, middle-stage cells, and advanced spermatids.

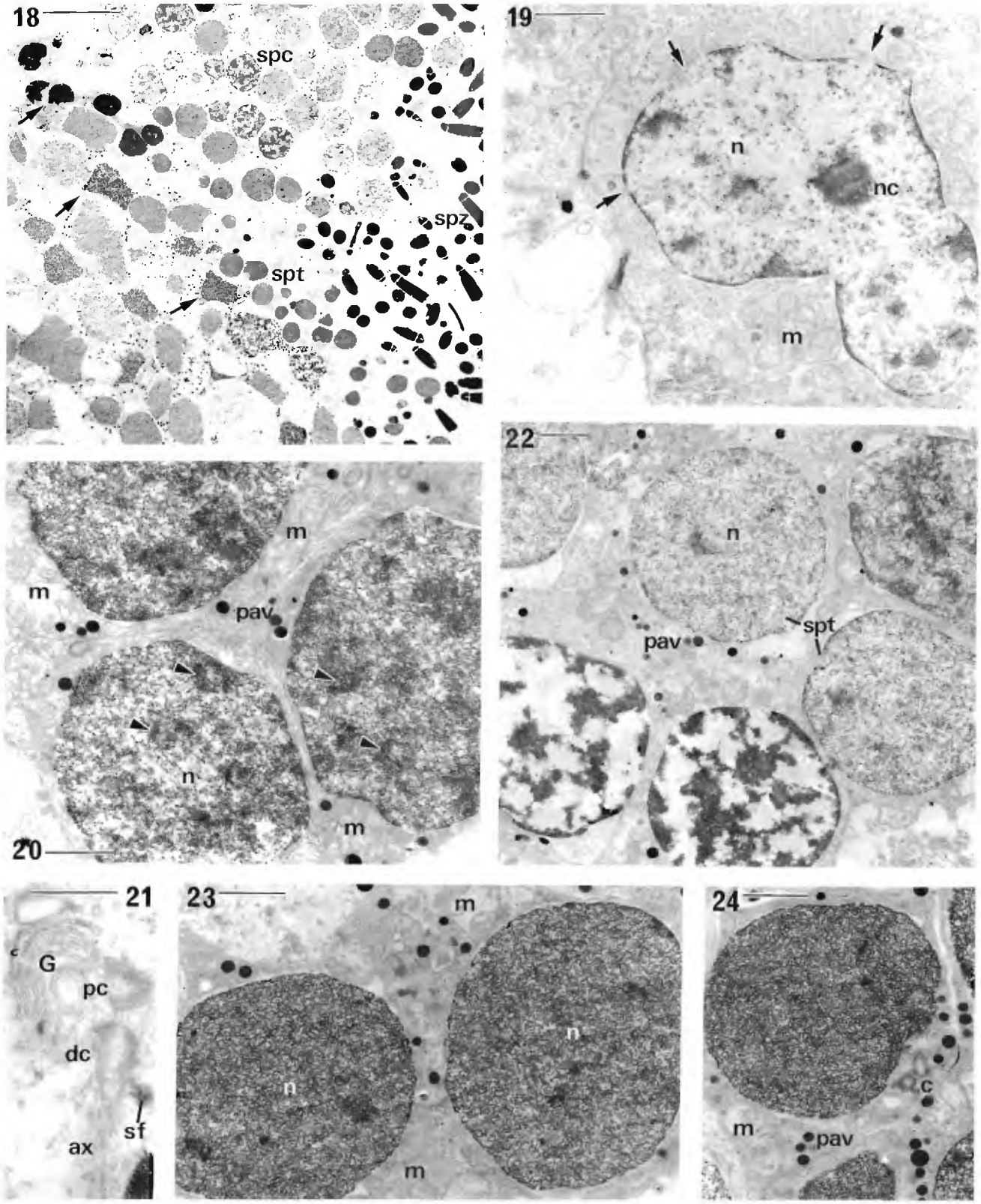
In early spermatids the nucleus (fig. 22, n) is spherical with pale-staining, fibrous contents. Middle-stage spermatids (figs. 23,24) are distinguished from earlier cells by a marked increase in the electron density of the nuclear fibers, and by a tendency of the mitochondria and centrioles to move toward the incipient posterior pole of the nucleus. Although multiple proacrosomal vesicles are still apparent within the cytoplasm of middle-stage spermatids (figs. 24, 25, pav), it is during this phase of spermiogenesis that the definitive acrosomal vesicle is formed by fusion of proacrosomal vesicles. In advanced spermatids, mitochondria and the acrosomal vesicle come to lie in shallow depressions of the nucleus, while the nucleus itself becomes oblong and its constituent fibers more condensed (figs. 26,27,36,37). In addition, the acrosomal vesicle undergoes pronounced changes in shape and substructure. Initially, the acrosomal vesicle is round and underlain by a thin disjointed layer of subacrosomal material (fig. 26, sm). As seen in figure 26, the site of first contact between the definitive acrosomal vesicle and nucleus may occur close to where the mitochondria are situated. Following attachment of the acrosomal vesicle to the condensing nucleus, vesicle contents become differentiated into a cluster of coarse granules (fig. 27, g) and a more extensive homogeneous portion (fig. 27, h). These granules become partitioned into two deposits that occupy anterior and posterior depressions in the homogeneous portion (fig. 28). Subsequently, an invagination of the homogeneous portion, but not the acrosomal membrane, begins to form anteriorly (fig. 29, arrowhead). The anterior cluster of granules transforms into a finely ridged layer (figs. 28–34, rl). As this layer grows, it extends into a deepening invagination of the homogeneous portion (figs. 29–31, arrowhead). The posterior cluster of granules forms the electron-lucent basal rim of the acrosomal vesicle. A thin deposit of dense material defines the basal region of the acrosomal membrane (figs. 28,29, dm). Late in spermiogenesis, the basal invagination of the acrosomal vesicle develops and is filled with subacrosomal material (fig. 31, sm). The anterior invagination of the homogeneous portion, which is not an invagination of the vesicle membrane, and the basal invagination of the vesicle are distinct and unconnected structures. The anterior invagination ultimately disappears, perhaps by a process of eversion, leaving the ridged electron-lucent layer (figs. 31,34,35, rl) and a small electron-lucent plate (figs. 31,34,35, asterisk).

Nuclear lacunae, so clearly apparent in mature testicular spermatozoa, only become evident in the very last stage of spermiogenesis. These spaces are not in contact with the exterior of the spermatid. The centriolar fossa (fig. 36, arrowhead) forms through invagination of the nuclear extension that lies between the posteriorly positioned mitochondria. Origins of the pericentriolar matrix and centriolar rootlet were not determined. Presumably the centrioles play some role in the growth of these structures.

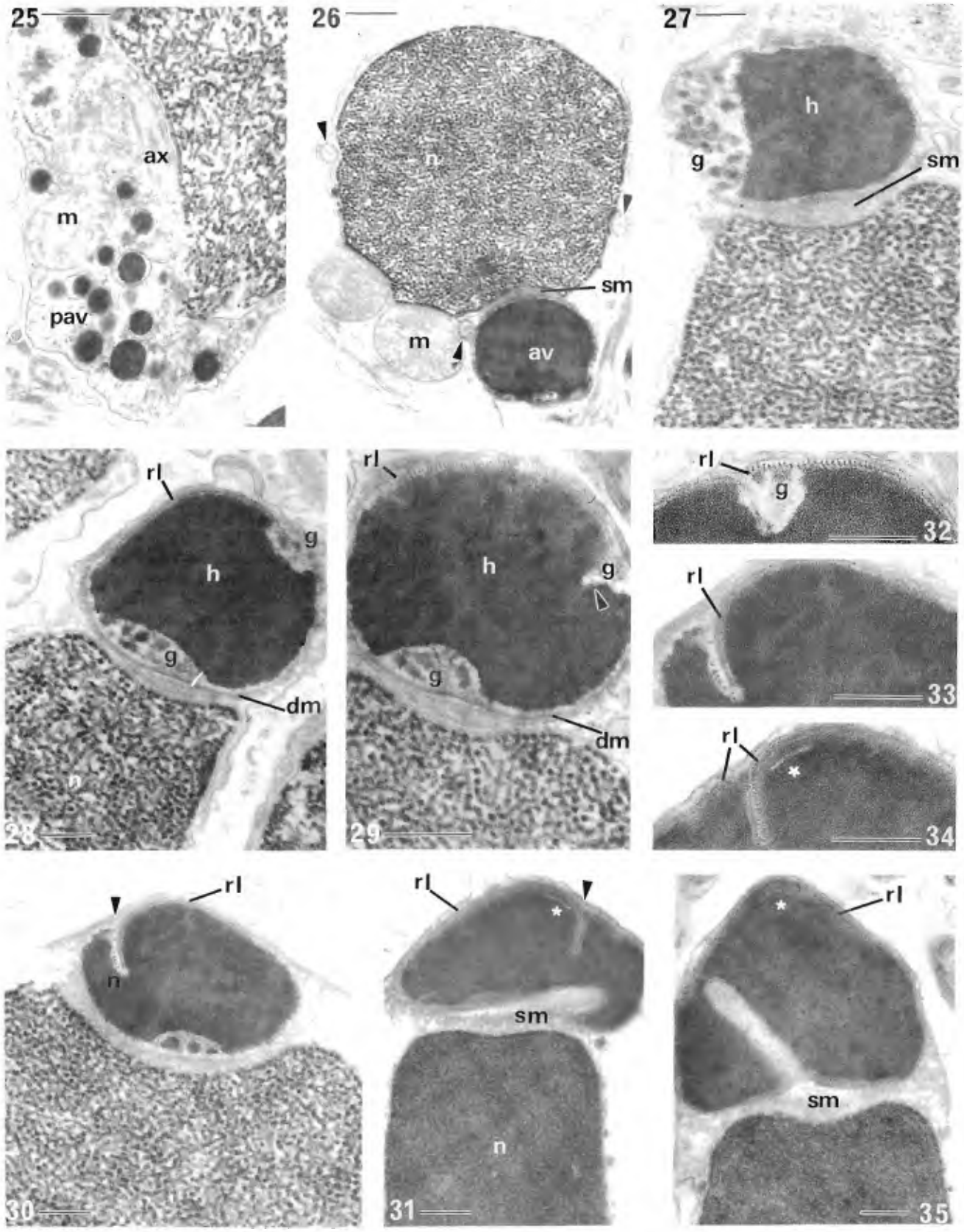
**Aberrant spermiogenic cells:** In addition to spermatogonia, spermatocytes and spermatids, the testes also contained numerous abnormally developing spermatocytes and spermatids. Some of these spermatocytes appear moribund (fig. 38). The spermatids, however, are clearly recognizable by their angular shape, evidently the result of cytoplasmic pressure from adjacent cells (figs. 18, spt; 39). Nuclear condensation and proacrosomal vesicle production seem to proceed as in normally developing cells. Gradually, however, the nucleus becomes oblong then angular and ultimately irregular in shape (figs. 39–42). Like normal spermatozoa, mature nuclei of the abnormal, presumably abortive, lines have numerous lacunae (fig. 42, nl) and a fibro-granulate substructure (figs. 40–42). The proacrosomal vesicles, rather than forming a definitive acrosomal vesicle, remain as a clump of unfused entities (Figure 40, inset, pav). Mitochondria, located in depressions of the nucleus, and axonemal profiles are often observed in developing and 'mature' aberrant spermatozoa (figs. 40, m; 41, ax). The position and number of centrioles was not determined.

## DISCUSSION

**Reproductive System:** The morphology of the male reproductive system of *Perotrochus quoyanus* agrees in all major features with that of *Mikadotrochus beyrichii*, the only other species of pleurotomariid for which the male reproductive system has been documented (Woodward, 1901). The female reproductive system of pleurotomariids differs from the male reproductive system in that the pallial portion of the right kidney, the urinogenital duct, is glandular. To date, only *M. beyrichii* (Woodward, 1901) and *Perotrochus midas* (Fretter, 1966) are confirmed to have glandular female urinogenital ducts. The duct of the holotype of *Perotrochus amabilis* (Bayer, 1963), an "immature" female on the basis of gonadal sections, lacked glandular elements, prompting Fretter (1964:179) to suggest that this was a young individual that had never spawned, and that glands may develop in the walls of this duct only as the gonad becomes mature. Examination of the shells of more than a dozen specimens collected in the intervening decades reveals that the holotype of *M. amabilis* is among the larger specimens known of this species. It is therefore unlikely that the holotype is an immature individual. Gonadal development of several western Atlantic pleurotomariids varies with season (Harasewych, unpublished observations), suggesting that the glandular lining of the urinogenital duct of females may develop and diminish cyclically. As evidenced by the three specimens used in this study, the urinogenital ducts of male pleurotomariids are not glandular, even during the spawning season. Nevertheless, absence of a glandular urinogenital duct may




**Figures 18–24.** *Perotrochus quoyanus*. Spermatogenesis. **18.** Survey section of testis showing spermatozoa (spz), developing spermatocytes (spc), and advanced spermatids (spt). Arrows indicate aberrant spermatids. **19.** Spermatogonium. Note lobulate nucleus (n), large nucleolus (nc), nuclear pores (arrows), and numerous small mitochondria (m). **20.** Spermatocytes. Note mitochondria (m), nucleus (n), presence of proacrosomal vesicles (pav), and putative synaptinemal complexes (arrowheads). **21.** Spermatocyte Golgi complex (G) close to proximal and distal centrioles (pc, dc) and axoneme (ax). Note satellite fibres (sf) associated with distal centriole. **22.** Early spermatids (spt) with homogeneously granular nuclei (n) and proacrosomal vesicles (pav). **23.** Middle stage spermatids with very electron dense fibrillar nuclei (n) and mitochondria (m). **24.** Middle stage spermatid showing pair of centrioles (c), proacrosomal vesicles (pav), and mitochondria (m).
Scale bars: **18** = 10 μm; **19,20,22–24** = 1 μm; **21** = 0.5 μm.




**Figures 25–35.** *Perotrochus quoyanus*. Acrosome development. **25.** Group of proacrosomal vesicles (pav) near mitochondrion (m) and axoneme (ax). **26.** Spermatid with acrosomal vesicle (av) contacting nucleus (n) near mitochondrion (m). Subacrosomal

not be a sufficient criterion for identifying male specimens.

**Spermatogenesis:** Despite the complicating factor of moribund and abnormally developing cells within the testis, spermatogenic stages of *Perotrochus quoyanus* resemble those reported for the Trochoidea (Kohnert & Storch, 1983; Azevedo *et al.*, 1985; Koike, 1985; Healy, 1989). Using museum-preserved tissues, Healy (1988b) was able to determine that acrosomal development in *Perotrochus westralis* involved the production of multiple proacrosomal vesicles. Fusion of proacrosomal vesicles into a definitive acrosomal vesicle has been demonstrated in many bivalves (Longo & Dornfeld, 1967; Kubo, 1977; Bernard & Hodgson, 1985; Hodgson & Bernard, 1986; Eckelbarger *et al.*, 1990), and, outside the Mollusca, in groups as disparate as the Polychaeta (Franzén, 1987) and Echinodermata (Dan & Sirakami, 1971; Chia & Bickell, 1983). In contrast, acrosome development in patelloidean gastropods centers on the production of a single, electron-lucent vesicle to which small vesicles from the Golgi cisternal edges fuse and contribute (Hodgson & Bernard, 1988). Our study has discovered details of acrosome development previously undescribed in the Vetigastropoda, including the differentiation of anterior and posterior extremities of the vesicle and formation of fine ridges in the anterior electron-lucent layer. There are reasons for believing that these events also occur in the Trochoidea. Mature acrosomes of trochids frequently show anterior and posterior electron-lucent layers (the anterior layer with ridges: Healy & Daddow unpublished). In spermatids of *Omphalius pfeifferi* (Philippi, 1846), the definitive acrosomal vesicle (Koike, 1985:plate 3D) closely corresponds to the stage illustrated herein for *Perotrochus quoyanus* (fig. 30). The origin of the subacrosomal material in *P. quoyanus* and in *Gibbula umbilicalis* (da Costa, 1778) (see Azevedo *et al.*, 1985) is unknown. A Golgian source seems unlikely, as this secretory organelle has migrated posteriorly by the time the definitive acrosomal vesicle has formed (the stage when subacrosomal material becomes visible). Possibly, the acrosomal vesicle itself is capable of organizing the accretion or polymerization of extravesicular materials within the cytoplasm. Takaichi & Dan (1977) proposed a similar origin for subacrosomal material in the pulmonate *Euhadra hickonis* (Kobelt, 1879). An interesting feature of spermiogenesis in *Perotrochus quoyanus* is the often distant positioning of the nuclear-contacted acrosomal vesicle relative to this vesicle's final position at the nuclear apex (see fig. 26). A comparable situation occurs in the trochid *Calliotropis glyptus* (Watson, 1879) (see Healy, 1989) and evidently in the turbinid *Lunella granulata* (Gmelin, 1791) (see micrographs of Koike, 1985). In *Perotrochus*, *Calliotropis*, and *Lunella*, however, the mature acrosomal vesicle lies at the nuclear apex, indicating that by some means, perhaps via nuclear shape change late in spermiogenesis or acrosomal movement, the vesicle attains its final position.

The pattern of nuclear condensation in *Perotrochus quoyanus* differs from that occurring in the Trochoidea in two respects: (1) the heterochromatin forms a homogeneous network of dense fibers, whereas the heterochromatin forms distinct granules in Trochoidea; and (2) nuclear lacunae appear only at the last stage of spermatid development, whereas the lacunae are well developed and visible at earlier stages in Trochoidea (Kohnert & Storch, 1983; Azevedo *et al.*, 1985; Koike, 1985; Healy, unpublished data). Unfortunately, no comparative information exists on nuclear condensation or, in fact, on any aspect of spermiogenesis, in the Haliotidoidea, Scissurelloidea, or Fissurelloidea. Initially the centriolar fossa of *Calliotropis glyptus* spermatids resemble the mature fossa of *Perotrochus* spp., but late in spermiogenesis, the solid rootlet and attached centrioles of *C. glyptus* become drawn into a greatly expanded fossa (Healy, 1989).

Incorporation of the future flagellar axoneme within the cytoplasm of spermatocytes and spermatids in *Perotrochus* warrants some comment. The same phenomenon can be seen in published micrographs of developing spermatids in the trochid *Monodonta turbinata* (Born, 1778) (see Kohnert & Storch, 1983) and in the turbinid *Lunella granulata* (see Koike, 1985). Unfortunately, neither Kohnert and Storch (1983) nor Koike (1985) offer a discussion of this positioning of the axoneme. In spermatids of the caudofoveate *Chaetoderma* sp., the proximal and distal centrioles each give rise to an axoneme within the cytoplasm (Buckland-Nicks & Chia, 1989). Of these two axonemes, only that associated with the future distal centriole survives into the mature spermatozoon. A similar situation has been reported by Eckelbarger *et al.* (1989) in paraspermatozoan development of the abyssal sea urchin *Phrissocystis multispina*, with the exception that both axonemes survive in the mature cell. Given the large number of abnormally developing spermatids observed in the ripe testes of our specimens of *Perotrochus quoyanus*, it cannot be ruled out that the intracellular axoneme in spermatids of this species may

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material (sm) is thin. Arrowheads indicate axonemal profiles. **27.** Acrosomal vesicle showing granule cluster (g) and homogeneous portion (h) of vesicle contents. Note also subacrosomal material (sm). **28.** Granules (g) distributed in anterior and posterior depressions of homogeneous portion (h). Note ridged layer (rl) and basal rim defined by dense material (dm). **29.** Beginning of invagination (arrowhead) of homogeneous portion (h). **30.** Penetration of ridged layer (rl) into deepening invagination (arrowhead) of homogeneous portion. **31.** Acrosome of late spermatid showing developing basal invagination of acrosomal vesicle as well as invagination of homogeneous portion (arrowhead). Asterisk indicates electron-lucent plate. **32–34.** Sequence of ridged layer (rl) development shown in detail. Granule cluster (g). Asterisk indicates electron-lucent plate. **35.** Nearly mature acrosome. Electron-lucent plate indicated by asterisk. Subacrosomal material (sm).
Scale bars: **25,26** = 0.5 μm; **27–35** = 0.25 μm.

be an aberrant rather than normal feature. Examination of testes from animals collected at the commencement of the reproductive season should resolve this question.

**Aberrations in spermatogenesis:** Few ultrastructural studies have dealt with the incidence of spermatogenic abnormalities in mollusks. Takaichi (1979) detailed radiation-induced malformations of the mitochondrial sheath and nucleus and duplication of the axoneme in spermatids of the pulmonate *Euhadra hickonis*. Dorange and Le Pennec (1989) noted binuclear spermatids and angularly dislocated axonemes in late spermatids of *Pecten maximus* (Linné, 1758) and regarded these features as true aberrancies. O'Foighil (1985) suggested that angular dislocation of the axoneme in testicular sperm of the bivalve *Lasaea subviridis* Dall, 1899 could be due to slight immaturity. In *Perotrochus quoyanus* we have observed numerous spermatocytes and spermatids that were undergoing a form of development clearly different from normal spermatogenesis. Leaving aside the phenomenon of sperm dimorphism (a well-documented and 'normal' occurrence in many Caenogastropoda—see Healy, 1988a for discussion), the irregular shape of the condensed nucleus (pressed into shape by abutting cells), and the apparent inability of proacrosomal vesicles to fuse into a single acrosomal vesicle, strongly suggest that these are abnormal cells. Bearing in mind that a certain background level of spermatogenic abnormality probably exists in many if not most animal species (Bryan & Wolosewick,1973; Baccetti & Afzelius, 1976), we believe the appearance of aberrant cells in *P. quoyanus* is probably a normal event heralding the end of the annual reproductive phase in this species. We base this view on the fact that all three males examined were either spent or contained principally mature spermatozoa in the testis (with isolated pockets of developing and abnormal spermatogenic stages).

**Spermatozoa:** Healy (1988b) has previously drawn attention to the structural similarities between spermatozoa of *Perotrochus westralis* and those of the Trochoidea, particularly Trochidae. Our observations on glutaraldehyde-fixed testis sperm of *P. quoyanus* have enabled us not only to confirm these similarities but also to expand on details of the *Perotrochus* spermatozoon as reconstructed by Healy from sea-water formalin/ethanol-preserved material.

The electron-lucent anterior layer of the *Perotrochus* acrosomal vesicle contains regularly spaced ridges. Similar ridges have elsewhere been observed in the Trochidae [*Austrocochlea constricta* (Lamarck, 1822), *Bankivia australis* (Menke, 1830); Healy & Daddow unpublished] and in the liotiid *Liotina peronii* (Kiener, 1839) (Healy & Ponder unpublished). It is interesting to note that the acrosomal vesicle of other pleurotomarioidean (*s.l.*) families (*Haliotis* spp.—Haliotidae, Lewis *et al.*, 1980; Sakai *et al.*, 1982; *Sinezona* sp.—Scissurellidae, Healy, 1990a) lack an electron-lucent anterior layer, whereas in the fissurellids *Scutus antipodes* Montfort, 1810 and *Montfortula conoidea* Reeve, 1842, a layer is present but exhibits no discernible ridged substructure (Healy, unpublished). The acrosomal complex in *Haliotis* and in *Sinezona* also differs from that of *Perotrochus* by having an extensive subacrosomal deposit similar to that seen in spermatozoa of some fissurellids (*Scutus antipodes*, *Montfortula conoidea*—see Healy, 1990a for illustrations) and many bivalve species (see references in Popham, 1979). The difference in appearance of subacrosomal material between *Perotrochus westralis* (rod-like) and *P. quoyanus* (diffuse, with some evidence of fibrous texture), may be due to use of different fixation methods (*P. westralis*—sea water formalin/ethanol; *P. quoyanus*—glutaraldehyde in cacodylate buffer). Azevedo *et al.* (1985) state that exposure of spermatozoa of *Gibbula umbilicalis* to sea water for five minutes resulted in a clearly defined rod (or perforatorium), derived from a formerly diffuse subacrosomal substance. It therefore seems possible that the subacrosomal rod of *P. westralis* may also be an end product of prolonged exposure to sea water. The dense layer of material visible within the subacrosomal material in the vicinity of the nuclear apex (see figs. 7, 8) may also be involved in rod formation. This layer was observed by Healy (1988b) in sea water-formalin/ethanol fixed sperm of *P. westralis* and interpreted as the possible remnants of nuclear membranes. Our observations, based on glutaraldehyde-fixed sperm of *P. quoyanus*, show that such material truly lies outside the intact nuclear and acrosomal membranes, and therefore constitutes part of the subacrosomal material.

The close resemblance of the crypt-like nuclear fossa of *Perotrochus* spp. (Healy, 1988b; this study) to the spermatid fossa of *Calliotropis glyptus* (Healy, 1989) has already been mentioned. In most vetigastropods and the Patellogastropoda, the centrioles are only superficially attached to a shallow nuclear invagination. In *Haliotis*, the proximal centriole itself sometimes occupies the shallow fossa (Lewis *et al.*, 1980; Sakai *et al.*, 1982), while in *Sinezona* (Scissurellidae) and *Calliotropis* (Trochidae) the centriole(s) and proximal portion of the axoneme are

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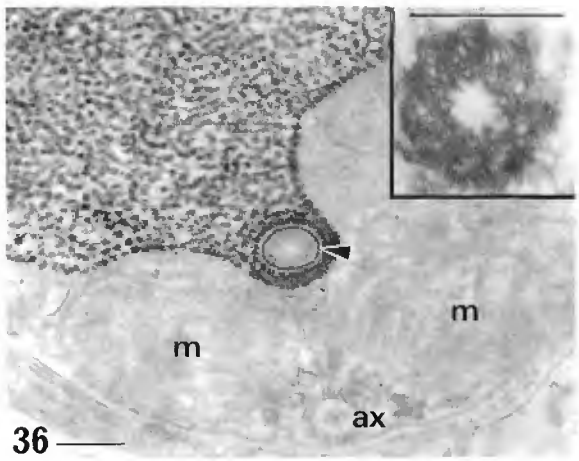
**Figures 36–42.** *Perotrochus quoyanus*. **36,37.** Developing nucleus (n), midpiece mitochondria (m), axoneme (ax), centriolar fossa (arrowhead), and proximal (pc) and distal centrioles (dc) of advanced spermatids. **36 Inset.** Triplet microtubules of centriole in advanced spermatid. **38.** Moribund spermatocyte showing synaptinemal complex (arrows), mitochondria (m), proacrosomal vesicles (pav), and axoneme profiles (arrowheads). **39.** Aberrant spermatid. Note angular shape of cell and its condensing nucleus (n), as well as the presence of proacrosomal vesicles and mitochondria (m). **40.** 'Mature' aberrant spermatozoon wedged between early, probably normal spermatids. Note mitochondria (m) in depressions at base of nucleus (n). **Inset.** Detail of unfused proacrosomal vesicles (pav) from aberrant spermatozoon. **41.** Fully 'condensed' nucleus (n) of aberrant sperm showing irregular shape and multiple axonemal profiles (ax). **42.** Nuclear lacunae (nl) of aberrant spermatozoon.
Scale bars: **36,37,40 Inset,41,42** = 0.25 μm; **38–40** = 1 μm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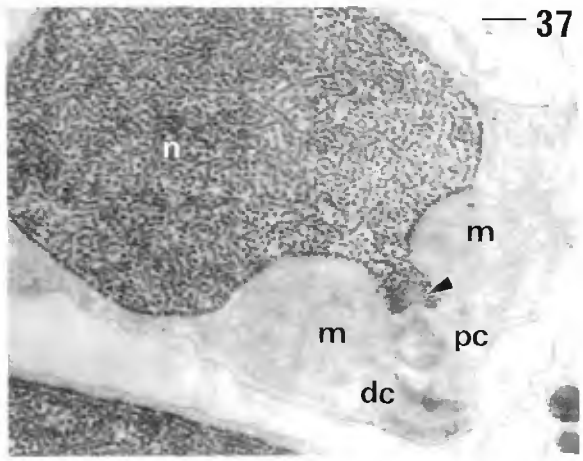
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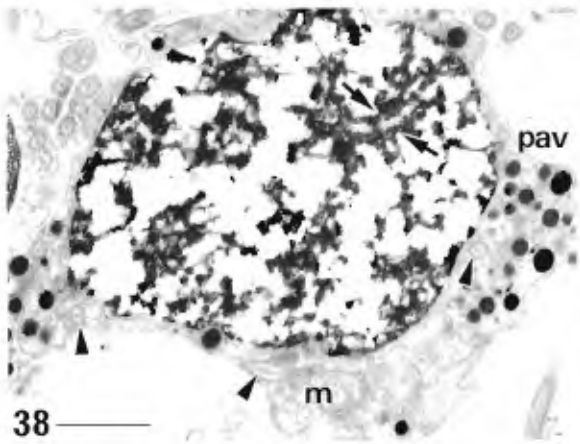
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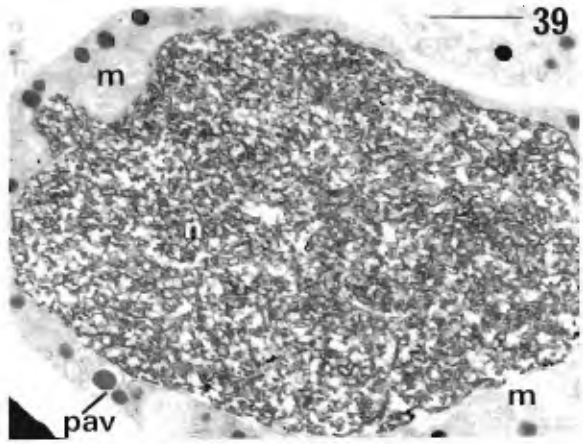
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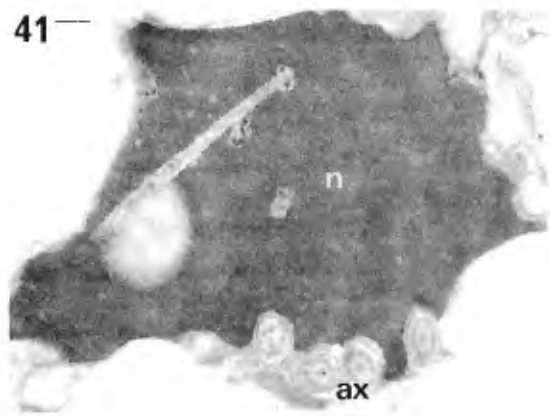
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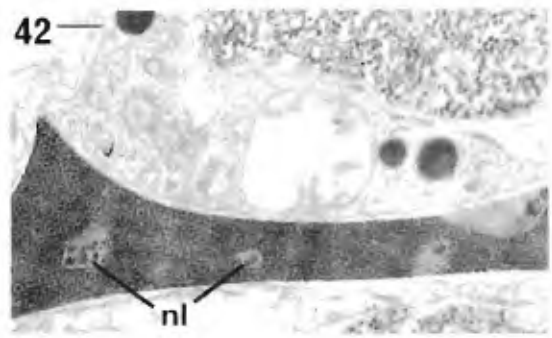
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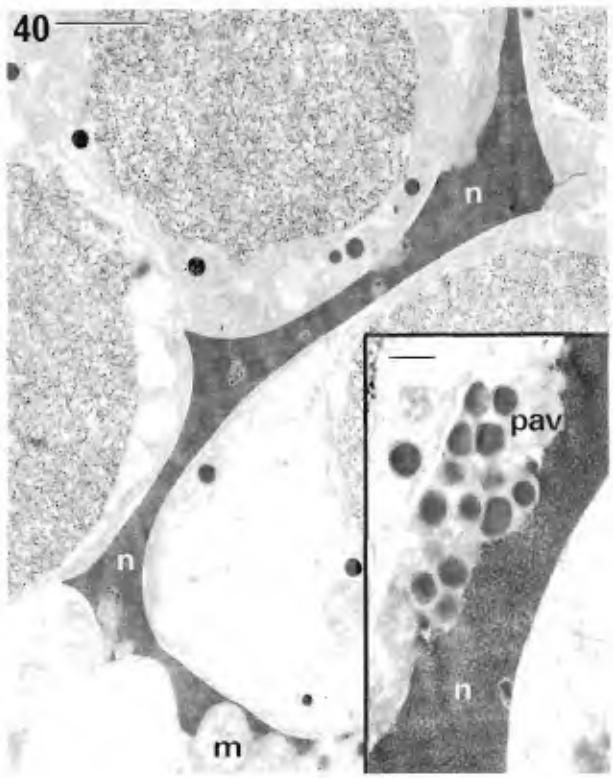
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actually contained within the fossa (Healy, 1989, 1990a). The ball-and-socket fitting of rootlet and centriolar fossa of *Perotrochus* spp. is unusual among gastropods, although a similar configuration occurs in the shipworm bivalve *Lyrodus bipartita* (Jeffreys, 1860) (see Figure 4 of Popham, 1974). Examination of other genera (*Mikadotrochus*, *Entemnotrochus*) may show this type of nuclear fossa to be a feature of all Pleurotomariidae.

Nuclear lacunae are widely reported in spermatozoa of externally fertilizing mollusks, polychaetes, brachiopods, echinoderms, as well as of some internally fertilizing groups (*e.g.* some teleosts, *Homo*) (Baccetti & Afzelius, 1976). Their occurrence or degree of development seems to be more closely linked with the mode of nuclear condensation than with the degree of modification of nuclear shape occurring during spermiogenesis. For example, in the trochoid *Zalipais laseroni*, the euspermatid nucleus undergoes marked elongation during condensation (fibro-granular pattern), but retains lacunae that ultimately fuse to form an axial tube within the mature, filiform nucleus (Healy, 1990b). Lacunae are usually not observed where nuclear condensation proceeds through either or both longitudinal fibrillar and lamellar phases (see Kaye, 1969; Horstman, 1970, Maxwell, 1983; Kohnert & Storch, 1984b; Koike, 1985).

The midpiece and satellite fiber/centriole complex of *Perotrochus* spp. are essentially as observed in the majority of Vetigastropoda and Patelloidea (Koike, 1985; Hodgson & Bernard, 1988; Healy, 1990a; Healy & Daddow unpublished), the Bivalvia (for references see Popham, 1979), Scaphopoda (Dufresne-Dube *et al.*, 1983) and Caudofoveata (Buckland-Nicks & Chia, 1989). The same arrangement of these organelles, clearly one associated with sperm tail attachment and stability, also occurs in spermatozoa of many other externally fertilizing animal species (for major references see Baccetti & Afzelius, 1976; Wirth, 1984).

The flagellum consists of an axoneme (9+2 microtubular substructure) sheathed by the plasma membrane. Our scanning electron micrographs reveal that the flagellum is narrower in diameter close to the nucleus. TEM observations suggest that this is probably the result of a more closely applied plasma membrane in this region of the flagellum, although slight narrowing of the axoneme does occur near the distal centriole (see figs. 4, 15). At present we cannot clarify the origin of the dense body sometimes observed within the flagellum (see fig. 17). It was not observed in longitudinal sections through the immediate post-nuclear region of the flagellum and could yet prove to be an artifact of fixation.

**Systematic Considerations:** If spermatozoa of *Perotrochus* spp. are representative of the Pleurotomariidae, then a closer relationship between this family and the Trochoidea (particularly Trochidae) than with the other pleurotomarioidean (*s.l.*) families Haliotidae and Scissurellidae seems evident. This conclusion accords both with Haszprunar's (1988, 1989) finding that no synapomorphies exist to unite the Pleurotomarioidea (*s.l.*), and with his decision to place the Haliotidae and Scissurellidae into separate superfamilies within the Vetigastropoda. The question as to whether ancestral vetigastropods were more like scissurellids than pleurotomariids (see Haszprunar, 1988, 1989 for discussion) cannot yet be resolved using sperm data alone because too many significant taxa (including the new hydrothermal vent groups) remain unstudied. Based on the present evidence, however, we suspect that spermatozoa of any stem vetigastropod would have resembled more closely the unmodified type of *Perotrochus* (Healy, 1988b; this paper) than the modified type of *Sinezona* (Healy, 1990a).

Vetigastropoda, Patellogastropoda and Neritimorpha can be distinguished on the basis of sperm features (especially acrosomal and nuclear) and features of spermiogenesis (dimorphic in the case of the Neritimorpha; rarely so in the Vetigastropoda) (Koike, 1985; Healy, 1988a, 1990a,b). It will be interesting to determine whether the cocculinids—once included in the Vetigastropoda (Salvini-Plawen, 1980) but since removed to a separate archaeogastropod suborder, Cocculiniformia (Salvini-Plawen & Haszprunar, 1987)—also show characteristic sperm and spermiogenic features.

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## LITERATURE CITED

Azevedo, C., A. Lobo-Da-Cunha, and E. Oliveira. 1985. Ultrastructure of the spermatozoon in *Gibbula umbilicalis* (Gastropoda, Prosobranchia), with special reference to acrosomal formation. Journal of Submicroscopic Cytolology 17:609–614.

Baccetti, B. and B. A. Afzelius. 1976. The biology of the sperm cell. Karger, Basel. 254 pp.

Bernard, R. T. F. and A. N. Hodgson. 1985. The fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna*. South African Journal of Zoology 20: 5–9.

Bouvier, E. L. and H. Fischer, 1899. Étude monographique des Pleurotomaires actuels. Bulletin of the Museum of Comparative Zoology, Harvard University 32:193–249.

Bouvier, E.L. and H. Fischer. 1902. L'organisation et les affinités des gastéropodes primitifs d'apres l'étude anatomique du *Pleurotomaria beyrichi*. Journal de Conchyliologie 50(2):117–272, pls. 2–6.

Buckland-Nicks, J. and F.-S. Chia. 1989. Spermiogenesis in

*Chaetoderma* sp. (Aplacophora). Journal of Experimental Zoology 252:308–317.

Bryan, J. H. D. and J. J. Wolosewick. 1973. Spermatogenesis revisited. II. Ultrastructural studies of spermiogenesis in multinucleate spermatids of the mouse. Zeitschrift Zellforschung 138:155–169.

Chia, F.-S. and L. R. Bickell. 1983. Echinodermata. *In*: Adiyodi, K.G. and R.G. Adiyodi (eds.). Reproductive biology of invertebrates. Volume II: Spermatogenesis and sperm function. John Wiley and Sons, Chichester, p. 545–620.

Cox, L.R. 1960. Thoughts on the classification of the Gastropoda. Proceedings of the Malacological Society of London 33:239–261.

Daddow, L.Y.M. 1983. A double lead stain method for enhancing contrast of thin sections in electron microscopy: a modified multiple staining method. Journal of Microscopy 129:147–153.

Dall, W.H. 1889. Reports on the results of dredgings, under the supervision of Alexander Agassiz, in the Gulf of Mexico (1877–78) and in the Caribbean Sea (1879–80), by the U.S. Coast Survey Steamer "Blake," Lieut. Commander C. D. Sigsbee, U.S.N., and Commander J. R. Bartlett, U.S.N., commanding. XXIX.—Report on the Mollusca. Part 2. Gastropoda and Scaphopoda. Bulletin of the Museum of Comparative Zoology, Harvard 18:1–492, pls.10–40.

Dan, J.C. and A. Sirakami. 1971. Studies on the acrosome. X. Differentiation of the starfish acrosome. Development, Growth and Differentiation 13:37–52.

Dorange, G. and M. Le Pennec. 1989. Ultrastructural characteristics of spermatogenesis in *Pecten maximus* (Mollusca, Bivalvia). Invertebrate Reproduction and Development 15:109–117.

Dufresne-Dube, L., B. Picheral, and P. Guerrier. 1983. An ultrastructural analysis of *Dentalium vulgare* (Mollusca, Scaphopoda) gametes with special reference to early events at fertilization. Journal of Ultrastructure Research 83:242–257.

Eckelbarger, K. J., C. M. Young, and J. L. Cameron. 1989. Ultrastructure and development of dimorphic sperm in the abyssal echinoid *Phrissocystis multispina* (Echinodermata: Echinoidea): implications for deep sea reproductive biology. Biological Bulletin 176:257–271.

Eckelbarger, K. J., R. Bieler, and P. M. Mikkelsen. 1990. Ultrastructure of sperm development and mature sperm morphology in three species of commensal bivalves (Mollusca: Galeommatoidea). Journal of Morphology 205:63–75.

Franzén, A. 1987. Spermatogenesis. *In*: Giese, A.C., J. S. Pearse, and V.B. Pearse (eds.). Reproduction of marine invertebrates. Volume IX: General aspects: seeking unity in diversity. Blackwell Scientific Publications and The Boxwood Press, California, p.1–47.

Fretter, V. 1964. Observations on the anatomy of *Mikadotrochus amabilis* Bayer. Bulletin of Marine Science of the Gulf and Caribbean 14(1):172–184.

Fretter, V. 1966. Biological investigations of the deep sea. 16. Observations on the anatomy of *Perotrochus*. Bulletin of Marine Science 16(3):603–614.

Giusti, F. 1971. L'ultrastruttura dello spermatozoo nella filogenesi e nella sistematica dei molluschi gasteropodi. Atti della Societa Italiana di Scienze Naturali e Museo Civico di Storia Naturale Milano, 112:381–402.

Harasewych, M.G., Pomponi, S.A. & Askew, T.M. 1988. Spongivory in pleurotomariid gastropods. The Nautilus 102 (3): 92–98.

Harasewych, M.G., Pomponi, S.A. & Askew, T.M. 1992. The ecology of western Atlantic pleurotomariid gastropods. The Nautilus, in press.

Haszprunar, G. 1988. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. Journal of Molluscan Studies 54:367–441.

Haszprunar, G. 1989. New slit-limpets (Scissurellacea and Fissurellacea) from hydrothermal vents. Part 2. Anatomy and relationships. Contributions in Science, Natural History Museum of Los Angeles County. Number 408. 17pp.

Healy, J.M. 1983. Ultrastructure of euspermatozoa of cerithiacean gastropods (Prosobranchia: Mesogastropoda). Journal of Morphology 178:57–75.

Healy, J.M. 1986. Ultrastructure of paraspermatozoa of cerithiacean gastropods (Prosobranchia: Mesogastropoda). Helgoländer Meeresuntersuchungen 40:177–199.

Healy, J.M. 1988a. Sperm morphology and its systematic importance in the Gastropoda. *In*: Ponder, W.F. (ed.). Prosobranch phylogeny. Malacological Review Supplement 4:251–266.

Healy, J.M. 1988b. Ultrastructural observations on the spermatozoa of *Pleurotomaria africana* Tomlin (Gastropoda). Journal of Molluscan Studies 54:309–316.

Healy, J.M. 1989. Ultrastructure of spermiogenesis in the gastropod *Calliotropis glyptus* Watson (Prosobranchia: Trochidae), with special reference to the embedded acrosome. Gamete Research 24:9–20.

Healy, J.M. 1990a. Sperm structure in the scissurellid gastropod *Sinezona* sp. (Prosobranchia, Pleurotomarioidea). Zoologica Scripta 19: 189–193.

Healy, J.M. 1990b. Euspermatozoa and paraspermatozoa in the trochoid gastropod *Zalipais laseroni* (Trochoidea: Skeneidae). Marine Biology 105:497–407.

Hickman, C.S. 1984. *Pleurotomaria*: pedigreed perseverance. *In*: N. Eldridge and S.M. Stanley (eds). Living fossils. Springer-Verlag, New York, N.Y., p. 225–231.

Hodgson, A.N. and R. T. F. Bernard. 1986. Ultrastructure of the sperm of three species of Mytilidae (Mollusca, Bivalvia). Gamete Research 15:123–135.

Hodgson, A.N. and R. T. F. Bernard. 1988. A comparison of the structure of the spermatozoa and spermiogenesis of 16 species of patellid limpet (Mollusca: Gastropoda: Archaeogastropoda). Journal of Morphology 195:205–223.

Hodgson, A.N., J. M. Baxter, M. G. Sturrock, and R. T. F. Bernard. 1988. Comparative spermatology of 11 species of Polyplacophora (Mollusca) from the suborders Lepidopleura, Chitonina and Acanthochitonina. Proceedings of the Royal Society of London (B) 235:161–177.

Horstman, E. 1970. Structures of caryoplasm during the differentiation of spermatids. Morphological Aspects of Andrology 1:24–28.

Jamieson, B. G. M. 1987. A biological classification of sperm types, with special reference to annelids and molluscs, and an example of spermiocladistics. *In*: H. Mohri (ed.). New horizons of sperm cell research . Japan Scientific Societies Press, Tokyo/Gordon and Breach Science Publications, New York, p. 311–332.

Kaye, J. 1969. The ultrastructure of chromatin in nuclei of interphase cells and spermatids. *In*: A. Lima-De-Faria (ed.). Handbook of molecular cytology. North Holland Publishing Company, Amsterdam, p. 361–380.

Kohnert, R. and V. Storch. 1983. Ultrastrukturelle Untersuchungen zur Morphologie und Genese der Spermien von Archaeogastropoda. Helgoländer Meeresuntersuchungen 36:77–84.

Kohnert, R. and V. Storch. 1984a. Vergleichend-ultrastrukturelle Untersuchungen zur Morphologie eupyrener Spermien der Monotocardia (Prosobranchia). Zoologische Jarhbucher 111:51–93.

Kohnert, R. and V. Storch. 1984b. Elektronenmikroskopische Untersuchungen zur Spermiogenese der eupyrenen Spermien der Monotocardia (Prosobranchia). Zoologischer Jarhbucher 112:1–32.

Koike, K. 1985. Comparative ultrastructural studies on the spermatozoa of the Prosobranchia (Mollusca: Gastropoda). Science Report of the Education Department of Gunma University 34:33–153.

Kubo, M. 1977. The formation of a temporary-acrosome in the spermatozoon of *Laternula limicola* (Bivalvia, Mollusca). Journal of Ultrastructure Research 61:140–148.

Lewis, C.A., D. L. Leighton, and V. D. Vacquier. 1980. Morphology of abalone spermatozoa before and after the acrosome reaction. Journal of Ultrastructural Research 72: 39–46.

Longo, F.J. and E. J. Dornfeld. 1967. The fine structure of spermatid differentiation in the mussel, *Mytilus edulis*. Journal of Ultrastructure Research 20:462–480.

Maxwell, W.L. 1983. Mollusca. *In*: Adiyodi, K.G. and R.G. Adiyodi (eds). Reproductive biology of invertebrates. Volume II. Spermatogenesis and sperm function. John Wiley and Sons Ltd., Chichester, p. 275–319.

McLean, J.H. 1989. New slit-limpets (Scissurellacea and Fissurellacea) from hydrothermal vents. Part 1. Systematic descriptions and comparisons based on shell and radular characters. Contributions in Science, Natural History Museum of Los Angeles County. Number 407. 29 pp.

Nishiwaki, S. 1964. Phylogenetical study on the type of the dimorphic spermatozoa in Prosobranchia. Science Report of the Tokyo Kyoiku Daigaku (B) 11:237–275.

O'Foighil, D. 1985. Fine structure of *Lasaea subviridis* and *Mysella tumida* sperm (Bivalvia, Galeommatacea). Zoomorphology 105:125–132.

Pelseneer, P. 1899. Recherches morphologiques et phylogénétiques sur les mollusques archaiques. Memoires couronnés et Memoires des savants etrangés, l'Academie Royale des Sciences de Belgique 57:1–112, Plates I-XXIV.

Ponder, W. F. 1973. The origin and evolution of the Neogastropoda. Malacologia 12(2):295–338.

Popham, J.D. 1974. Comparative morphometrics of the acrosomes of the sperms of "externally" and "internally" fertilizing sperms of the shipworms (Teredinidae, Bivalvia, Mollusca). Cell and Tissue Research 150:291–297.

Popham, J.D. 1979. Comparative spermatozoon morphology and bivalve phylogeny. Malacological Review 12:1–20.

Sakai, Y. T., Y. Shiroya, and K. Haino-Fukushima. 1982. Fine structural changes in the acrosome reaction of the Japanese abalone, *Haliotis discus*. Development, Growth and Differentiation 241:531–542.

Salvini-Plawen, L.v. 1980. A reconsideration of systematics in the Mollusca (phylogeny and higher classification). Malacologia 19:249–278.

Salvini-Plawen, L. v. and G. Haszprunar. 1987. The Vetigastropoda and the systematics of streptoneurous Gastropoda. Journal of Zoology, London (A) 211:747–770.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31–43.

Takaichi, S. 1979. Spermiogenesis in the pulmonate snail, *Euhadra hickonis* IV. Effect of X-rays on the spermiogenesis. Development, Growth and Differentiation 21:87–98.

Takaichi, S. and J. C. Dan. 1977. Spermiogenesis in the pulmonate snail *Euhadra hickonis*. I. Acrosome formation. Development, Growth and Differentiation 19:1–14.

Wagner, H. P. and H. E. Coomans. 1990. Review of the *Perotrochus africanus*-complex, with a note on the nomenclature of the western Australian species. Gloria Maris 29(3):41–52.

Wirth, U. 1984. Die Struktur der Metazoen-Spermien und ihre Bedeutung für die Phylogenetik. Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg (NF) 27:295–362.

Woodward, M.F. 1901. The anatomy of *Pleurotomaria beyrichi* Hilg. Quarterly Journal of Microscopical Science 44(2): 215–268.

Yonge, C. M. 1973. Observation of the pleurotomariid *Entemnotrochus adansoniana* in its natural habitat. Nature 241(5384):66–68.