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Spermatogenesis in the harvestman *Vonones sayi* (Simon) (Opiliones: Laniatores: Cosmetidae)

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Summary

Transmission and scanning electron microscopes in addition to light microscopic techniques were used to study spermatogenesis and spermatozoa of the harvestman *Vonones sayi*. Spermatogenesis is characterized by formation of a shell stage, nuclear invagination, elongation, and development of a covering of numerous microvilli. Encysted non-viable spermatozoa are phagocytized in the testis. The unusual covering of microvilli on the spermatozoa are also reported for the cosmetid harvestman genera *Cynorta* and *Paecilaemana*.

Introduction

Arthropodan spermatozoa range from elongated forms with long flagella to spheroid encysted shapes lacking flagella. It is not surprising then that a progression of motility in spermatozoa is found from non-motile to highly motile types. Spermatozoa are often used in studies of phylogeny (Baccetti, 1979; Juberthie & Manier, 1976a, 1978; Phillips, 1976; and others cited therein).

Studies of spermatogenesis and spermatozoa in Opiliones remain incomplete. Spermatogenesis of the major groups of Opiliones follows a typical pattern including spermatids which lack flagella or axonemes, and maintaining a kinetic centre composed of centrioles (Tripepi, 1983). Despite this supposed common trend among Opiliones, many variations in developmental patterns and eventual morphology of mature spermatozoa are found. Spermatogenesis in Cyphophthalmi is characterized by formation of "flagellated" spermatids with two types of development. The first is considered normal, and the second atypical or aberrant with the formation of spermatic spheres (Juberthie, Manier & Boissin, 1976). Palpatores have a single type of sperm development and their spermatozoa are spheroid and aflagellate (Juberthie & Manier, 1976b, 1977a, 1977b; Reger, 1969; Sokolow, 1929, 1930; Tripepi, 1983; Warren, 1933). Laniatores have spermatogenic development similar to the Palpatores, lacking flagella and possessing a kinetic centre with two centrioles of the 9 + 0 type (Juberthie & Manier, 1977c; Sotelo &

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Trujillo, 1954; Warren, 1933; Wettstein & Sotelo, 1965).

The subject of this study is *Vonones sayi* (Simon), a member of the Cosmetidae (suborder Laniatores). Parthasarathy & Goodnight (1958) report some observations on the spermatogenesis of this species. In this communication we describe, using SEM, TEM, and light microscopy, spermatogenesis and spermatozoa of *V. sayi*.

Materials and Methods

Mature males of *Vonones sayi* were collected 6.4 km east of Kerrville, Kerr County, Texas, USA. The male reproductive organs were dissected in insect Ringer's solution and immediately placed in 5% gluteraldehyde in 0.2 M cacodylate buffer for 24 hours at about 5°C. They were post-fixed in 1% osmium tetroxide in 0.2 M buffer for three hours. Specimens were washed in buffer for five minutes, dehydrated in an ethanol series (70%, 90%, 100%), and embedded in Spurr's medium. Sections 0.25-0.4 μm thick were stained with toluidine blue and cover-slipped for light microscopic examination. Thin sections (500-900 Å) were made with glass knives on a Reichert OM U2 microtome, and stained in uranyl acetate and lead citrate. Thin sections were observed and photographed using a transmission electron microscope (Hitachi model HS-8-2).

The reproductive system of one male was examined by a Scanning Electron Microscope (Hitachi model S-570). Following dissection, the reproductive system was preserved in 80% ethanol, critically point dried, and then gold coated. Following observations of external morphology, testes were cut open, remounted, and gold coated for examination of sperm.

The vasa deferentia were dissected from living animals and crushed in Ringer's solution to obtain spermatozoa. Mature spermatozoa were examined for motility with a phase contrast microscope.

Additional material used for light microscopic examinations were whole mounts of spermatozoa (from vasa deferentia) that were stained and mounted in 1% aqueous toluidine blue. Specimens used for these preparations were: *Cynorta* sp. (Madden Dam area, north of Panamá, Panamá), *Paecilaemana quadripunctata* Goodnight & Goodnight (Madden Dam area), *Vonones* sp. (Copala, Sinoloa, México), *Vonones ornata* (Gainesville, Alachua County, Florida, USA), and *Vonones sayi* (8 km east of Nashville, Davidson County, Tennessee, USA, and 4.8 km south of Reynolds, Jefferson County, Nebraska, USA).

Results

Three sections were made through the testis: two subdistal and one medial. Observed spermatogenic stages from these areas are discussed collectively because all developmental stages were present in all sections. Spermatids of similar developmental stages were always observed together in a bunch, with several of these bunches occurring throughout the testis. Groups of early stage spermatids were often bordered by groups of later developmental stages. No linear or belted arrangements of developing sperm were observed.

Primary spermatocytes occurred in large numbers and were tightly packed together. Numerous gap junctions were observed between the spermatids. Each spermatid contained a large spheroid nucleus with evenly dispersed chromatin. Many randomly distributed small mitochondria occurred outside the nuclear envelope (Fig. 1). As development progressed, portions of the nuclear envelope became dense. The chromatin underwent condensation and compaction with subsequent migration to form a dense layer underneath the nuclear envelope surrounding a less dense area (Fig. 2). A few distinct pores were visible in the nuclear envelope (Fig. 3). Reduction in cytoplasmic volume surrounding the nucleus began during this time with a slight reduction of cell diameter. Cell organelles migrated towards the area of nuclear invagination, forming a polarized cell. No distinct centrioles were observed in the area of polarization. Cell organelle migration, and chromatin compaction continued, resulting in a later stage spermatid with obvious cytoplasmic reduction (Fig. 6). The cells were spherical, with the nucleus somewhat flattened on one side. By this stage approximately half of the nuclear envelope had become dense, possibly due to membrane fusion. A Golgi complex was present at the site of polarization. Much of the cytoplasmic matrix had been eliminated by pinocytosis, leaving open spaces between spermatids (Fig. 6). The nuclear chromatin again became less compacted and more dispersed throughout the nucleus. A small amount

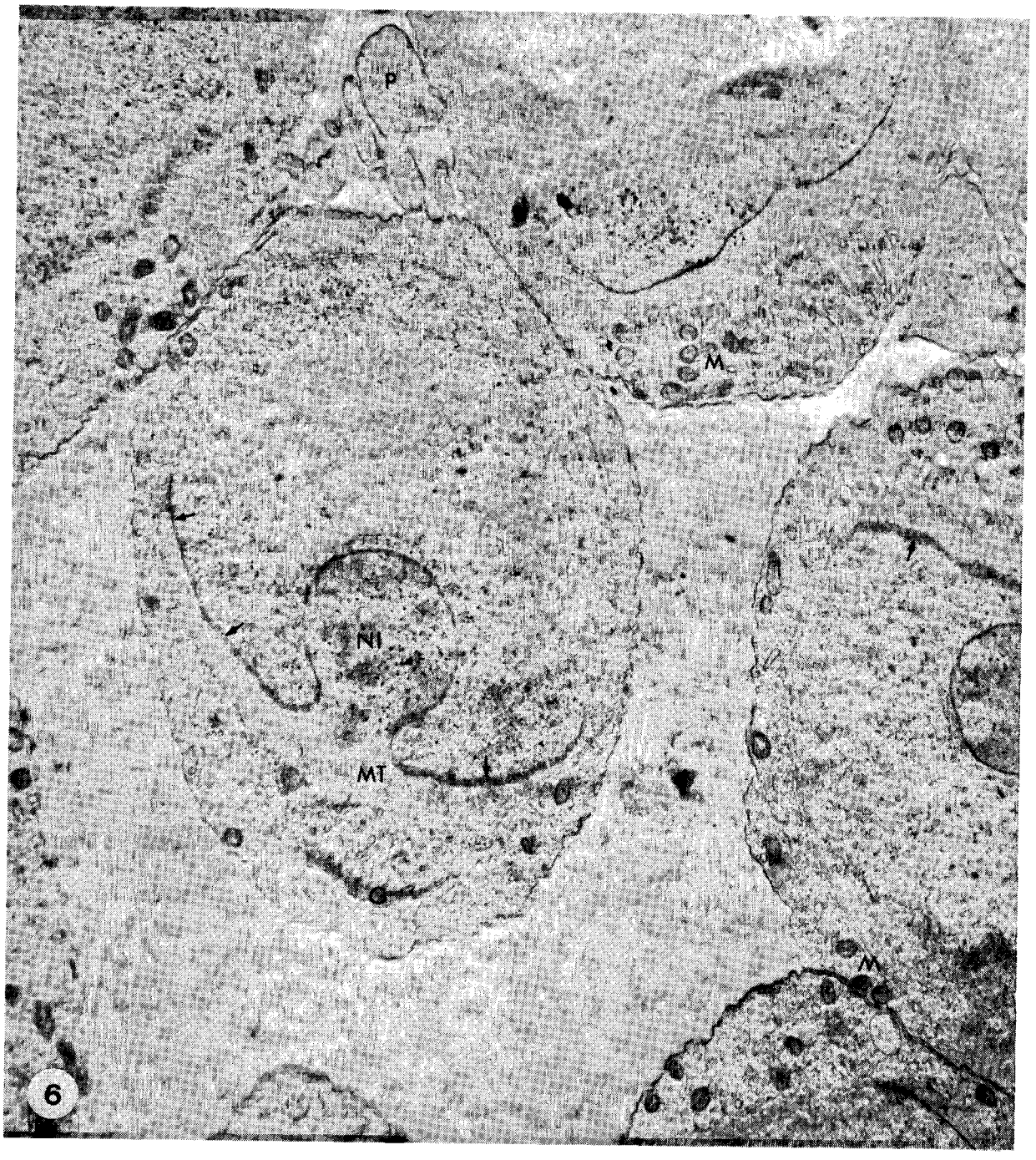
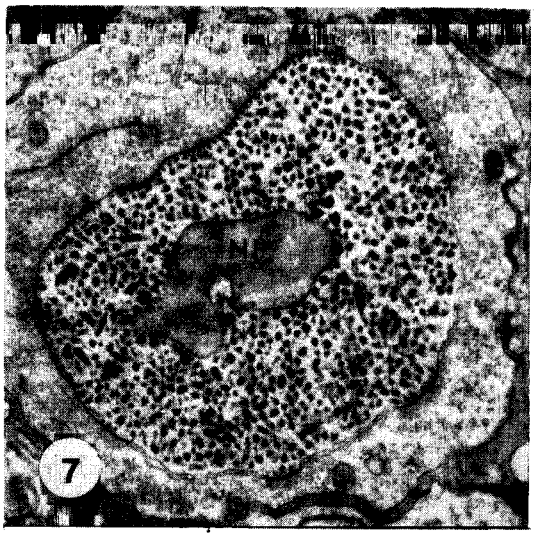
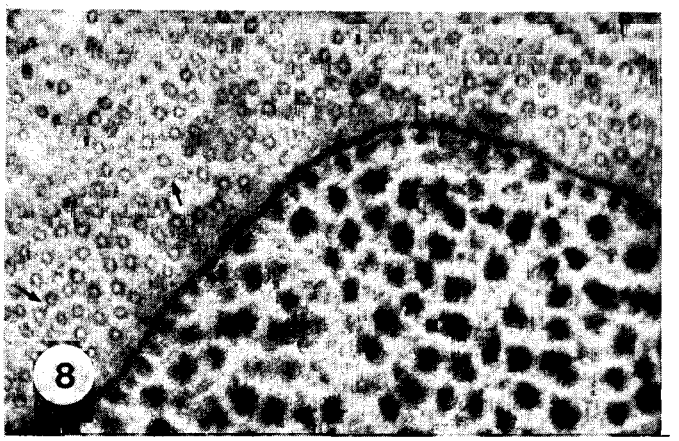
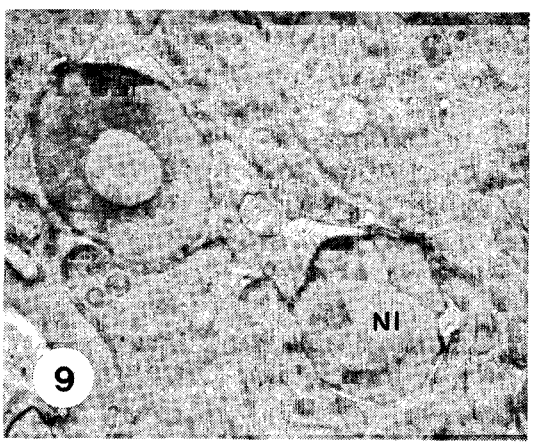
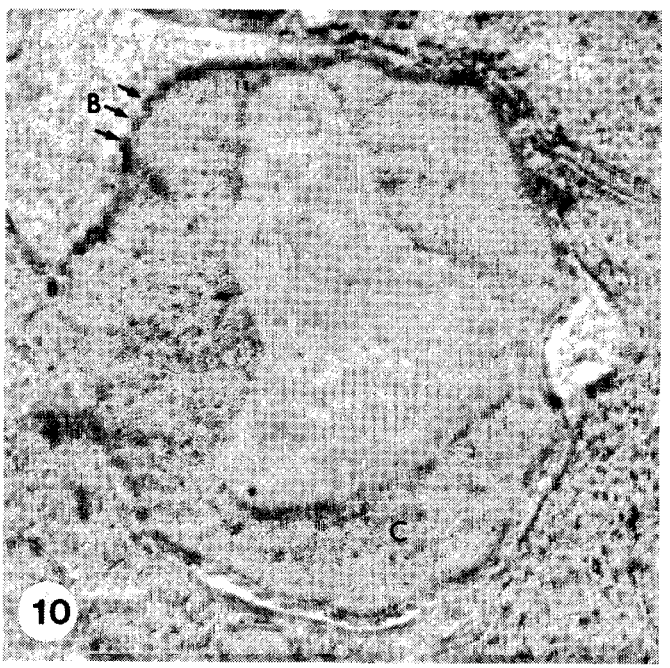


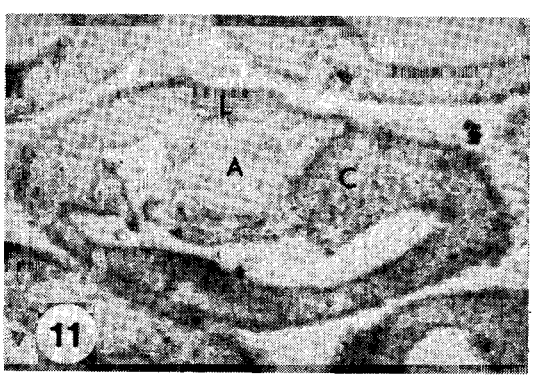
Fig. 6: Spermatid of *Vonones sayi* showing nuclear invagination, arrows = possible nuclear envelope fusion. x 16,800. Abbreviations used: G = Golgi apparatus, M = mitochondria, MT = manchette tubules, NI = nuclear invagination, P = area of pinocytosis.

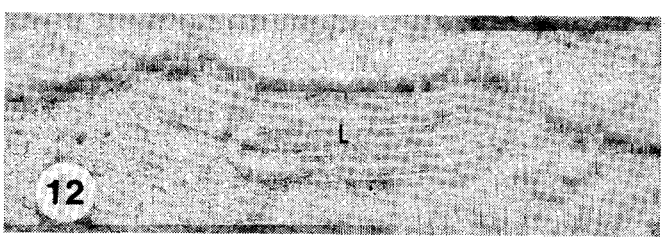












Figs. 7-12: Spermatids and early spermatozoa of *Vonones sayi*. **7** Spermatid cross-section surrounded by manchette tubules. x 17,000; **8** Enlargement of manchette tubules and condensed chromatin, arrows = manchette tubules bridges. x 57,500; **9** Early spermatozoa. x 17,000; **10** Enlargement of early spermatozoa showing bud formation. x 57,500; **11** Early spermatozoon with lamellate structure. x 11,000; **12** Enlargement of lamellate structure. x 40,300. Abbreviations used: A = acrosome, B = buds, C = condensed chromatin, L = lamellate structure, MT = manchette tubules, NI = nuclear invagination.

of chromatin remained near the nuclear envelope, leaving a lighter central area. Manchette tubules occurred at the site of nuclear flattening. At this stage, the nucleus began to invaginate. Manchette tubules increased in number at the area of invagination and extended approximately halfway around the nucleus (Fig. 6). At some point during nuclear invagination, but before completion of the invagination, the chromatin condensed drastically forming thickened strands (Figs. 4, 5). This condition persisted until well after nuclear invagination was complete. Only cytoplasmic matrix of uniform density was observed to be incorporated into the nucleus during invagination, and this matrix later became more electron-dense (Fig. 7). After completion of the nuclear invagination, the manchette tubules increased in number and completely surrounded the nucleus (Figs. 7, 8). Manchette tubules remained in greater numbers at the area where nuclear invagination occurred. Bridges were observed between some tubules (Fig. 8). Following invagination, the spermatid became somewhat elongated (Figs. 4, 5).

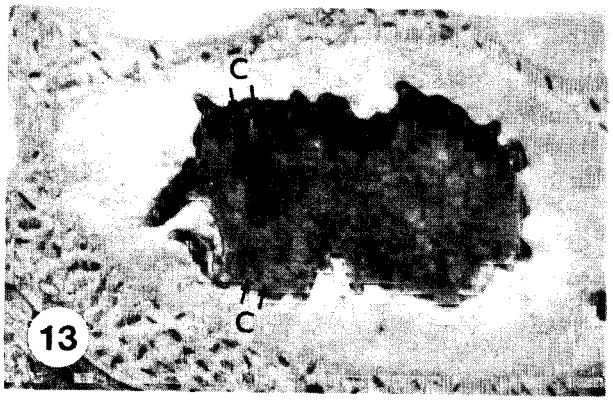
The spermatozoa continued to eliminate cytoplasm and organelles, thereby becoming more isolated. The thick strands of chromatin began to disperse, forming an electron-dense perimeter surrounding a less dense centre (Figs. 9, 10). The manchette tubules remained during chromatin dispersion, but had disappeared when the chromatin reached uniform density (Fig. 10). The nearly mature spermatozoa had eliminated all cytoplasm and organelles exterior to the nucleus, resulting in a cell that possesses three external membranes: an outer bi-layered cell membrane and an inner dense nuclear envelope (Figs. 9, 10). Small buds appeared in the outer cell wall membrane of the spermatozoa (Fig. 10), and these buds later developed into microvilli surrounding the mature sperm. Between 70 to 85 of these buds occurred per spermatid, corresponding to the number of microvilli found on the mature sperm at its widest point. At a similar stage, a lamellate structure was observed near the acrosome (Figs. 11, 12).

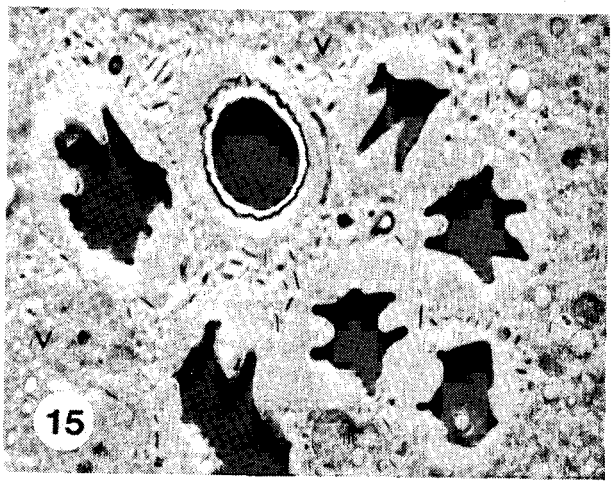
Encysted or highly condensed sperm occurred commonly throughout the testis, and were never observed in any other part of the reproductive system. These cysts had electron-dense centres surrounded by a transparent area with no distinct membrane (Figs. 13, 15). Cysts were always surrounded by a matrix containing cytoplasmic fragments (Figs. 13-15), and were always adjacent to areas containing many vesicles (Fig. 15). The appearance of the cytoplasmic fragments within vesicles (Figs. 15, 17) and the amoeboid shape of the cyst centres suggested that these non-viable spermatozoa were being phagocytized. Observed cysts were somewhat spindle-shaped (Fig. 16), but never attained the elongated form of the viable sperm (Fig. 21). The dense nuclear material underwent compaction and a coiled structure within the cyst appeared (Figs. 13, 14). The coiled structure may be composed of heterochromatin. Early cysts contained approximately 13 coils, later cysts had fewer.

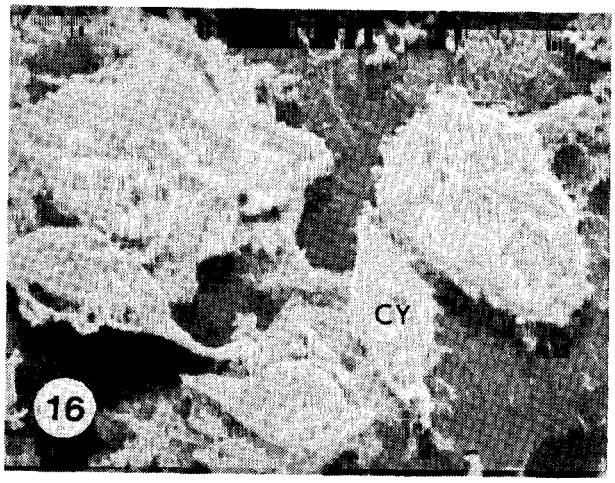
Mature spermatozoa were elongate (about 9 or 10 times longer than wide) and covered with concentric rings of microvilli (Figs. 18-22) and were observed to be non-motile. Length of microvilli serves as an indication of spermatozoon age, with older sperm having longer microvilli. Only spermatozoa with short microvilli were observed in the testis, and spermatozoa with short and long microvilli were present in the vas deferens.

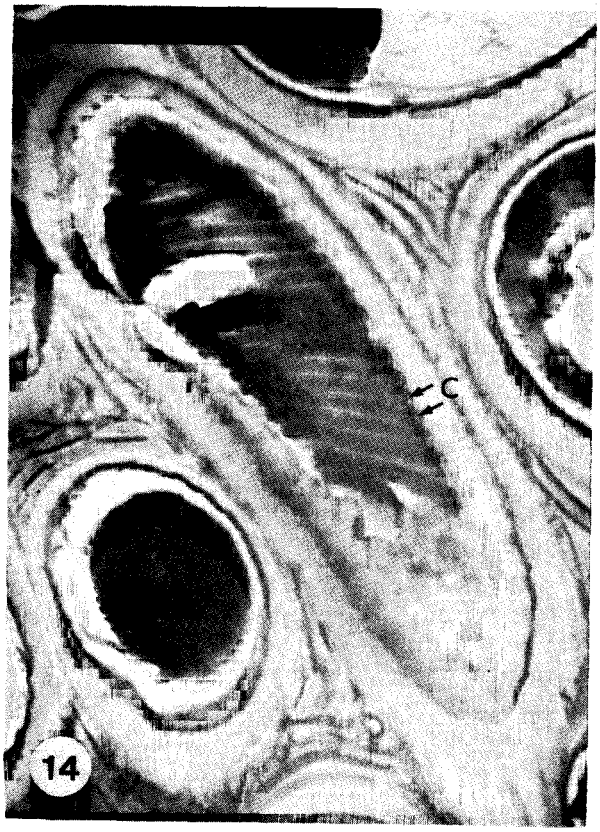
Acrosomal complexes were readily observed in mature spermatozoa, but their development was not observed. Acrosomes consisted of electron-dense material with an associated near-translucent dome (Fig. 22). The dense and less dense areas were separated by periacrosomal material which also separated the acrosome from the nucleus. The entire complex was surrounded by a thin retaining membrane.

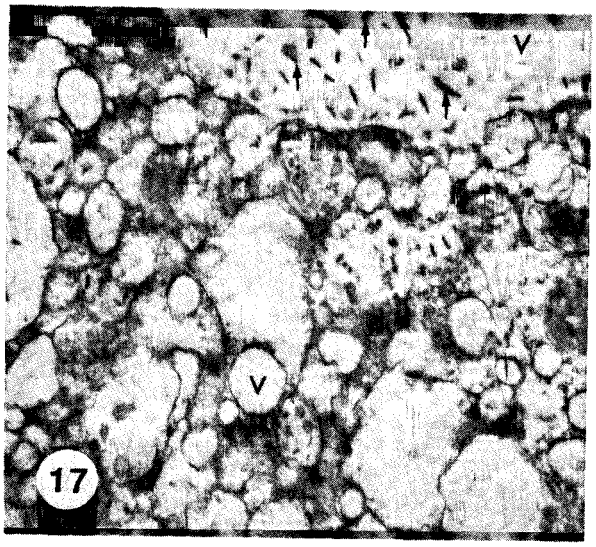
The vas deferens and seminal reservoir differed from the testis in several respects. As already mentioned, only mature and near-mature spermatozoa were contained in the vas deferens. No cysts were observed in the vas deferens and seminal reservoir. The vas deferens was composed of epithelial cells surrounding a cavity or lumen. This layer contained numerous mitochondria which occurred in greatest numbers near the base of a profusion of microtubules which extended into the lumen (Fig. 23). Mature and near-mature spermatozoa lay within the lumen, usually parallel to the vas deferens and to each other. Rarely did a spermatozoon lay perpendicular to the others (Figs. 18, 21). The lumen matrix in which the spermatozoa were bathed was an electron-transparent area of uniform density.



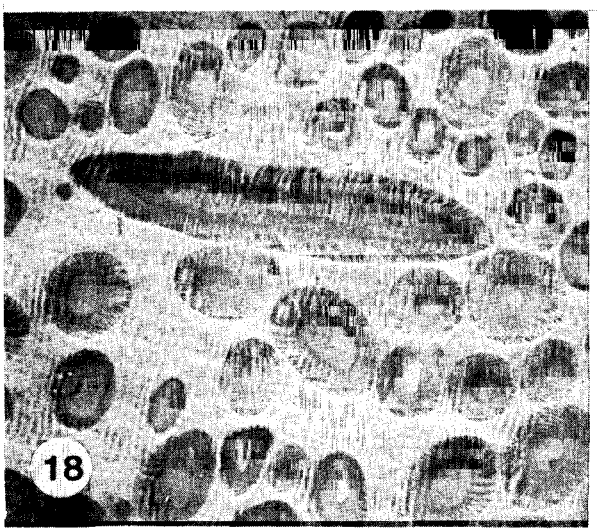


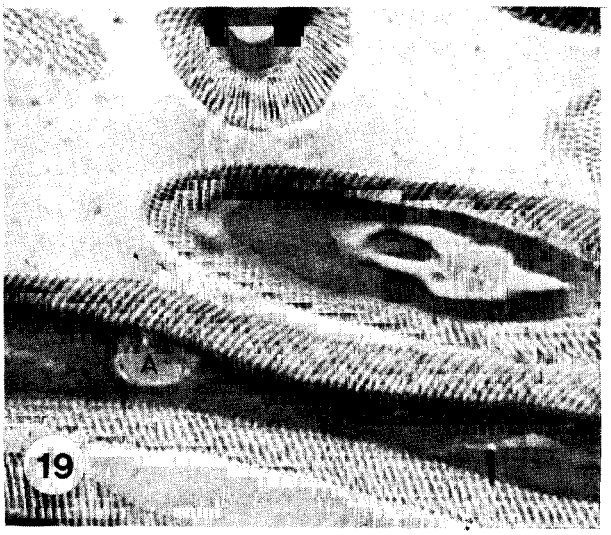


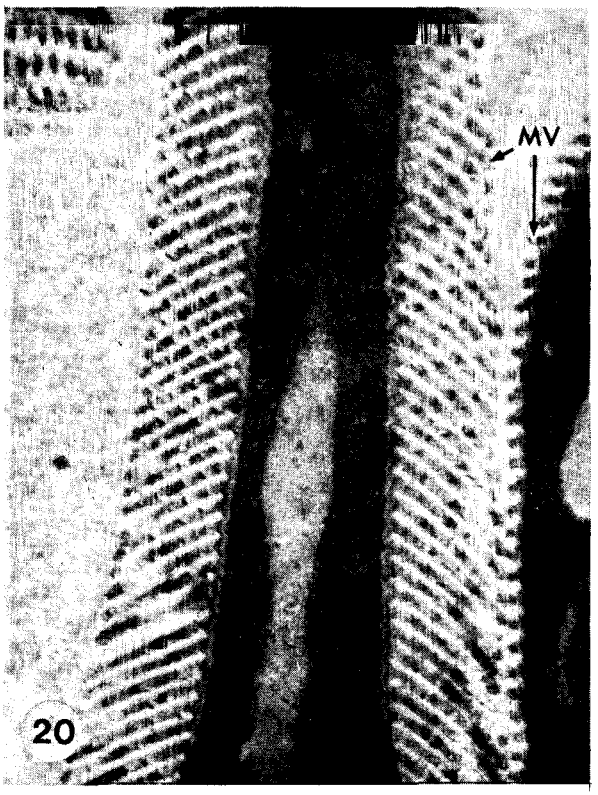




Figs. 13-17: Encysted spermatozoa of *Vonones sayi.* **13** Late encysted spermatozoon showing row of tubules. x 37,000; **14** Early encysted spermatozoon showing coiled structure. x 25,800; **15** Early and late encysted spermatozoa. x 8,900; **16** Scanning electron micrograph of encysted spermatozoa. x 3,000; **17** Area showing digestive vesicles, arrows = cytoplasmic fragments. x 17,000. Abbreviations used: CY = cyst, C = coiled structure, V = vesicles.







Figs. 18-21: Mature spermatozoa of *Vonones sayi*. **18** Longitudinal and cross-sections of mature spermatozoa. x 4,000; **19** Longitudinal and cross-section of mature spermatozoa showing microvilli and acrosome. x 11,000; **20** Longitudinal sections of mature spermatozoa with short and long microvilli. x 17,800; **21** scanning electron micrograph showing three-dimensional shape of mature spermatozoa. x 3,500. Abbreviations used: A = acrosome, MV = microvilli.

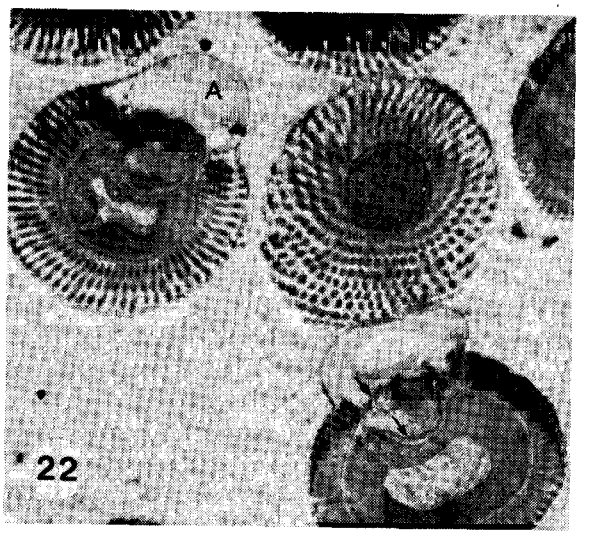
Discussion

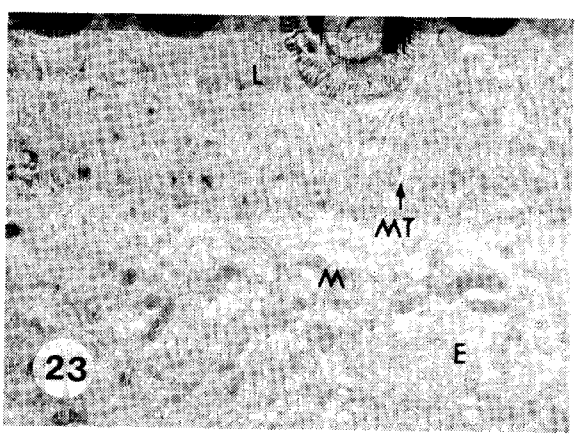
The development of *V. sayi* spermatozoa is similar in some respects to that of the Palpatores (Reger, 1969) and Diplopoda (Reger & Cooper, 1968). Similarities include the apparent fusion of the nuclear membrane at one side of the nucleus and invagination of the nucleus at that point. Like the four other species of Laniatores thus far studied (Juberthie & Manier, 1977c), *V. sayi* has a microtubular apparatus which is apparently used for shaping during spermiogenesis. The function of the manchette tubules may be cytoplasm redistribution during spermiogenesis (Phillips, 1974).

A "shell stage" is fairly common in many animals and plants. In this stage chromatin is concentrated in a dense layer under the nuclear envelope and the interior of the nucleus is occupied by electron-transparent proteinaceous material (Wolfe, 1972). The significance of this stage is unknown. A shell stage was reported in the development of spermatozoa in *Phalangium opilio* L. (Tripepi, 1983), and is present in *V. sayi*.

Cyst formation in *V. sayi* is apparently a mechanism for removal of non-viable spermatozoa. The exterior membrane is slowly phagocytized leaving a transparent area around the nucleus. Simultaneously, the coiled structure is dispersed, coil by coil, and acquires an amoeboid shape. Eventually, the entire cyst is phagocytized and presumably re-used or eliminated from the system. Numerous cysts were observed in the testis. Cosmetid harvestmen live several years as adults, and because our specimens were of unknown age, it is possible that the number of non-viable encysted sperm increases with the age of the animal. This could account for the large number of cysts which we found. Cyst formation has been described in the Phalangiidae (*P. opilio*) but those cysts do not appear to contain coiled structures (Tripepi, 1983). The cysts in *V. sayi* are similar in appearance to some encysted sperm described in Pseudoscorpiones by Legg (1973), however the coiled structures around the nucleus in Pseudoscorpiones are mitochondrial in origin and are important in later movement following de-encystment within the female. Up to now, no encysted sperm have been reported as non-viable in the Opiliones. The atypical spermatozoa of the Cyphophthalmi, *Siro rubens* Latreille, lack an acrosome and nuclear DNA. The sperm are stored in the vas deferens along with normal sperm as a "spermatic sphere": a ball of normal spermatids surrounded by atypical forms (Juberthie, Manier & Boissin, 1976). In the Cyphophthalmi, the atypical sperm are eliminated from the system during copulation. Szöllösi (1982) reports phagocytosis of mature sperm in some Orthoptera.

Cyphophthalmi are known to produce flagellate spermatids, whereas Laniatores and Palpatores do not (Juberthie & Manier, 1978). According to Baccetti





Figs. 22-23: Cross-section of mature spermatozoa and vas deferens. **22** Cross-section of mature spermatozoa, arrows = periacrosomal material. x 15,000; **23** Cross-section of a portion of the vas deferens. x 7,300. Abbreviations used: A = acrosome, L = lumen, MT = microtubules, M = mitochondria, E = epithelium.

(1979), the encysted condition of Cyphophthalmi certainly results in a functionally aflagellate sperm. The coiled structure found in *V. sayi* cysts is not a flagellum because cross-sectional views of these elements revealed no axial complex.

Associated with the spermatid nucleus is a lamellate structure which is a possible annulate lamella. This structure conforms in size to other described annulate lamellae (Kessel, 1983). However, the numerous pores typically associated with annulate lamellae are not obvious in the single micrograph obtained of this structure. Furthermore, the position of the lamellate structure corresponds to that of mitochondria observed in *Cynorta* by Juberthie & Manier (1977c). It may thus be a modified mitochondrium. The only annulate lamellae thus far reported from the Arachnida are from öocytes of the argasid tick, *Ornithodorus moubata* Murray (Kessell, 1983).

The acrosome of *V. sayi* is apparently an electron-translucent structure protruding from the side of the spermatozoon. This translucent structure is associated with a more dense structure embedded in the spermatozoon wall, but separated from it by perinuclear material. Legg (1973) described a possible acrosome from Pseudoscorpiones consisting of a myelin-like structure with many layers. A similar structure from *V. sayi* was observed and may be the precursor for the acrosome found in the mature spermatozoon. However, no clear developmental progression of acrosomes was observed. A clear, membranous organelle was described in *Leiobunum* sp. by Reger (1969), which may be analagous to the structure observed here. In order to determine the definite function of this organelle, spermatozoa-öocyte fusion must be observed. Like *Cynorta,* the acrosomal complex is not accompanied by an acrosomal rod and occurs as a dome. The five or six mitochondria which characteristically surround laniatorid acrosomes (Juberthie & Manier, 1977c) are not obvious in our preparations.

The concentric rings of microvilli on the sperm of *V. sayi* differ greatly in size, number and structure from the microvilli of Cyphophthalmi (Juberthie, Manier & Boissin, 1976), the protuberances of Trogulidae (Juberthie & Manier, 1977b) and the regular cristae of *P. opilio* (Tripepi, 1983). The microvilli of *V. sayi* are almost certainly the same as the "spines" on the spermatozoa of the gonyleptid *Acanthopachylus aculeatus* (Kirby) [= *Heteropachyloidellus robustus* Roewer] (Sotelo & Trujillo, 1954). Juberthie & Manier (1977b) did not report microvilli coatings on *Cynorta cubana* sperm, however their presence was confirmed by C. Juberthie (pers. comm.). We verified the presence of microvilli in spermatozoa of *Vonones* sp., *V. ornata, Cynorta* sp. and *P. quadripunctata* with light microscopy. Parthasarathy & Goodnight (1958) also did not report microvilli, but this oversight could easily be explained by the low magnifications used during their study.

Mature spermatozoa of the ixodid tick *Hyalomma asiaticum* Schulze & Scholettke resemble those of *V. sayi* due to the covering of "tubular elements" and the elongated aflagellate shape of the cell, but they are clearly not homologous. These elements start as buds externally on *V. sayi* spermatocytes, whereas the tubular elements of *H. asiaticum* develop internally and evert to form the surface coat only after they are transported via a spermatophore into the female genital tract (Raikhel, 1983: 230-234, figs. 397-415).

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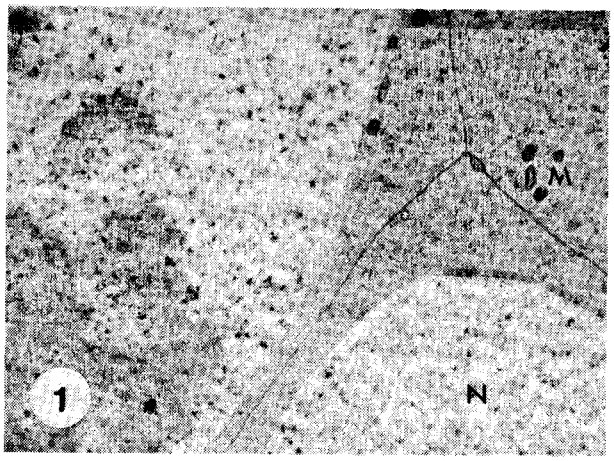

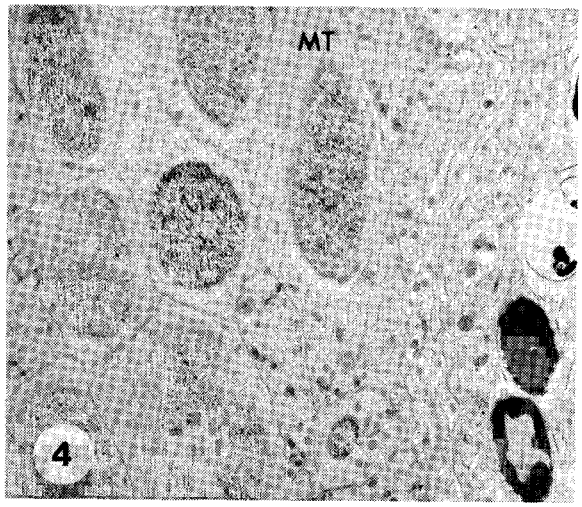

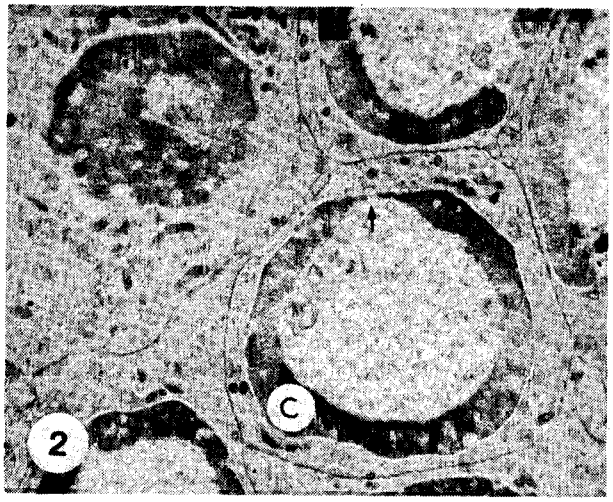

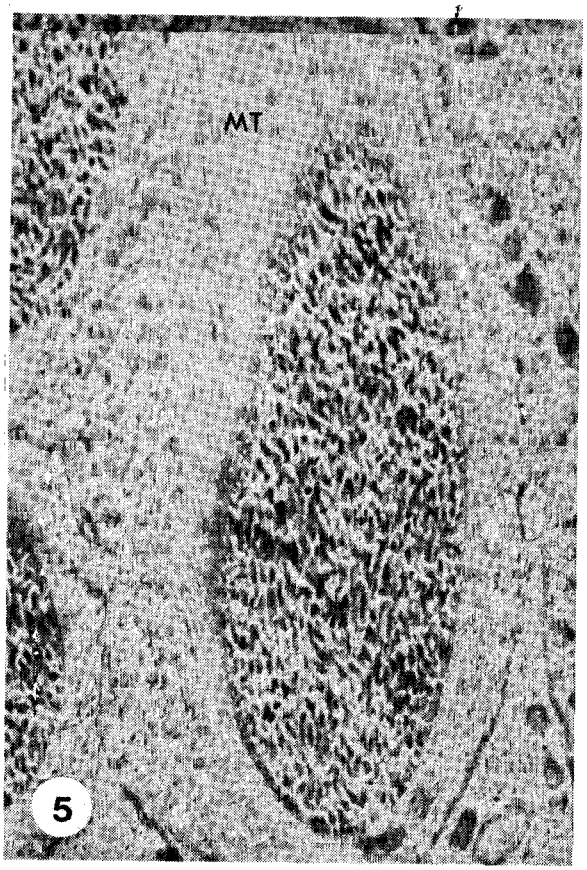

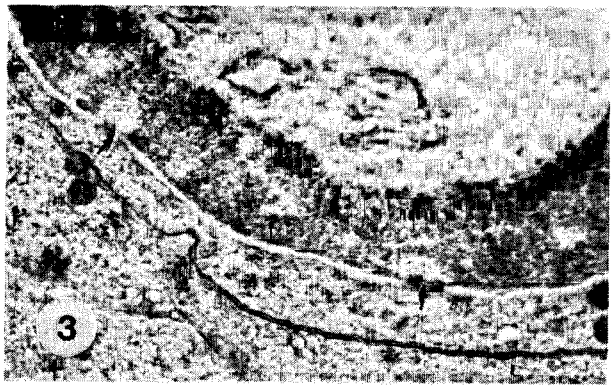

Figs. 1-5: Spermatocytes of *Vonones sayi*. **1** Primary spermatocytes. x 11,000; **2** Shell stage spermatocytes, arrow = area of reduced chromatin. x 7,300; **3** Enlargement of shell stage, arrows = nuclear envelope pores. x 23,900; **4** Early spermatids showing condensed strands of chromatin. x 4,500; **5** Enlargement of early spermatid showing condensed chromatin. x 14,900. Abbreviations used: C = condensed chromatin, M = mitochondrion, MT = manchette tubules, N = nucleus.