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STUDIES OF *NAUTILUS BELAUENSIS* IN PALAU

Ultrastructural Changes in the Formation of Spermatozoa of *Nautilus belauensis* in Palau

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Abstract

Morphological changes in the formation of spermatozoa of *Nautilus belauensis* in Palau were studied. The nucleus undergoes considerable elongation during early spermiogenesis and is surrounded by extensive microtubules to make the manchette structure. The mitochondria migrate to one pole of the secondary spermatocyte to become two mitochondrial masses which elongate and eventually lie in two grooves of the nuclear envelope on the opposite side of the nucleus. The acrosomal complex is a pointed apical sac. The complex contains anterior and posterior acrosomal rods and an irregular vesicles situated in the front tip of the nucleus. At the posterior end of the nucleus proximal and distal basal bodies lie parallel in the basal body fossa. Each basal body is composed of nine sets of doublet microtubules. These morphological features are compared with the ultrastructure of *Nautilus pompilius* in the Philippines, Fiji and Papua New Guinea and it is suggested that the differences found among them are not considerable enough to distinguish the species.

Introduction

There have been several ultrastructural studies of the formation of spermatozoa of *Nautilus pompilius* (Arnolds and Williams-Arnolds, 1978, Tsukahara, 1985, 1988, Tsukahara *et al.*, 1991). Tsukahara *et al.* (1991) compared ultrastructural features of spermatozoa of *Nautilus pompilius* from Fiji and Papua New Guinea and showed that there was only a little geographical difference between them.

The species level taxonomy of chambered *Nautilus* has long been obscure. Recently, Saunders (1987) proposed the division of *Nautilus* into five or possibly six species (*N. pompilius, N. macromphalus, N. scrobiculatus, N. stenomphalus, N. belauensis* and possibly *N. repertus*). However, some malacologists regard the latter three putative species as geographic variants of *N. pompilius* (e.g. Habe, 1980, Abbott & Dance, 1983). Tanabe *et al.* (1990) carried out comparative morphological studies of living *Nautilus* from the Philippines, Fiji and Palau, and have suggested that the Palau population, previously distinguished as *N. belauensis*, and the other two populations belong to the same, wide ranging species, *N. pompilius*, or that otherwise they are closely related sibling species, *N. belauensis* and *N. pompilius* respectively.

This paper describes ultrastructural changes during spermatogenesis and spermiogenesis of *N. belauensis* in comparison with the morphological features of the formation of spermatozoa of *N. pompilius*.

Materials and Methods

Six specimens of mature male *N. belauensis* captured in Mutremdiu Bay, Palau during August and September were used in this study. Their soft parts were dissected and testes and spermatophores in the spermatophore sacs were removed. Small pieces were prefixed with 2.5% glutaraldehyde in buffered saline (1% $K_2Cr_2O_7$-KOH buffer (pH7.4) containing 0.5M NaCl) for 3 hours at room temperature. After rinsing three times with buffered saline, post fixation was carried out for 1 hour with 1% OsO_4 in buffered saline at 0℃. Tissues were then dehydrated and embedded in Spurr resin. Ultra-thin sections of the specimens were stained with uranyl acetate and lead citrate, and were observed by a Hitachi H-600 transmission electron microscope.

Results

Spermatocyte and spermatid in the testis

Many spermatogonia and young spermatocytes are seen near the basal lamina of the seminiferous tubule. Primary spermatocytes undergo first meiotic division to the zygotene stage and studded synaptonemal complexes are observed in its nucleus (Fig. 1, 2a, 2b). Numbers of mitochondria are scattered in the cytoplasm. The Golgi apparatus and a few electron dense granules are also observed in the cytoplasm.

When the youngest spermatid begins spermiogenesis, many mitochondria crowd near one side of the nucleus and fuse gradually with one another to become two large oval mitochondrial masses (Fig. 3). Some spermatids have a cytoplasmic bridge to connect to the neighboring cell throughout their differentiation into mature spermatozoon (Fig. 6, Fig. 11). A pair of centrioles is found in the peripheral cytoplasm near the mitochondrial mass (Fig. 3, arrowhead), and then moves toward the nuclear envelope (Fig. 4, arrowhead). One of them changes into the distal basal body to prepare an elongated flagellum (Fig. 5, db), while the other changes into the proximal basal body and moves closely into the pit of the envelope (Fig. 5, pb). These two basal bodies are set on a slightly acute angle to each other.

The spherical nucleus elongates gradually to a pear shape as subsequent development progresses (Fig. 6). Proximal and distal basal bodies are almost parallel to each other in a implantation fossa of the nuclear envelope. On the nearly opposite side of these basal bodies around the nucleus the Golgi apparatus makes a number of small Golgi vesicles, and many of these appear to gather like a cap on the anterior surface of the nucleus (Fig. 7). Two mitochondrial rods are elongated along the nuclear envelope on the opposite side to the fossa. Many microtubules begin to develop around the nucleus and mitochondrial rods (Fig. 6, arrowhead). In cross section it is easy to observe many parallel microtubules are in a row around the nucleus and mitochondrial rods (Fig. 8).

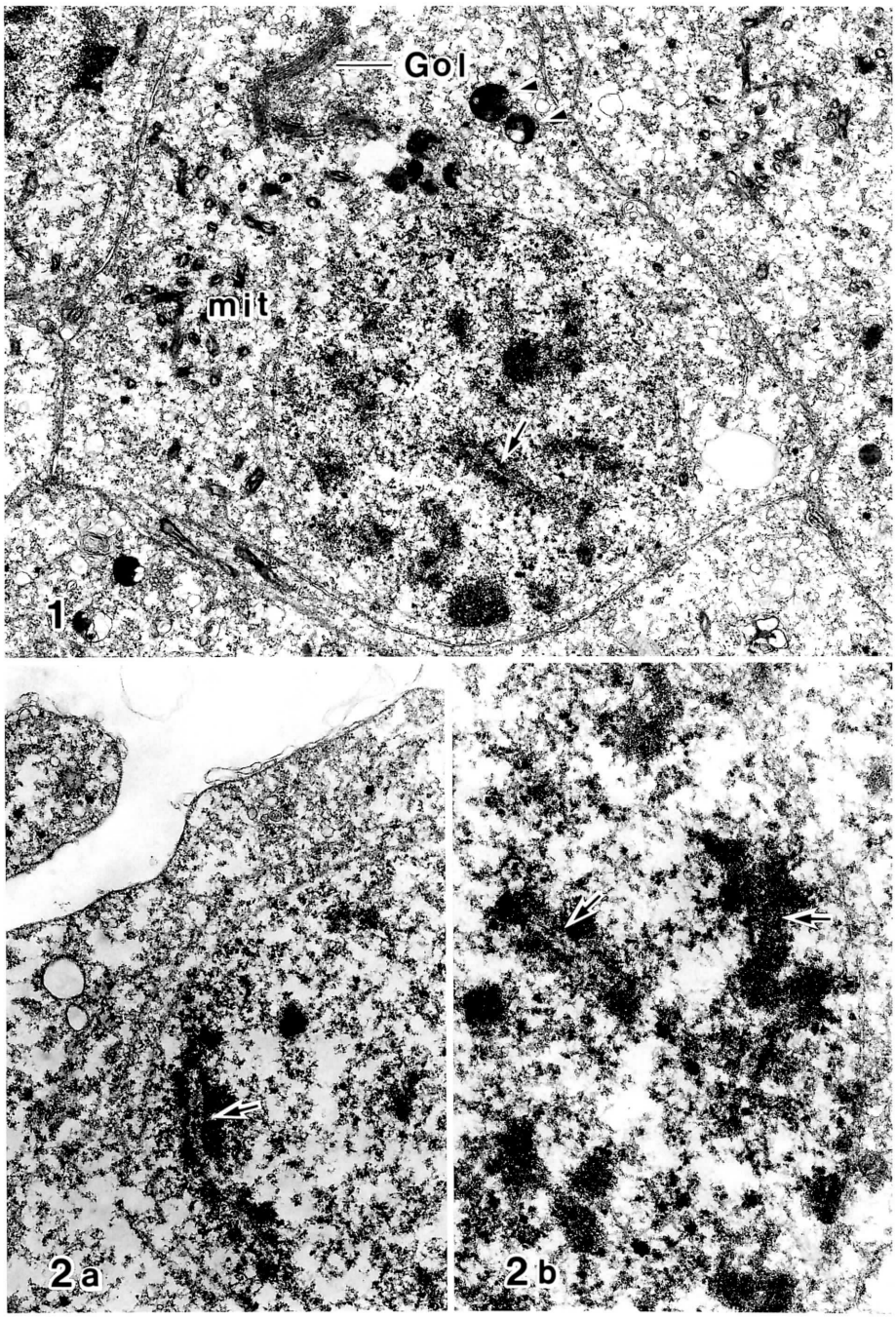

Fig. 1. Primary spermatocyte in which synaptonemal complexes in the nucleus (arrow), and mitochondria (mit), Golgi apparatus (Gol) and electron dense granules (arrowhead) in the cytoplasm. ×8,100.

Fig. 2a. A synaptonemal complex (arrow) in the nucleus of a primary spermatocyte. ×14,400.

Fig. 2b. Synaptonemal complexes (arrow) in the nucleus of a primary spermatocyte. ×18,000.

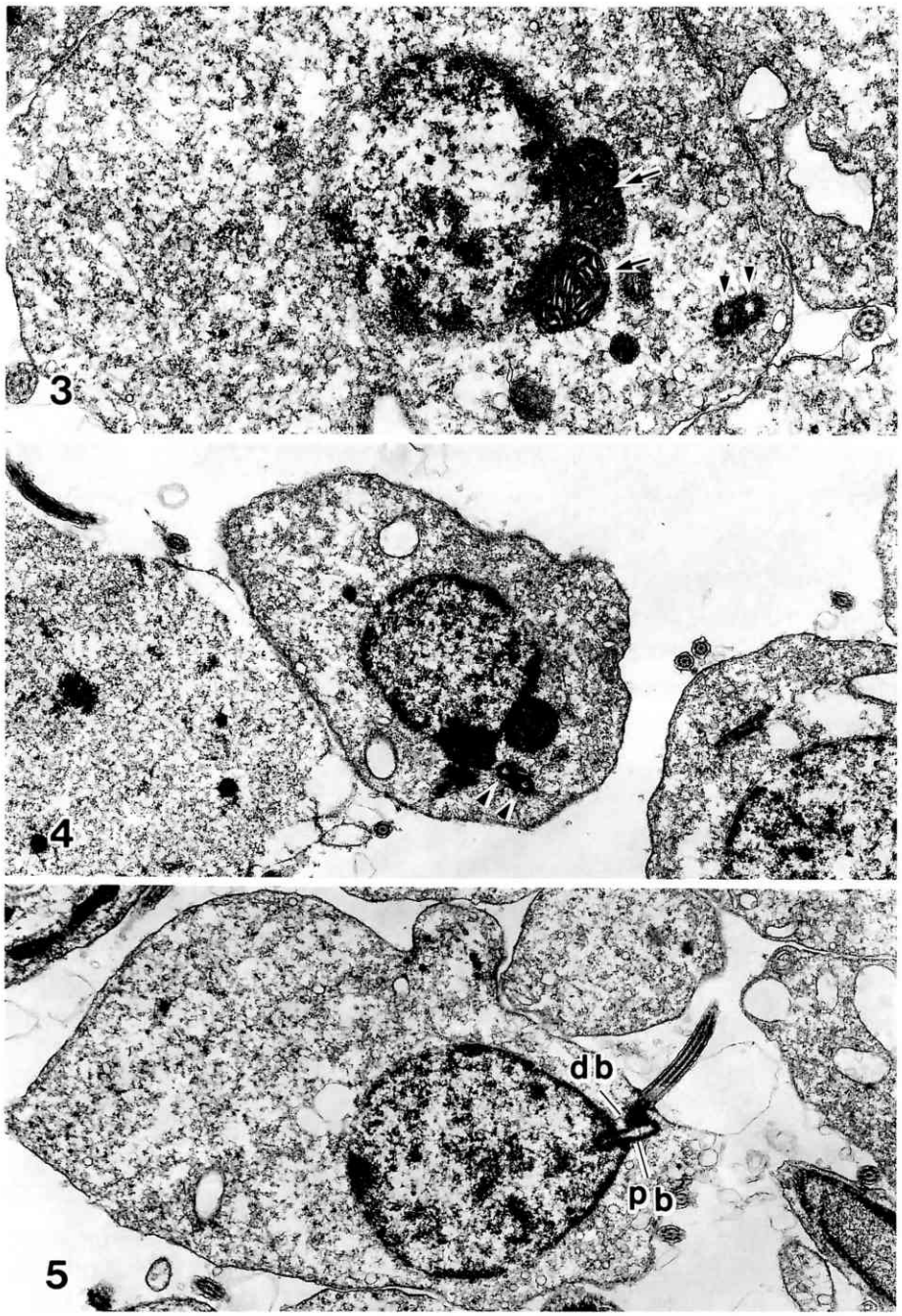

Fig. 3. Early spermatid in which the mitochondria (arrow) have clumped to one side of the nucleus. A pair of parallel centrioles (arrowhead) in the peripheral cytoplasm. ×18,000.

Fig. 4. Early spermatid in which the two mitochondrial masses and two centrioles (arrowhead) are located to one side of the nucleus. ×9,000.

Fig. 5. Spermatid with two basal bodies. The proximal basal body (pb) penetrates part way into the fossa and the distal basal body (db), which is set on a slightly acute angle, forms an elongated flagellum. ×9,000.

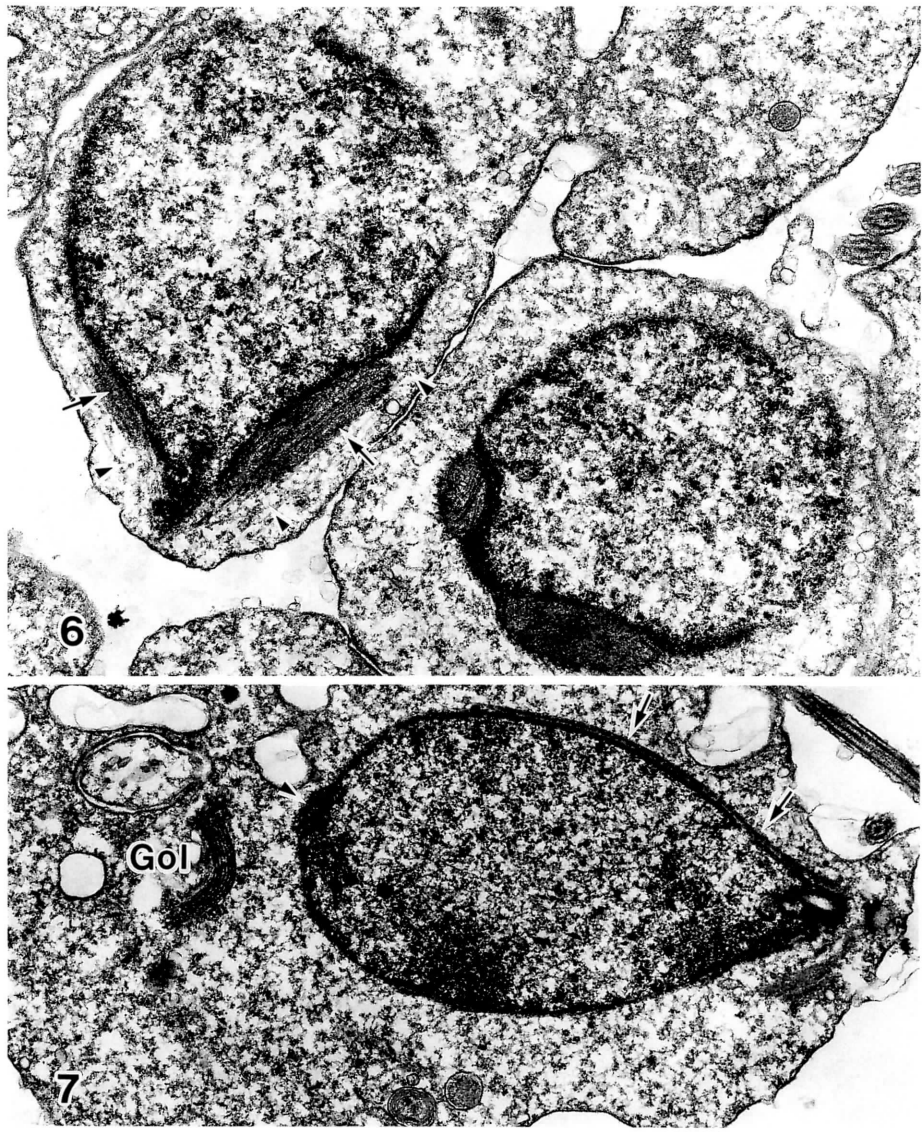

Fig. 6. The mitochondria (arrow) elongate on either side of the pear-shaped nucleus of the spermatid. Many microtubules (arrowhead) have extended along the nucleus and mitochondrial rods. ×16,200.

Fig. 7. Mitochondria elongate notably along the nuclear envelope (arrow). Golgi apparatus (Gol) is situated on the future anterior of the spermatid. The electron dense mass (arrowhead) adheres to the future apex of the nucleus. ×13,500.

The nucleus elongates further to become a cylindrical shape (Fig. 9). Chromatin condensation begins to appear in the periphery of the nucleus tip. Two or three rows of parallel microtubules surround the nuclear envelope and mitochondrial rods as the manchette structure (Fig. 10). The number of microtubules amounts to more than 200 around the nucleus of about 1 μm in diameter. Two mitochondrial rods are situated in the groove of the nuclear envelope on opposite

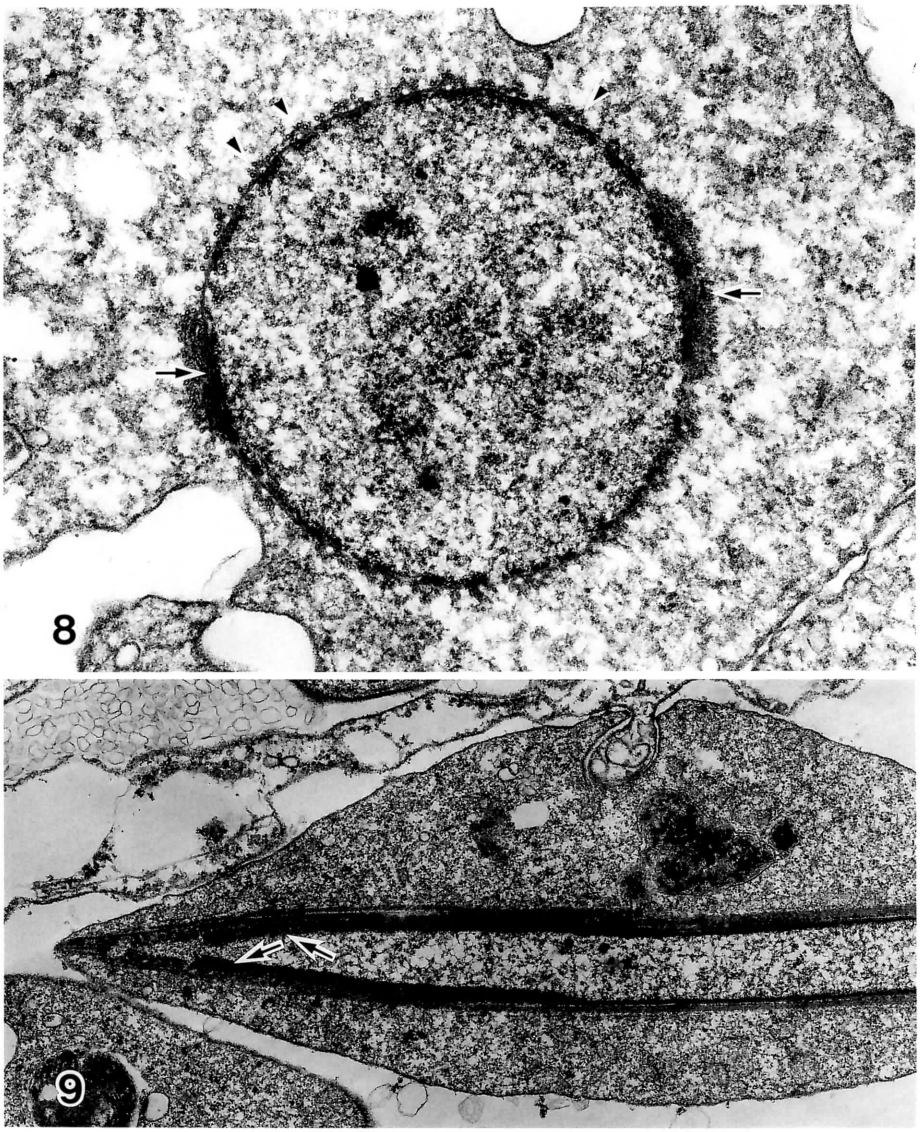

Fig. 8. Cross section of the elongating nucleus with a row of microtubules (arrowhead) around the nucleus and mitochondrial rods (arrow) of the spermatid. ×28,800.
Fig. 9. Anterior side of the cylindrical nucleus of the spermatid. Condensation of the chromatin begins in the periphery of nucleus (arrow). ×14,400.

sides of the nucleus.

The fully grown spermatid in the testis have small masses of cytoplasm around near the nucleus tip (Fig. 11). Some of these are still linked by cytoplasm bridges. The apical end of the nucleus presents as a disc below the acrosomal complex (Fig. 12). The acrosomal region is a pointed apical sac with granular, dense material. There are two acrosomal rods. The anterior electron dense rod is about 250 nm in length and about 30 nm in width at the tip of the sac, and the posterior,

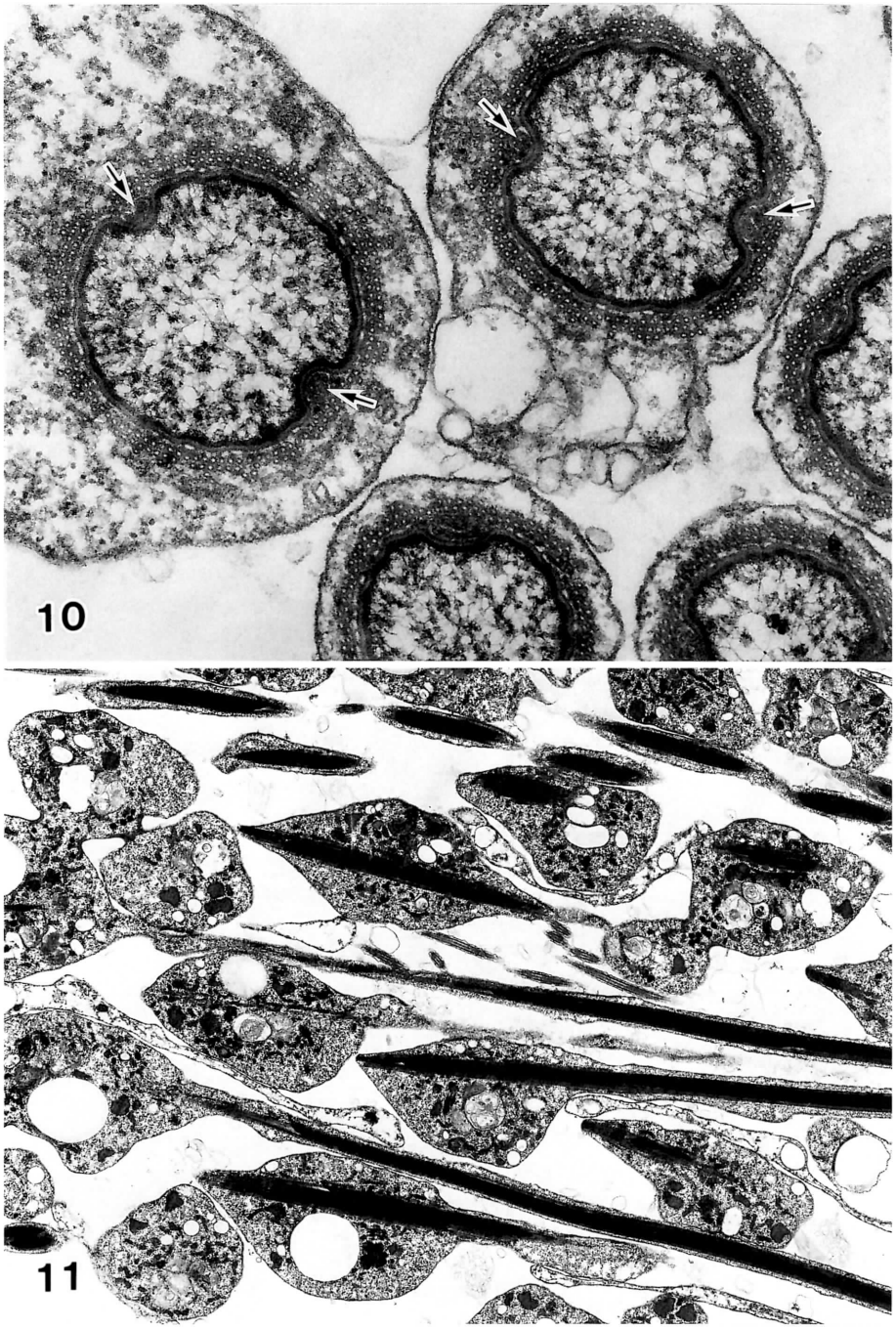

Fig. 10. In a cross section of a slightly older spermatocyte, the well developed manchette structure surround the nucleus. Two mitochondrial rods are situated in a groove of the nuclear envelope (arrow). ×43,200.

Fig. 11. Anterior side of a chromatin showing the well-condensed nucleus of a fully grown spermatid with a small mass of cytoplasm. ×4,860.

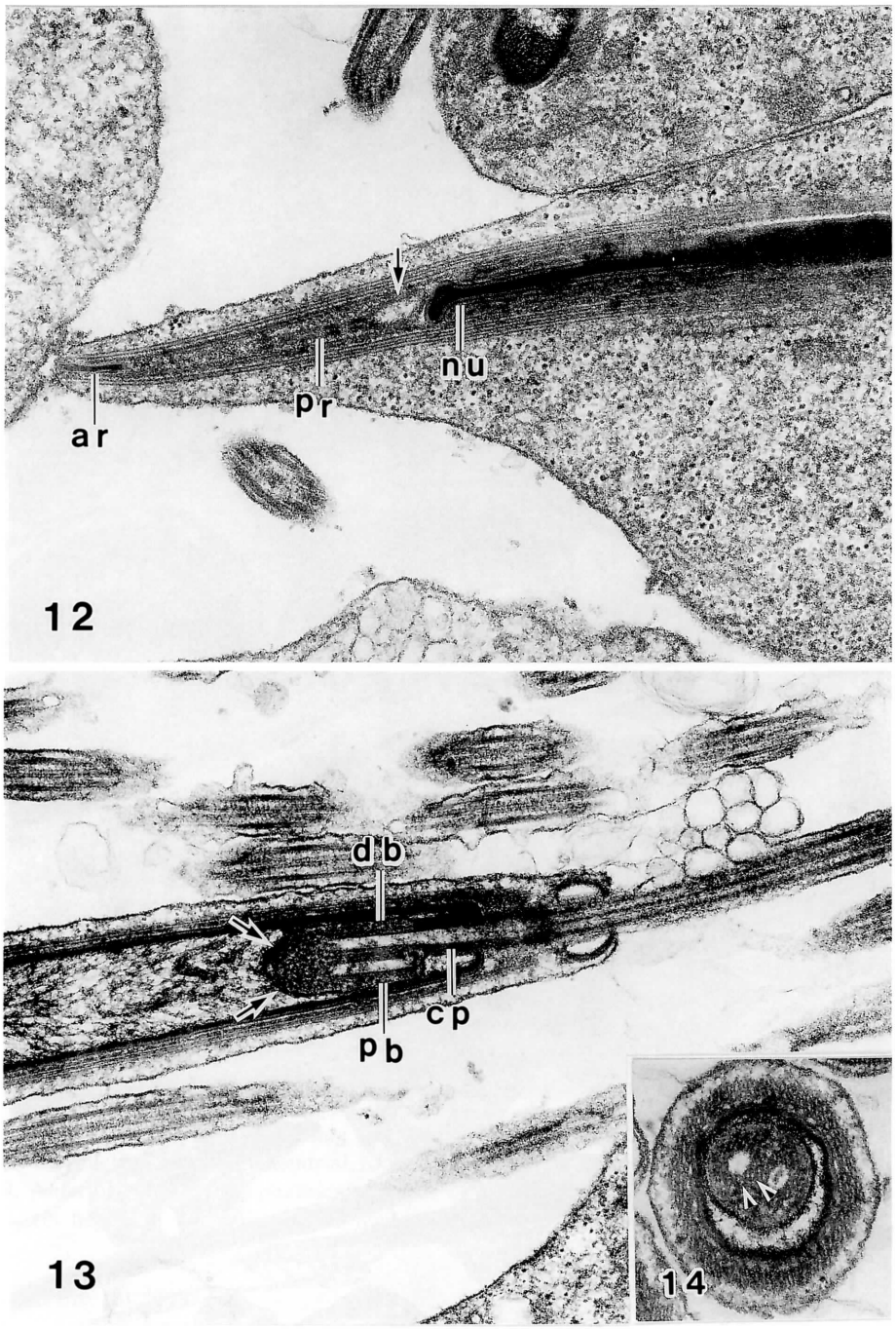

Fig. 12. The pointed tip of the nucleus and acrosomal region with an anterior rod (ar), a posterior rod (pr) and a irregular vesicle (arrow) in front of the nuclear end (nu). ×36,000.
Fig. 13. Parallel basal bodies (pb, db) with electron dense cap (arrow) lie in a deep fossa of the posterior end of the nucleus of a spermatid. A connecting piece (cp) is inserted between the basal body and axoneme. ×27,000.
Fig. 14. Parallel basal bodies with doublets of microtubules (arrowhead). ×43,200.

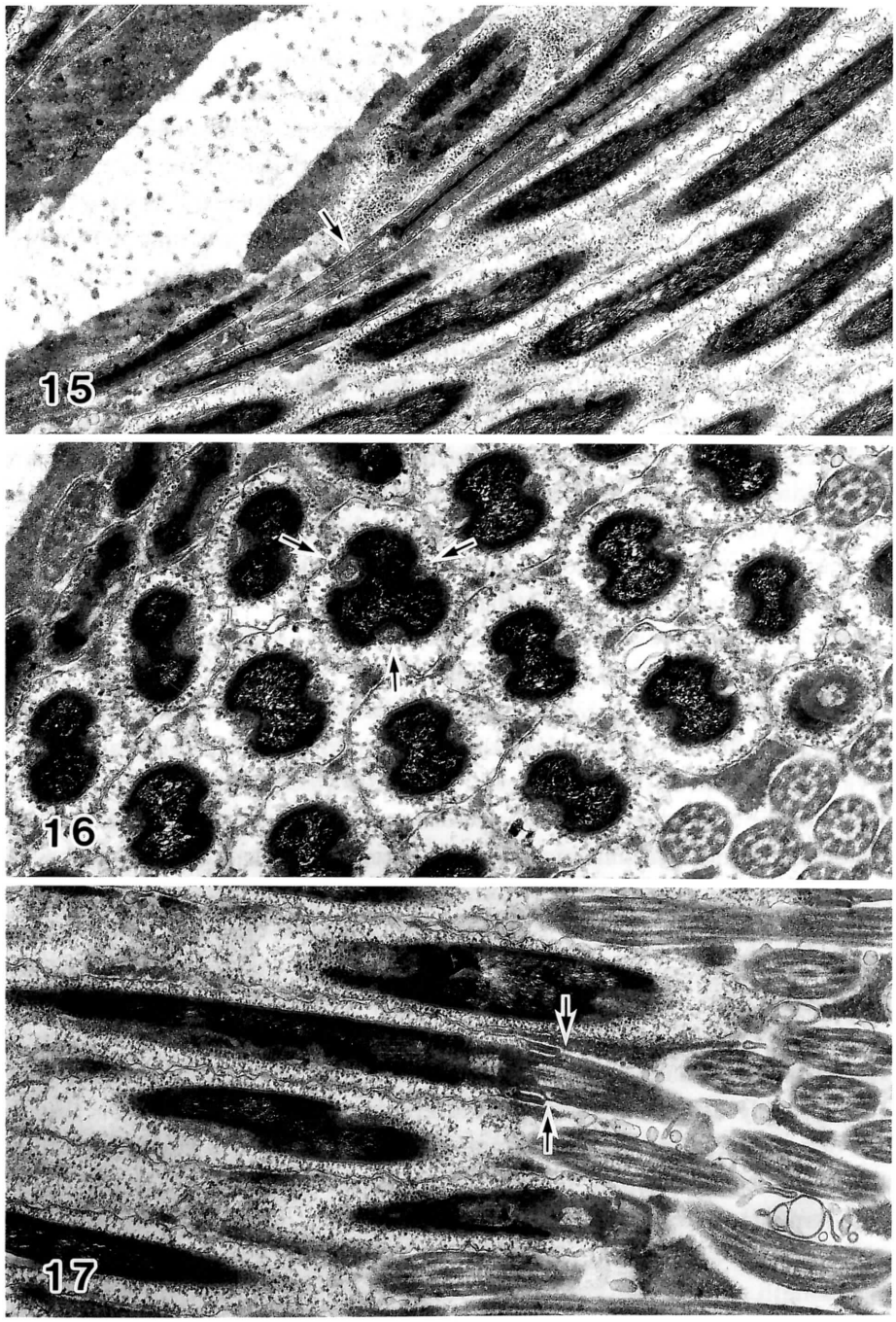

Fig. 15. Dense mature spermatozoa are arranged in the spermatophore; a conical nucleus tip (arrow) ×18,000.

Fig. 16. Cross section of mature spermatozoa. A rare cloverleaf-shaped nucleus with three grooves (arrow). ×27,000.

Fig. 17. Posterior end of mature spermatozoa. The width of the axoneme increases slightly just under the annular atrium (arrow). ×21,600.

slightly less dense rod is about 550 nm in length and about 40 nm in width. An irregular electron light vesicle is located in front of the nuclear disc. Many long filaments of nuclear material run along the long axis of the nucleus (Fig. 13). At the end of the nucleus two parallel basal bodies occur on the electron dense cap on the anterior side. Each body lies deep in the basal body fossa and is about 0.4 μm in length and about 0.15 μm in diameter. A connecting piece is found between the posterior basal body and the axoneme. There are nine set of doublet microtubules on each basal body, in cross section (Fig. 14).

Mature spermatozoa in the spermatophore

Numerous mature spermatozoa arrange their heads toward the outer layer of the electron dense mass (Fig. 15). The nucleus reaches about 38 μm in length. In cross section, the nucleus is 0.5×0.3 μm in diameter with two grooves situated on its opposite sides (Fig. 16). Rarely, a nucleus has three mitochondrial grooves around its envelope and appears like a clover leaf in cross section (Fig. 16, arrow). The elongated axoneme increases in width from about 0.2 μm to about 0.3 μm just under the annular atrium (Fig. 17, arrow).

Discussion

Franzen (1956) has theorized that animals with internal fertilization or complicated copulation have relatively specialized spermatozoa with highly modified morphology. Arnold and Williams-Arnold (1978) described spermiogenesis of *Nautilus pompilius* in the Philippines by electron microscope and compared this to the ultrastructures of other cephalopods. They speculated that the differences found are greater than might be expected if the mechanisms of sperm transfer and fertilization were the only controlling forces determining the evolution of spermatozoan ultrastructure.

Tsukahara (1985) and Tsukahara *et al.* (1991) analyzed the ultrastructure of the spermatozoa of *Nautilus pompilius* in Fiji and Papua New Guinea, comparing morphological differences with samples from the Philippines, and showed clearly that there is only a little geographical difference among *Nautilus* from these three areas. For instance, the mature spermatozoon of *N. pompilius* from the Philippines has a nucleus about 35 μm in length and about 0.3 μm in diameter (Arnold and Williams-Arnold, 1978), those from Fiji about 37 μm in length and about 0.5×0.3 μm in diameter (Tsukahara, 1985), while those from Papua New Guinea about 36 μm in length and 0.5×0.3 μm in diameter (Tsukahara *et al.*, 1991). These values may not be outside the range of individual variation in each region.

Ultrastructural studies of male gametes during spermatogenesis and spermiogenesis of *Nautilus belauensis* from Palau, indicate no considerable difference in morphological changes during the formation of the spermatocytes and spermatids between *N. belauensis* and *N. pompilius*, including elongation of the nucleus, emergence of the manchette structure, behavior of mitochondria, and the

features of the basal body, which is composed of nine sets of doublet microtubules, and the acrosomal complex. However, some small differences were observed; for instance, the nucleus of mature spermatozoa of *N. belauensis* is about 38 μm in length, while this is about 35-37 μm in length in *N. Pompilius*. The acrosomal rods of the two putative species are also slightly different. For instance, the anterior rod is about 0.25 μm in length and the posterior rod is about 0.55 μm in length in *N. belauensis*, while the former is about 0.36 μm and the latter is about 0.6 μm in *N. pompilius*. The maximum average number of microtubules in the manchette is 212 in *N. belauensis*, while 190 in *N. pompilius*. These differences between the two species, however, may be not considerable enough to serve as a basis to distinguish species. It is suggested that the Palau population, *N. belauensis* , and the populations belonging to *N. pompilius* have a wide variety of the morphological features exhibited during the formation of spermatozoa that overlap each other. At the most, these populations belong to closely related sibling species, *N. belauensis* and *N. pompilius* respectively.

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