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ULTRASTRUCTURE OF SPERM AND SPERMATOGENESIS OF *ANOPLODISCUS CIRRUSSPIRALIS* (PLATYHELMINTHES, MONOGENEA, MONOPISTHOCOTYLEA)

N. A. WATSON, K. ROHDE

SUMMARY

The mature spermatozoon of *Anoplodiscus cirrusspiralis* is long and filiform with a nucleus, mitochondrion and single axoneme pressed tightly together for most of its length. In contrast to most other platyhelminthes which have a solid central unit in the complex central element of the sperm axoneme (Trepaxonemata), the central unit in *Anoplodiscus* is a hollow cylinder. There are no peripheral microtubules in the sperm, and the arrangement conforms to sperm pattern 4 in the scheme of Justine *et al.* (1985) for the Monogenea (found in monopisthocotyleans from the orders Dactylogyridea and Tetraonchidea). However, spermatogenesis is distinctly different in *Anoplodiscus.* A short free axoneme rotates and fuses with the spermatid body, then continues to grow alongside the elongating nucleus and the gradually fusing mitochondria. There is no cytoplasmic outgrowth in the original direction of the axoneme, and the nucleus and mitochondria do not migrate past the basal body of the axoneme, as happens in other monopisthocotylean Monogenea. As other evidence suggests that *Anoplodiscus* is a monogenean, it is concluded that spermiogenesis is aberrant and a case of temporary deviation in ontogeny, not affecting the outcome of mature sperm structure.

RÉSUMÉ : **Ultrastructure du sperme et de la spermatogenèse d'*Anoplodiscus cirrusspiralis* (Plathelminthes, Monogenea, Monopisthocotylea).**

Le spermatozoïde mûr d'*Anoplodiscus cirrusspiralis* est long et filiforme avec un noyau, la mitochondrie et le seul axonème serrés de presque toute sa longueur. Par contraste avec la plupart des autres plathelminthes qui possèdent une unité solide au centre de l'élément central complexe de l'axonème du spermatozoïde (Trepaxonemata), l'unité centrale dans l'*Anoplodiscus* est un cylindre creux. Il n'y a pas de microtubules périphériques dans le sperme, et la disposition se conforme au modèle du sperme n° 4 dans la classification de Justine *et al.* (1985) du Monogène (se trouvant dans les monopisthocotyléens de l'ordre des Dactylogyridées et Tétraonchidées). Pourtant, la spermatogenèse est nettement différente dans l'*Anoplodiscus.* Un axonème court et libre tourne et s'unit avec le corps spermatide, et continue à se développer à côté du noyau qui s'allonge et des mitochondries qui se mettent peu à peu en fusion. Il n'y a aucune excroissance cytoplasmique dans la direction originale de l'axonème, et le noyau et les mitochondries ne dépassent pas le corps basal de l'axonème, comme il arrive à d'autres monogènes monopisthocotyléens. Comme d'autres observations suggèrent que l'*Anoplodiscus* est un monogène, on en conclut que la spermiogenèse est aberrante et un cas d'une déviation provisoire dans l'ontogénie, ne déterminant pas la structure éventuelle du spermatozoïde mûr.

INTRODUCTION

The ultrastructure of mature sperm and the processes of spermatogenesis have been studied in various groups of free-living and parasitic platyhelminths (see reviews by Davis and Roberts, 1983; Hendelberg, 1983; Mohandas, 1983; Ehlers, 1985*a* and references in Rohde *et al.*, 1988; Sopott-Ehlers, 1989) and are useful for phylogenetic considerations (see Ehlers, 1985*a*; Hendelberg, 1986; Rohde, 1990; Justine, 1991*a* and references therein). In this paper we describe spermatogenesis of *Anoplodiscus cirrusspiralis* from the fins of the snapper, *Chrysophrys auratus* (see Roubal *et al.*, 1983). The adult lacks hooks, but the genus is regarded as belonging to the Monogenea because, like Monogenea, it has two pairs of eyes and Ogawa and Egusa (1981) have demonstrated the presence of 16 hooks in the oncomiracidium and immature worms of *A. spari.* Rohde *et al.* (in press) studied the ultrastructure of the protonephridial system of *A. cirrusspiralis* and found it to resemble that of monogeneans in most respects. However, it lacks cytoplasmic cords along the flame bulb and the septate junction in the smallest protonephridial capillaries is rudimentary. Thus it is an aberrant monogenean. Spermatogenesis of *A. cirusspiralis* was studied to determine whether this process conforms to the pattern for monogeneans or whether the species is aberrant in this respect also.

MATERIAL AND METHODS

Infected snapper, *Chrysophrys auratus,* were aquacultured in a cage at the Fisheries Research Institute, Cronulla, Sydney. Worms removed from the fins or still attached to the fins were sent alive

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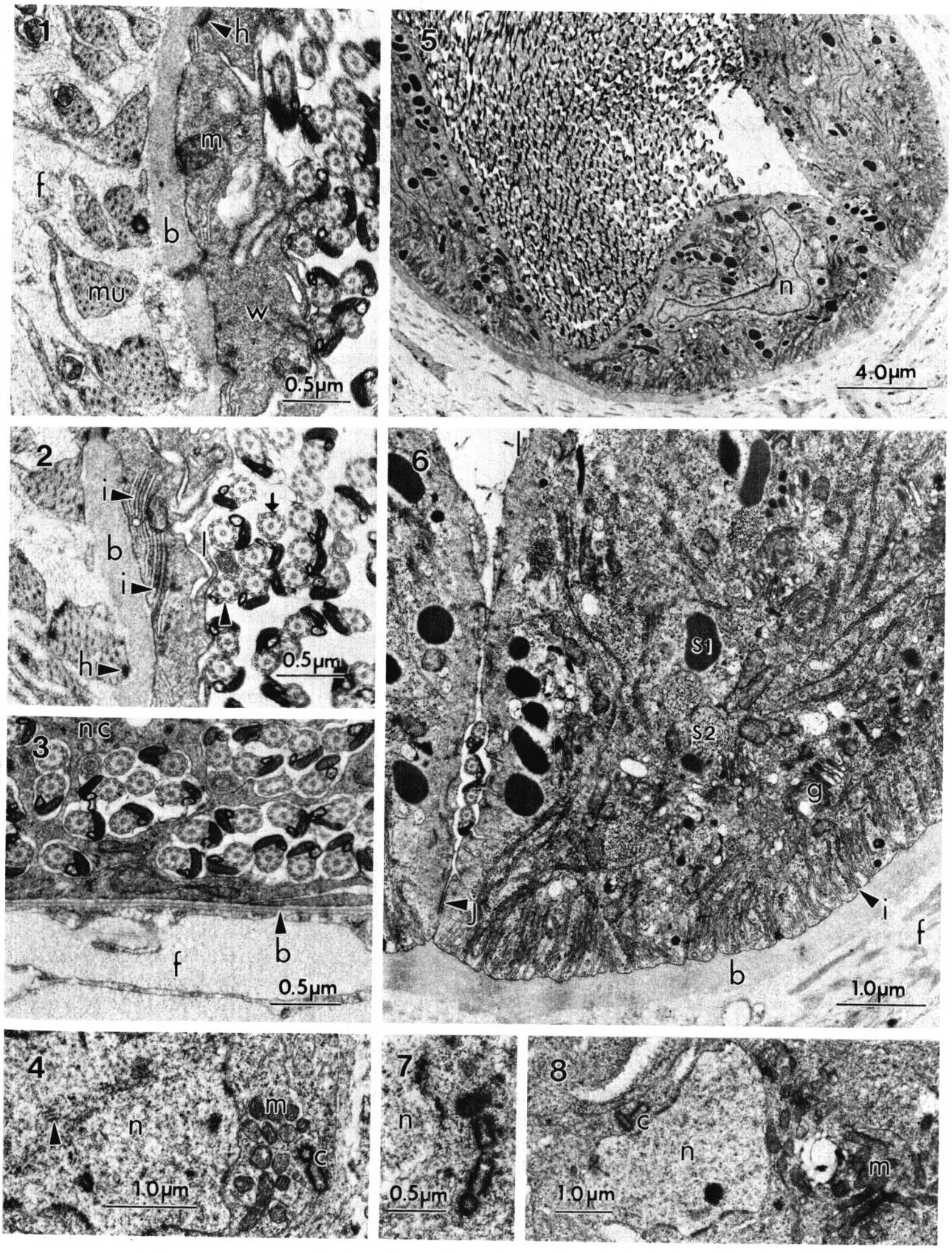
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FIGS. 1-8.

in cooled seawater by air from Sydney to Armidale, and fixed for 1.5-2 h at 4° C in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, made with filtered seawater, washed for 30 min in the same buffer at 4° C, post-fixed for 30 min at room temperature in 1 % OsO_4 in the same buffer, dehydrated in an ethanol series and embedded in Spurr's resin. Some specimens were fixed for 2 h immediately after removal from the fins of live fish, buffer washed, sent in cold buffer by air (approximately 2 h) and further processed as above. Sections of 60-70 nm were stained with alcoholic uranyl acetate and lead citrate and examined under a Jeol 1200 EX electron microscope at 60 kV.

RESULTS

Mature sperm

Mature sperm from the testis, sperm duct and ejaculatory duct are very long and filiform. For most of their length a single axoneme and a single mitochondrion lie tightly pressed alongside the nucleus, with no other inclusions, no peripheral cytoplasm, nor any peripheral microtubules *(Figs 1-3, 5, 6, 20-23)*. A short region, possibly at each end of the sperm, contains only the axoneme and the nucleus *(Fig. 2)*, then the nucleus ends and the axoneme tapers by first losing the central element, followed by one microtubule from each doublet. Finally the number of single microtubules diminishes *(Figs. 22, 23)*. Examination of a large number of transverse sections of mature sperm did not reveal any evidence for the persistence of a centriole nor any sperm containing two axonemes. The sperm axoneme has a « 9+1 » arrangement of the microtubules. However, while the complex central element in tangential longitudinal section *(Fig. 16)* shows the double helical organisation typical of Trepaxonemata, as illustrated in Burton (1967), it can be seen from cross sections *(Figs. 2, 3, 21-23)* that the hub of the central element is not a solid core, but a hollow cylinder.

Sperm duct

The lumen of the duct was tightly packed with sperm. The duct wall *(Figs. 1, 2)* is composed of a thin layer of cytoplasm 400-500 nm thick, with numerous lamellae extending into the lumen, and large numbers of evaginations by the surrounding basal lamina into the wall. The cytoplasm contains mitochondria, and there are junctions in the wall, but nuclei were not observed although they are likely to be present. The cytoplasmic layer is tightly surrounded by a layer of dense fibrous matrix (basal lamina) and by numerous small circular muscle bundles in a loose fibrous matrix *(Figs. 1, 2)*. Hemi-desmosomes attach muscle bundles and the cytoplasmic layer of the wall to the basal lamina *(Figs. 1, 2)*.

Ejaculatory duct

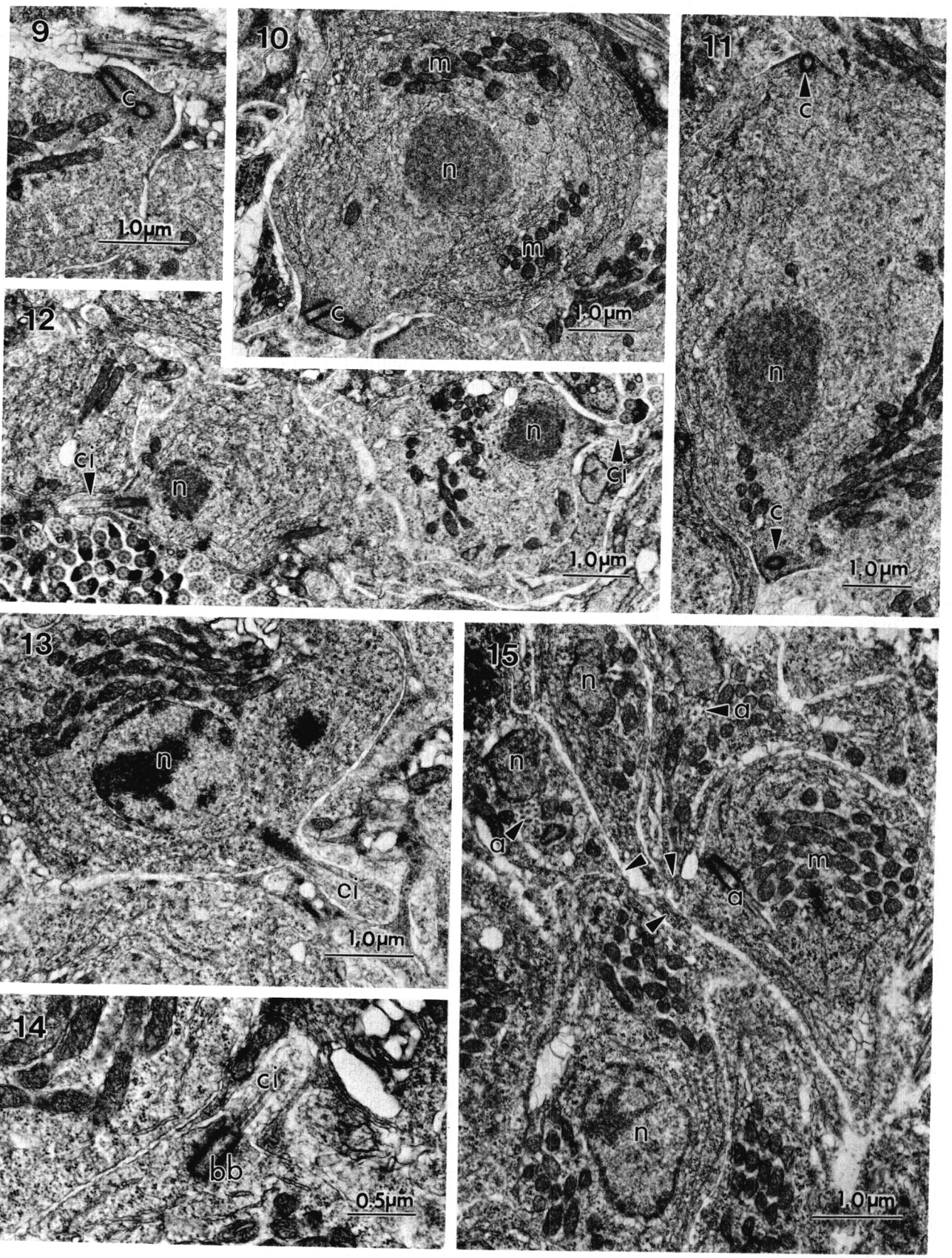
This organ, shaped like a bent elbow and located just posterior to the cirrus (see Roubal *et al.*, 1983) was also tightly packed with sperm *(Fig. 5)* with no secretion apparent between them. The wall is composed of several cells which are up to 8 µm thick in the middle but tapering such that adjacent wall cells are only in contact with each other by septate junctions approximately 0.5 µm long *(Fig. 6)*. These cells have short lamellae extending into the lumen, and many invaginations by the basal cell membrane, irregularly-shaped nuclei and numerous inclusions: Golgi apparatus, mitochondria, endoplasmic reticulum, ribosomes and two kinds of secretion—one uniformly opaque and the other of granular appearance. At the base of these cells is a basal lamina surrounded by a loose fibrous matrix *(Figs. 5, 6)* and a layer of circular muscles (not illustrated).

Spermatogenesis

The large, single, anterior testis of *Anoplodiscus* is thin-walled and surrounded by a basal lamina and many layers of fibrous tissue interspersed with cell processes *(Figs. 3, 20)*. All stages of spermatogenesis are tightly packed within the lumen, with no regular arrangement of cell types in relation to peripheral or central location. Late spermatogonia occur in clusters and are characterized by a high nucleus/cytoplasm ratio, a prominent nucleolus, loose

FIGS. 1-8. — Sections through sperm duct *(Figs. 1, 2)*, testis *(Figs. 3, 4, 7, 8)* and ejaculatory duct *(Figs. 5, 6)* of *Anoplodiscus cirrusspiralis*.

Fig. 1. Note cytoplasmic layer forming the wall (w), of the testis, mitochodrion (m), basal lamina (b), hemidesmosome (h) at a junction of the wall and the basal lamina, and muscle (mu) and fibrous matrix (f) external to the sperm duct. *Fig. 2.* Note hemidesmosome (h) attaching muscle to basal lamina (b), invaginations (i) by the basal plasma membrane into the wall, lamellae (l) in the duct lumen, and mature sperm tips with axoneme and nucleus only (arrowhead) and axoneme only (arrow). *Fig. 3.* Note mature sperm in the testis surrounded by cytoplasm of a nurse cell (nc), thin basal lamina (b) and fibrous matrix (f) external to the testis. *Fig. 4.* Primary spermatocyte—note pair of centrioles near to the cell boundary, bundle of mitochondria (m), and nucleus (n) with nuclear pores and synaptonemal complex (arrow). *Fig. 5.* Note ellipsoid shape of cells forming the wall of the ejacularoty duct and irregular shape of nucleus (n). *Fig. 6.* Higher magnification of *Figure 5* to show the junction between two of the cells forming the wall of the ejaculatory duct. Note septate junction (j), lamella (l), Golgi apparatus (g), two types of secretion (s1 and s2), many invaginations by the basal plasma membrane (i), and basal lamina (b) and fibrous matrix (f) external to the seminal vesicle. *Fig. 7.* Two adjacent pairs of centrioles near the nucleus (n) of a primary spermatocyte. *Fig. 8.* Primary spermatocyte—note electron-lucent nucleoplasm, indented margins of the nucleus (n), mitochondria (m) and a pair of centrioles (c) close to the nucleus.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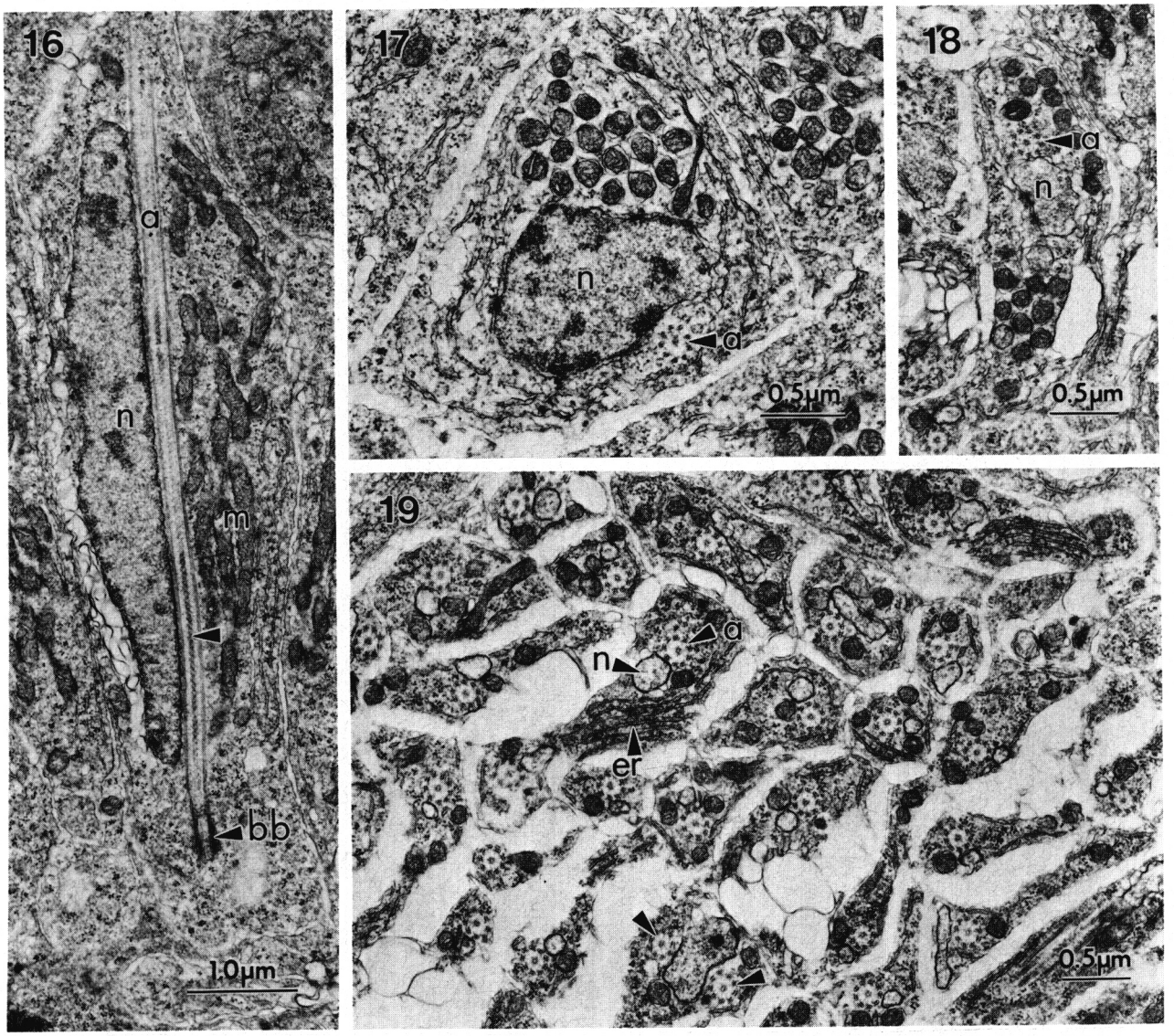
FIGS. 9-15.

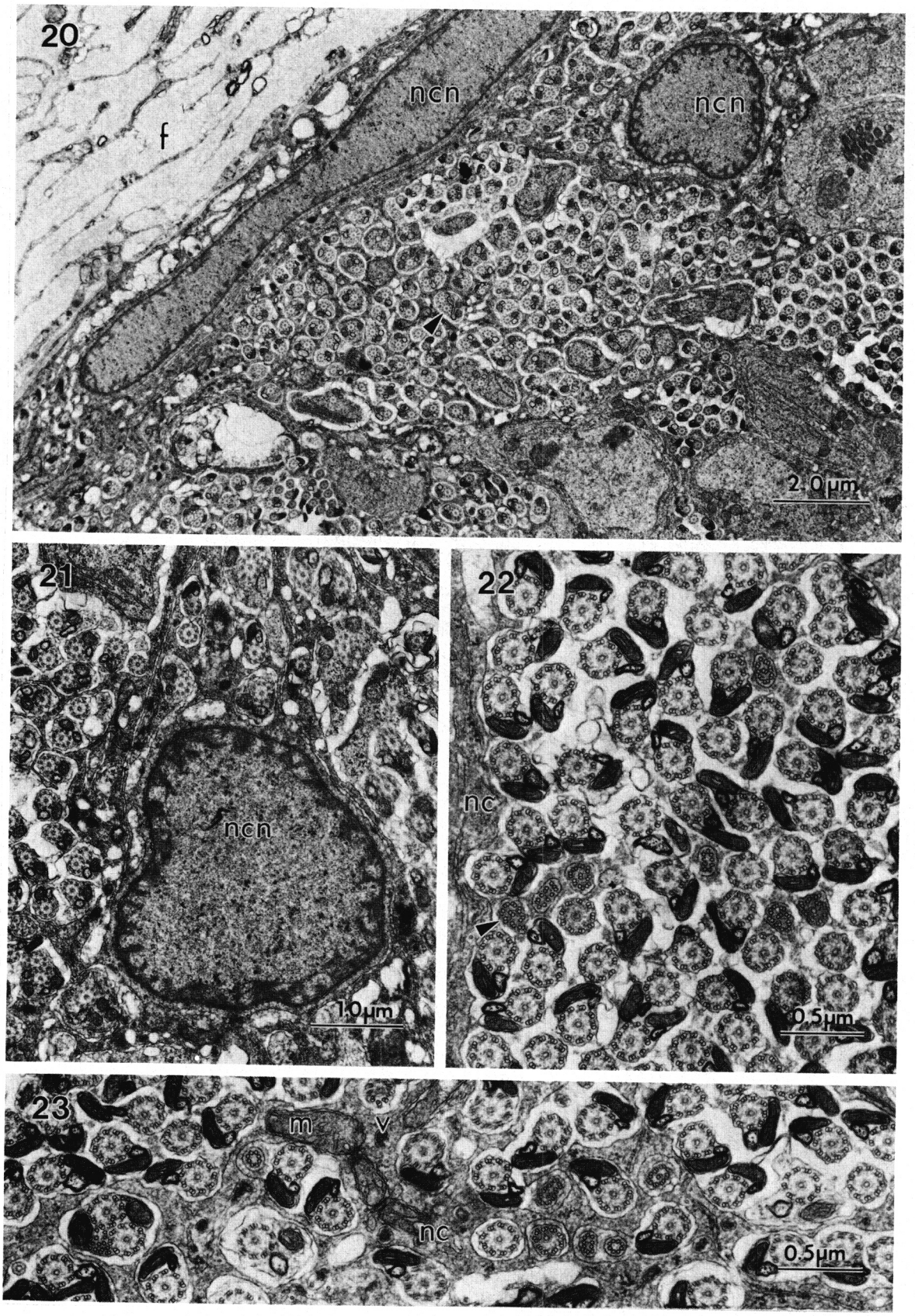


FIGS. 16-19. — Developing spermatids of *A. cirrusspiralis.*

Fig. 16. Longitudinal section—note basal body (bb) of axoneme (a) lies close to one pole of spermatid, double helical arrangement of complex central element of axoneme (arrowhead), elongating nucleus (n) and mitochondria (m). *Fig. 17.* Cross section—note nucleus (n) and axoneme (a). *Fig. 18.* Cross section of more advanced spermatid with narrower nucleus (n) and adjacent axoneme (a). *Fig. 19.* Cross sections of later spermatids—note nucleus (n) very narrow with dense periphery and few internal chromatin strands, axoneme (a) and persistent endoplasmic reticulum (er). Two arrowheads indicate two axonemes in one developing spermatid.

FIGS. 9-15. — Early spermatids of *A. cirrusspiralis.*

Fig. 9. Note two centrioles at right angle to each other (c) at the margin of the cell, dense layer beneath the plasma membrane near centrioles. *Fig. 10.* Note two centrioles at cell margin, bundles of mitochondria (m) and nuclear material (n) not yet bounded by a nuclear envelope. *Fig. 11.* Note centrioles (c) at opposite margins of the one cell, nuclear material (n) without nuclear envelope. *Fig. 12.* Two spermatids, each with a short bulbous cilium (ci) emerging from opposite poles, nuclear material (n) with nuclear envelope reforming. *Fig. 13.* Spermatid with short bulbous cilium (ci) and nucleus (n) well defined with patches of chromatin. *Fig. 14.* Note free cilium (ci) at an angle to the cell surface, basal body (bb) and no dark layer beneath the plasma membrane. *Fig. 15.* Cluster of early spermatids joined by thin cytoplasmic bridges (arrowheads). Note nuclei (n), mitochondria (m), and axonemes (a) growing within the spermatid body.



FIGS. 20-23.

clusters of mitochondria and electron dense cytoplasm. Primary spermatocytes, characterized by many synaptonemal complexes in each nucleus, occur in clusters and are readily recognised by the shape and electron-opacity of the nucleus *(Figs. 4, 7, 8)*. The margin of each nucleus has a scalloped appearance with an abundance of nuclear pores and increased membrane density in the indented regions. At least one pair of centrioles, oriented at right angles to each other, lies in one of these indentations. *Figure 7* shows two pairs of centrioles close to the nucleus of a primary spermatocyte, and in one instance, by examining consecutive serial sections, three pairs were located in the same cell. *Figure 4* shows a pair of centrioles close to the cell boundary of a primary spermatocyte. Primary spermatocytes contain discrete bundles of sausage-shaped mitochondria *(Figs. 4, 8)*.

Early spermatids have several rows of peripheral endoplasmic reticulum, clusters of ribosomes and discrete bundles of mitochondria, as do spermatocytes. Prior to the re-formation of the nuclear envelope, pairs of centrioles migrate to the cell boundary, remaining at right angles to each other, and a dark layer is visible beneath the plasma membrane adjacent to them *(Figs. 9, 10)*. No microtubules were seen in this layer. The occasional observation of centrioles at the cell surface widely separated from each other *(e. g. Fig. 11)* suggests that in these instances, the final cell division has not yet taken place. Spermatids are joined to each other by thin cytoplasmic bridges *(Fig. 15)*. Although pairs of centrioles were commonly observed, only one appears to give rise to a short bulbous cilium without a rootlet. The cilium is initially oriented at a right angle to the cell surface *(Figs. 12, 13)*. At this time the nuclear envelope reforms and chromatin patches appear *(Fig. 13)*. *Figure 14* shows a short cilium at a more acute angle to the cell surface. All observations of spermatids at a later stage of development show the basal body at or near the cell surface, with an axoneme growing within the spermatid mass, alongside the nucleus and mitochondria *(Figs. 15-19)*. This is taken to indicate that the short bulbous cilium has rotated and fused with the spermatid body, *i. e.* there is no cytoplasmic outgrowth in the direction of the free cilium, rather the cilium turns, fuses with the spermatid mass and then grows back alongside the nucleus. At least 100 sections through the testes of two individuals were examined. The whole spermatid elongates with the nucleus becoming progressively narrower, the axoneme continues to grow within the spermatid body and the numerous individual mitochondria eventually fuse into a single one *(Figs. 16-23)* as indicated by the lack of sections of the principal region of mature sperm without a mitochondrial profile *(Figs. 1-3, 5, 6, 20-23)*.

In several instances, spermatids were observed with two axonemes within the main body *(Figs. 19, 20, 23)*, although two free cilia were never seen, nor were any mature sperm from the sperm duct observed to have two axonemes. It is therefore assumed that such spermatids do not complete development or that the second axoneme is resorbed.

A conspicuous feature of the testis of *Anoplodiscus* is the tightly packed arrrangement of all the component cells. In addition to the germinal stages, there is a cell type interpreted as a nurse cell. These cells have a very large nucleus with a honeycombed arrangement of the peripheral chromatin *(Figs. 20, 21)*. Processes from these cells extend around the periphery of the testis *(Figs. 3, 20)* and ramify throughout the lumen enveloping all stages of spermatogenesis, leaving very little free space. They contain electron-dense cytoplasm, mitochondria and vesicles *(Figs. 3, 20-23)*.

DISCUSSION

The mature sperm of *A. cirrusspiralis* resembles that found in (at least) 11 monopisthocotylean Monogenea, belonging to the orders Dactylogyridea and Tetraonchidea (classification of Lebedev, 1988, see Justine, 1991*b*, Justine *et al.*, 1991) *i. e.* they possess a single axoneme, mitochondrion and nucleus and no peripheral microtubules. This configuration is designated sperm pattern 4 in the scheme of Justine *et al.* (1985) for the Monogenea and it appears to represent a monophyletic grouping. Further modifications on the basic pattern are found in several species within this group *e. g.* centriole adjunct, incomplete *b* tubules in the axoneme, lateral crest on the mature spermatozoon, coiled nucleus along part of the sperm (see Justine, 1991*b*). A single axoneme in the sperm is also found in Cestoda Cyclophyllidea, Caryophyllidea, Diphyllidea, Tetraphyllidea Phyllobothridae (Swiderski, 1974, 1976; Mokhtar-Maamouri and Swiderski, 1976; Euzet *et al.*, 1981; MacKinnon and Burt, 1984) but all Eucestoda examined lack a mitochon-

FIGS. 20-23. — Late spermatids, mature sperm and nurse cells.

Fig. 20. Note two nurse cell nuclei (ncn) with processes extending around developing spermatids and spermatocytes. Arrowhead indicates spermatid with two axonemes. *Fig. 21.* Enlargement of nurse cell perikaryon of *Figure 20,* showing cytoplasmic extensions enveloping spermatids. Note honeycombed peripheral chromatin in nurse cell nucleus (ncn). *Figs. 22, 23.* Mature sperm in the testis showing order of diminishing microtubules in the axoneme. Note hollow tubular core in centre of complex central unit, cytoplasm of nurse cell (nc), containing mitochondrion (m) and secretory vesicle (v).

drion (see Justine, 1991*a* for references). *Anoplodiscus* does not resemble the cestodes in this respect.

Ehlers (1985*a, b*) introduced the taxon Trepaxonemata incorporating the Polycladida, Seriata, "Typhloplanoida" (including Kalyptorhynchia), "Dalyellioida" and all Neodermata. The synapomorphy of this taxon is a central core of the axial unit with a unique structure: « a central electron-dense element, an intermediate transparent zone, and a peripheral electron-dense sheath, with 9 spoke-like radiations passing from this sheath to the A-subtubules of the peripheral doublets » (Ehlers, 1985*b*) (previously described by Burton, 1967). Examination of published micrographs of spermatozoa from a large number of species from these taxa confirms the electron-dense appearance of the central core. However, in the spermatids and sperm of *A. cirrusspiralis* the central element is distinctly hollow, thus resembling a larger than normal microtubule. It is interesting to note that a similar tubular central unit can also be seen in the micrographs of sperm from *Kronborgia isopodicola* (Williams, 1988 and personal observation) and in at least one of five species of *Pterastericola* examined, *i. e. P. asamushii* (Jondelius, in press). Neither Williams (1988) nor Jondelius (in press) commented on the hollow nature of the central unit shown in their micrographs. Within the Acoela, sperm from different species have ben demonstrated to have axonemes with a 9+2, 9+0, or two different kinds of 9+1 pattern (references in Hendelberg, 1983). *Childia groenlandica* was reported to have paired axonemes with a 9+1 pattern of the same type as is found in turbellarians, while *Paramecynostomum diversicolor* has a different 9+1 pattern, the core of the central structure being tubular and not electron-dense (Hendelberg, 1977), also found in *Pseudactinoposthia* sp. (Rohde *et al.*, 1988). This last description also applies to the sperm of *Anoplodiscus,* although the tubular structure in the acoels appears smaller than that found in *A. cirrusspiralis, K. isopodicola* and *Pterastericola asamushii.* All the observations of a hollow central core are of species which have incorporated axonemes, as characteristic of Neodermata.

While the appearance of the mature sperm appears to confirm the position of *Anoplodiscus* within the Monogenea Monopisthocotylea, the processes of spermiogenesis depict a significant departure from the pattern found within that group. The usual pattern for monopisthocotyleans, as described by Justine and Mattei (1984), whether they possess two axonemes, two reducing to one or one axoneme from the beginning, is for a common cytoplasmic mass to be formed following meiosis. « At first numerous protuberances jut out from the cytoplasmic mass in all directions; then the external protuberances disappear and numerous parallel canals develop in the mass ». Within these canals, the zones of differentiation develop. In most species, axonemes develop from one or two centrioles in this region and the nucleus and mitochondrion subsequently penetrate the zone, *i. e.* the axoneme is never free. In two species, *Heterocotyle* and *Loimosina,* the axonemes are free for a short period before incorporation in the cytoplasm, prior to migration of the nucleus and mitochondrion (Justine and Mattei, 1983, 1985). *Calceostoma* (see Justine and Mattei, 1986) and *Diplectanum* (see Justine and Mattei, 1984) show a modification of the development phase with the axoneme assuming a lateral position in relation to the zone of differentiation. This contrasts with the normal orientation in other Monogenea, and indeed in most Neodermata (major groups of parasitic platyhelminths), in which the axonemes assume a vertical position and the nucleus and mitochondria migrate directly into the protuberance, which lies at a normal angle to the spermatid surface.

The spermiogenetic process in *Anoplodiscus* is distinctly different from that described above. An initially free axoneme grows out from the spermatid surface, subsequently turns back towards the spermatid body, fuses with it and continues to grow within the elongating spermatid in the opposite direction to its initial outgrowth. At no stage is there a cytoplasmic outgrowth from the spermatid surface (or within an internal canal) in the direction of the cilium, nor does the nucleus elongate and migrate past the basal body of the axoneme. At least 100 sections from the testes of two individuals were examined and these phenomena were not observed.

To our knowledge, in all other cases of spermiogenesis studied so far in the Neodermata (except the aspidogastrean *Multicotyle purvisi*—Watson and Rohde, 1991), the nucleus (and mitochondrion when present) migrate into a zone of differentiation which includes the centriole(s) (and intercentriolar body and striated rootlets when present), *i. e.* these organelles grow past the basal body (bodies) and because of the length of time taken for elongation to occur, this stage is frequently seen and well documented in photographs. The vast majority of neodermatan species studied have initially free axoneme(s), and fusion with the median cytoplasmic process occurs from the proximal end of the spermatid where the centrioles are located, to the distal end where the tips of the free cilia are located. Thus, Justine (1991*a*) has proposed as a synapomorphy for the Cercomeridea (= Neodermata excluding the Udonellidea) spermiogenesis involving proximo-distal fusion of the axonemes. In contrast, in all other plathyhelminth group that have incorporated axonemes, *i. e.* in the Acoela, Kalyptorhynchia, Pterastericolidae and Fecampiidae, fusion of the initially free axonemes is in a non proximo-distal manner (Acoela-Hendelberg, 1969; Boyer and Smith, 1982; Rohde *et al.*, 1988; Kalyptorhynchia-Hendelberg, 1969; L'Hardy, 1988 and Pterastericolidae (personal observation), not yet observed in Fecampiidae). In these non-neodermatan orders, the two basal bodies give rise to axonemes which initially grow in opposite directions from each other. The zone of differentiation then elongates, carrying the basal bodies away from the main body of the spermatid. They then

rotate back towards the main body and fuse with the cytoplasmic process in a non proximo-distal direction. The nucleus and remainder of the spermatid elongate in the direction away from the basal bodies. In *Anoplodiscus,* spermiogenesis appear to be similar to the non-neodermatan taxa, although the free cilium is very short when it fuses with a still basically spherical/oval spermatid.

If it is accepted that *Anoplodiscus* is a monogenean, based on the possession of two pairs of eyes, 16 hooks in the oncomiracidium, prohaptor, adult opisthaptor similar to other Monogenea except in the lack of hooks, typical neodermis in the adult (personal observation), sensory receptors with electron-dense collars (personal observation), and similarity of protonephridial system in most respects (Rohde *et al.,* 1992), then the unusual spermiogenesis must be regarded as aberrant. It either represents a reversal of previously established proximal-distal fusion, *i. e.* is apomorphic for *Anoplodiscus,* or alternatively, is plesiomorphic, *i. e.* a « left-over » of the « primitive » turbellarian state. Other aberrant characteristics, including the lack of hooks in the adult and the lack of complete junctions along the flame bulb and smaller protonephridial capillaries (Rohde *et al.,* in press) appear to be the result of secondary loss and suggest that the aberrant spermiogenesis may be a secondary phenomenon, an apomorphy as well.

A third possibility would be acquisition by *Anoplodiscus* of several features from other taxa by horizontal gene transfer, *i. e.* mosaic evolution (Rohde, 1990). In other words, the species would be a mosaic of turbellarian (spermiogenesis) and monogenean (larval hooks, etc.) characteristics not as a result of common ancestry but due to a mixing of genes.

A particularly interesting result of our study is the demonstration that sperm characteristic of certain Monogenea (single axoneme, no peripheral microtubules) can arise in fundamentally different ways, by proximo-distal fusion in most species and by distal-proximal fusion in *Anoplodiscus.* This clearly shows that spermiogenesis is not always more reliable in establishing phylogenetic relationships than mature sperm structure.

Development of identical mature sperm in different ways is a case of « temporary deviation » in the sense of Rensch (1959), *i. e.* mutations have brought about changes of certain ontogenetic stages without affecting the outcome. In such cases, comparison of the end result may be more relevant in establishing phylogenetic relationships than comparison of the intermediate stage leading to it. However, in general, identical structure of the fully developed character and of ontogeny leading to it would give stronger support for phylogenetic relationship than only one of the two.

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