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Ultrastructure of spermiogenesis in the gastropod *Heliacus variegatus* (Architectonicidae), with description of a banded periaxonemal helix

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Abstract. Spermiogenesis and spermatozoa of the marine gastropod *Heliacus variegatus* Gmelin were examined using transmission electron microscopy (TEM). Mature spermatozoa are composed of an acrosomal vesicle, helical nucleus, elongate midpiece, annulus, glycogen piece and short end piece. The midpiece consists of a 9+2 axoneme, nine coarse fibres, and a banded helix, all enclosed by a continuous mitochondrial sheath (with multiple, helically coiled grooves). Anterior extensions of the mitochondrial sheath and banded helix form a double sheath around the basal half of the nucleus – an arrangement possibly unique in the Mollusca. During spermiogenesis, dense plaques delineating the anterior and posterior poles of the spermatid nucleus become attachment sites for the acrosomal vesicle and the axial complex (respectively). As the nucleus condenses and elongates, midpiece formation involves fusion of numerous, oblong mitochondria along the length of the axoneme. The coarse fibres and banded helix of the midpiece probably are derived through centriolar activity. Results of the study support inclusion of the Architectonicidae within the Heterobranchia, but in view of midpiece specializations, do not clarify the precise relationship of the family within this subclass.

Introduction

Architectonicids ("sundial shells") form a small but systematically important group of gastropods which feed on zooantharians (Robertson 1967) or corals (Robertson et al. 1970) and possess characteristically low-spired, umbilicate shells (planispiral in some genera – Garrard 1977, Boss 1982, Bieler 1988). Although they are regarded by some authors as true caenogastropods (e.g. Golikov and Starobogatov 1975, Gosliner 1981, Fretter and Graham 1982, Robertson 1985) or as an offshoot of the prosobranch-heterobranch transition (Robertson 1973), the weight of anatomical evidence increasingly favours their inclusion within the Heterobranchia (Haszprunar 1985a, b, 1988, Ponder and Warén 1988). Partly with the intention of resolving the systematic position of the Architectonicidae, Healy (1982a) showed that spermiogenesis in the architectonicid *Philippia* (*Psilaxis*) *oxytropis* A. Adams followed a pattern similar to that occurring in opisthobranchs and pulmonates. In addition, paraspermatozoa (apyrene or oligopyrene sperm – common in caenogastropods, including the Janthinoidea) were shown to be absent from this species, and have yet to be observed in the family (see also Healy 1988a, 1991). In the present account we describe sperm and spermiogenic features (some previously unrecorded in the Mollusca) in a representative of a second architectonicid genus, *Heliacus*, and contrast the results obtained with those available for other investigated members of the family, and, more generally, other molluscs. Systematic implications of sperm ultrastructure in the Architectonicidae have already been dealt with in the preceding paper (Healy 1991).

Materials and methods

Specimens of *Heliacus variegatus* Gmelin were collected at low tide from *Palythoa* sp. colonies near Mossman (northern Queensland) in May 1983 and Myora, Stradbroke Island (southern Queensland) in March 1988. Although the hermaphrodite duct of collected individuals contained few spermatozoa, squashes of ovotestis tissue revealed mature and developing spermatozoa and developing oocytes. Small (1 to 2 mm^3) pieces of ovotestis tissue were excised and fixed for 2 h in 3% glutaraldehyde (prepared with 0.2 *M* phosphate buffer containing 10% sucrose). Subsequently, tissues were rinsed for 30 min in phosphate buffer (sucrose-adjusted), postfixed for 80 min in 1% osmium tetroxide (prepared in phosphate buffer, sucrose-adjusted), rinsed again in buffer (1 h), dehydrated using an ascending series of ethanols, and finally embedded in Spurr's epoxy resin. All stages of processing up to and including 70% ethanol were maintained at 0 to 4°C, and thereafter at room temperature. Semithin and ultrathin sections were cut using an LKB 2128 UM IV ultramicrotome and collected on uncoated 200-mesh copper grids. Specimen-bearing grids were stained with 6% aqueous uranyl acetate and

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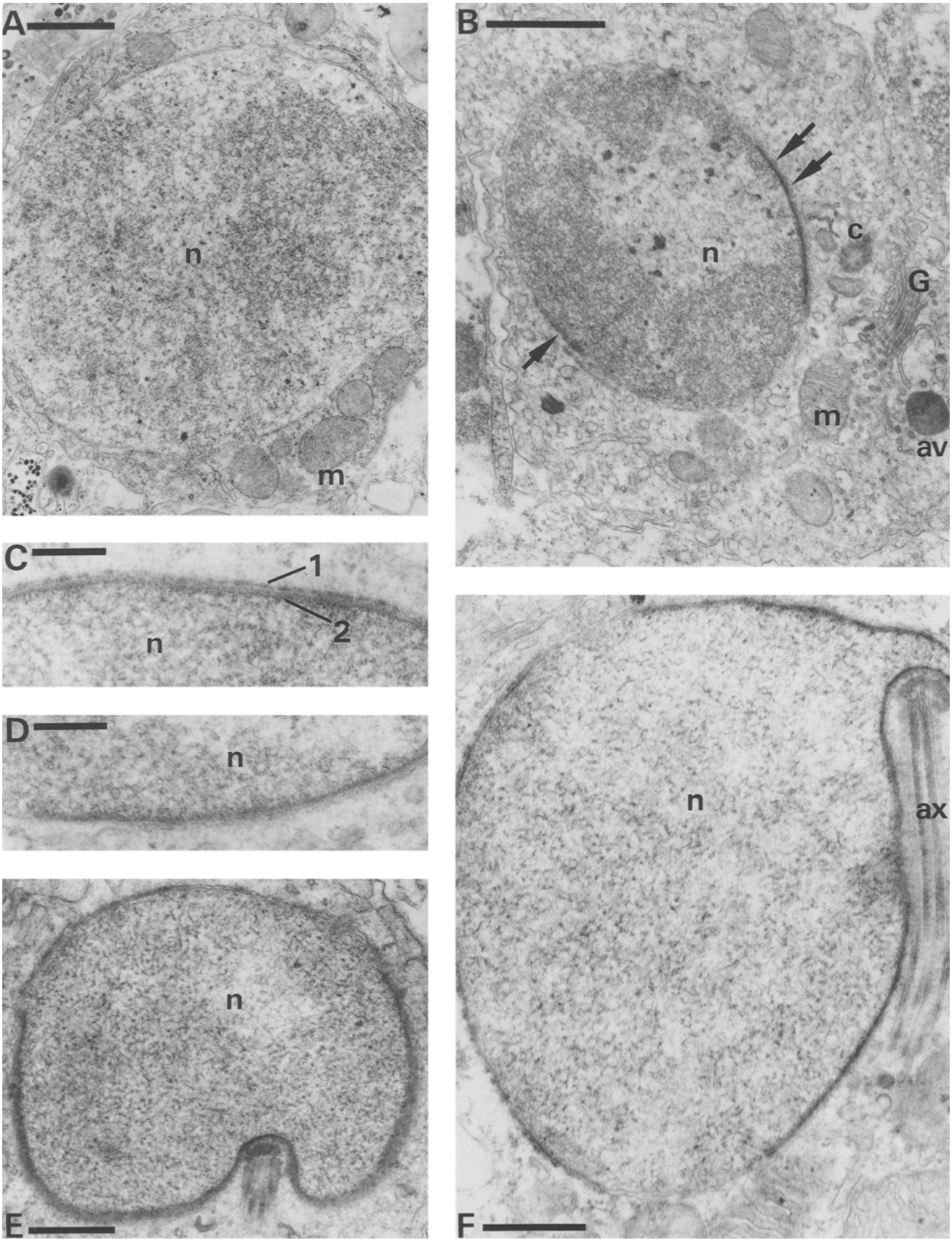

Fig. 1. *Heliacus variegatus*. (A) Early spermatid, showing numerous mitochondria (m) and uncondensed nucleus (n). (B) Appearance of posterior nuclear plaque (double arrow) associated with centriole (c) and anterior plaque (arrow); note also Golgi complex (G) and developing acrosomal vesicle (av). (C) Detail of anterior plaque, showing granular material lining outer nuclear membrane (1) and adjacent layer of condensed nuclear material (2). (D) Posterior plaque. (E) Relocation of centriolar complex within invaginated base of nucleus. (F) Oblique longitudinal section through developing basal invagination of nucleus and attaching centriolar complex and axoneme (ax). Scale bars: in (A), (B) = 1.0 μm, in (C), (D) = 0.2 μm, in (E), (F) = 0.5 μm

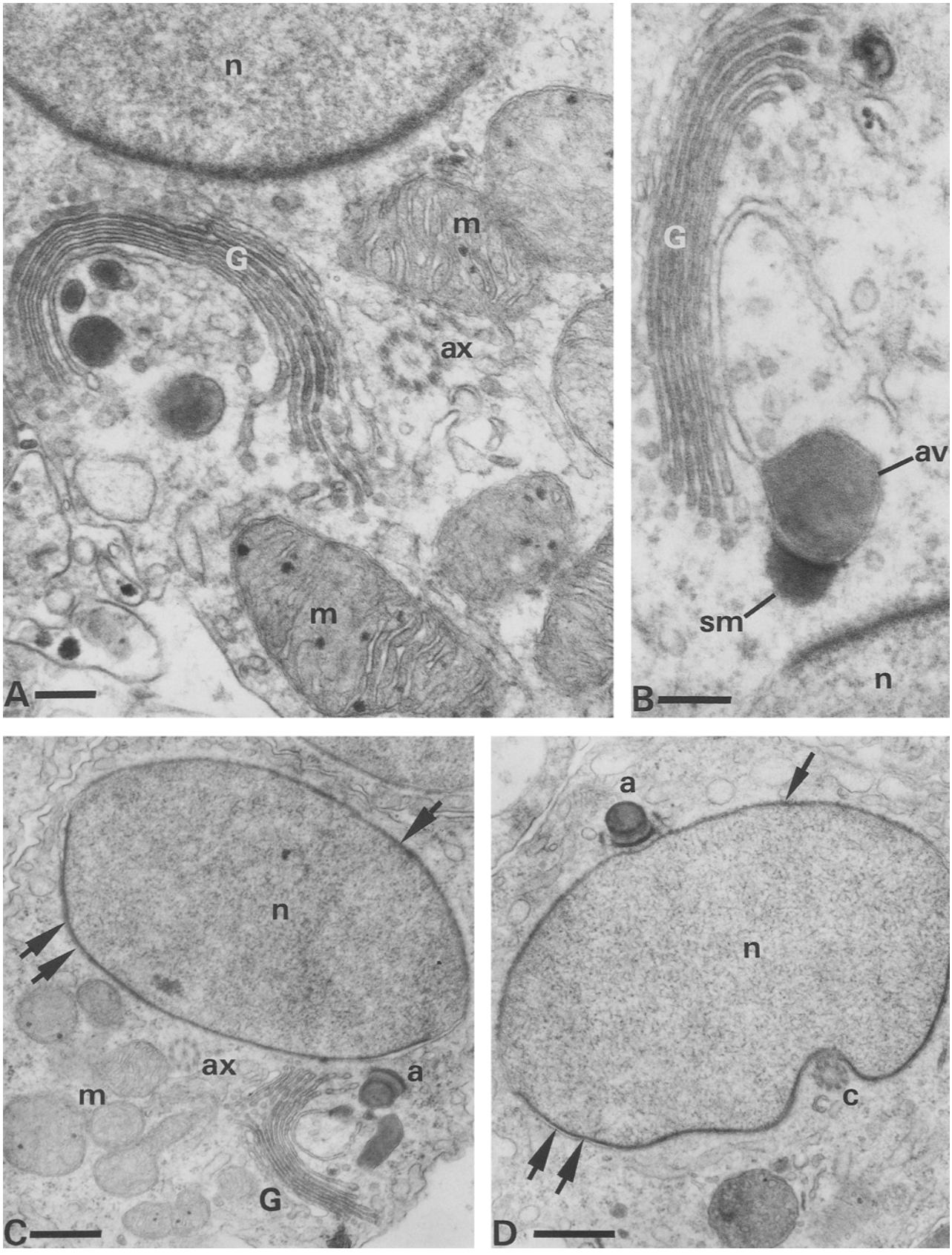

Fig. 2. *Heliacus variegatus*. (A) Earliest observed stage of acrosome development; note Golgi complex (G) and four secretory vesicles, mitochondria (m) with dense intramitochondrial deposits, axoneme (ax) and condensing nucleus (n) (showing anterior plaque). (B) Definitive acrosomal vesicle (av) attached to Golgi cisterna; base of the acrosomal vesicle is periodically banded and associated with a large deposit of subacrosomal material (sm). (C) Spermatid, showing relative positioning of developing acrosome (a), Golgi complex, axoneme, mitochondria and condensing nucleus [anterior plaque (arrow) and posterior plaque (double arrow)]. (D) Spermatid with acrosomal complex attaching to anterior nuclear plaque (arrow); note also posterior plaque (double arrow) and centriolar complex (c) within nuclear invagination. Scale bars in (A), (B) = 0.2 μm, and in (C), (D) = 0.5 μm

Reynold's lead citrate, then examined using a Siemens Elmiskop I or Hitachi 300 transmission electron microscope (TEM) operated at 60 or 75 kV. Voucher specimens of *Heliacus variegatus* have been deposited at the Queensland Museum (Brisbane) (Registration Nos. MO. 15919, MO. 28786).

Results

Spermiogenesis

Nuclear condensation

Initially the spermatid nucleus of *Heliacus variegatus* is spherical (diameter of about 5 μm), and its contents evenly granular in appearance (Fig. 1 A). Formation of dense plaques, firstly at the posterior and later the anterior poles of the nucleus (Fig. 1 B), mark the onset of visible chromatin condensation. Although the two plaques are similar in extent (Fig. 1 F), the anterior plaque is distinguished by a layer of coarsely granular material lining the adjacent portion of the outer nuclear membrane (cf. C and D in Fig. 1). Both plaques act as attachment sites for developing organelles. As the acrosomal complex settles on the anterior plaque (Fig. 2 D), the posterior plaque and nuclear base invaginate to form a socket for the centriolar derivative and attached axonome (Fig. 1 E). As these events take place, the nuclear contents change from a granular to fibro-reticular fabric (Figs. 1 E; 2 C, D). The next phase of condensation involves substantial elongation of the nucleus (from 2 to 10 μm) and modification of the fibres into lamellae (Figs. 3 C; 4 A, B) through lateral fusion of fibres. A single row of microtubules running anterio-posteriorly, surround the spermatid nucleus at this stage of development and remain in place until sperm are fully mature (Fig. 4 D). As lamellae become compacted, electron-lucent lacunae within the condensing nucleus (Fig. 4 A, D) are gradually eliminated. Mature sperm nuclei show no trace of lacunae or the lamellar substructure (Fig. 5 A, B).

Acrosome development

Development of the acrosomal complex may occur anywhere within the cytoplasm of early spermatids. Typically, however, it commences at the same time as the nuclear plaques appear (Fig. 1 B), but immediately prior to formation of the centriolar fossa. Cisternae on the concave side of the Golgi complex are responsible for secretion of the acrosomal vesicle and possibly its associated subacrosomal deposit (Fig. 2 A, B). In the earliest observed stage of acrosome development (Fig. 2 A), at least four secretory vesicles are clustered within the concave face of the Golgi cisternae. A later stage (Fig. 2 B) shows the acrosomal vesicle continuous with a Golgi cisterna and bearing an electron-dense extravesicular deposit. Contents of the vesicle lying adjacent to the extravesicular deposit differentiate into layers (Fig. 2 B). Subsequently, the vesicle contents further differentiate into a spherical anterior portion (defined by a dense peripheral layer) and the banded basal portion (Figs. 2 C, D; 3 A, B). The subacrosomal deposit flattens to form a thin, curved layer lining the base of the acrosomal vesicle (Fig. 3 A, B). Fig. 3 A shows the acrosomal vesicle connected to a similarly dense, oblong vesicle – presumably also Golgi-derived and contributing in some way to acrosomal development (although the acrosomal vesicle has already reached its definitive size). The acrosomal complex eventually migrates towards the anterior nuclear plaque, if it is not already so positioned, and settles there (Figs. 2 D; 3 B). A dense, poorly defined cylinder, here termed a support cylinder, now girdles the acrosomal vesicle (Figs. 2 D; 3 B). As nuclear condensation progresses through fibroreticular, fibrous and lamellar stages, the acrosomal complex contacts the plasma membrane (Fig. 3 C, D) and the support cylinder elongates slightly. The internal substructure of the acrosomal vesicle does not change once attached to the condensing nucleus.

Midpiece formation

In early spermatids, mitochondria are scattered throughout the cytoplasm (Fig. 1 A, B). They are oblong, cristate and exhibit prominent intramitochondrial granules (Fig. 2 A). After the centriole/axoneme apparatus attaches to the nuclear invagination, mitochondria gather posteriorly along the length of the axoneme (Fig. 4 A). As the nucleus passes from fibrillar to lamellar stages of condensation, mitochondria begin to wrap and fuse around the axoneme (Fig. 3 E, F). Excess mitochondrial material, extruded from the developing mitochondrial sheath (e.g. Fig. 3 F), is possibly utilized further posteriorly where fusion has yet to take place. The role of the Golgi complex in midpiece formation of *Heliacus variegatus* is not clear. Commonly it is observed in the vicinity of fusing mitochondria, suggesting that the fusion process may to some degree be Golgi-facilitated. During formation of the mitochondrial sheath, the cristate substructure of participating mitochondria is totally remodelled (Fig. 3 G). The completed sheath is cylindrical and characterized by numerous grooves (? modified cristal surfaces) which face the coarse fibres (Fig. 4 E, F). A ring of microtubules which surrounds the midpiece late in development (Fig. 4 E) is probably continuous with that sheathing the late spermatid nucleus.

Fig. 3. *Heliacus variegatus*. (A) Detail of developing acrosome shown in Fig. 2 C; acrosomal vesicle (av), positioned close to Golgi complex (G) and nucleus (n), is attached to an oblong secretory vesicle; base of the acrosomal vesicle shows periodic banding, while subacrosomal material (sm) is transformed into a curved plate. (B) Detail of Fig. 2 D, showing acrosomal complex attaching to nucleus; a support cylinder (s) envelops the complex. (C) Acrosomal complex, partly sheathed by plasma membrane (pm), of advanced spermatid (lamellar stage of nuclear condensation). (D) Acrosomal complex of almost mature spermatozoon; note curved indentation of nucleus to accomodate acrosome and support cylinder. (E), (F) Early stage in formation of midpiece; mitochondria (m) enclose coarse fibres (cf) and axoneme, then fuse. (G) Midpiece of late spermatid prior to formation of periaxonemal helix; mitochondrial sheath (ms) shows grooves (? modified cristae). All scale bars = 0.2 μm

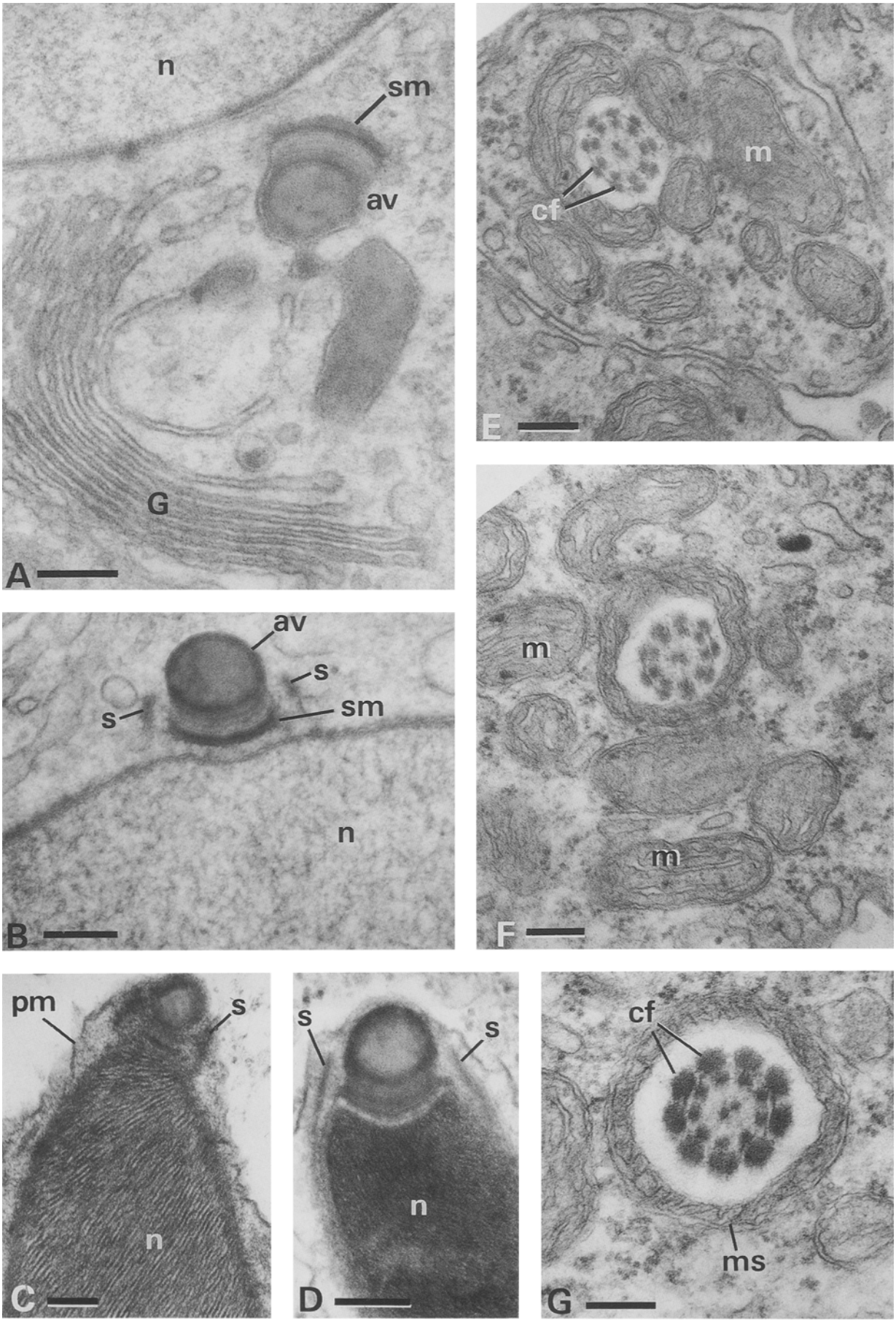

Fig. 3(A)–(G)

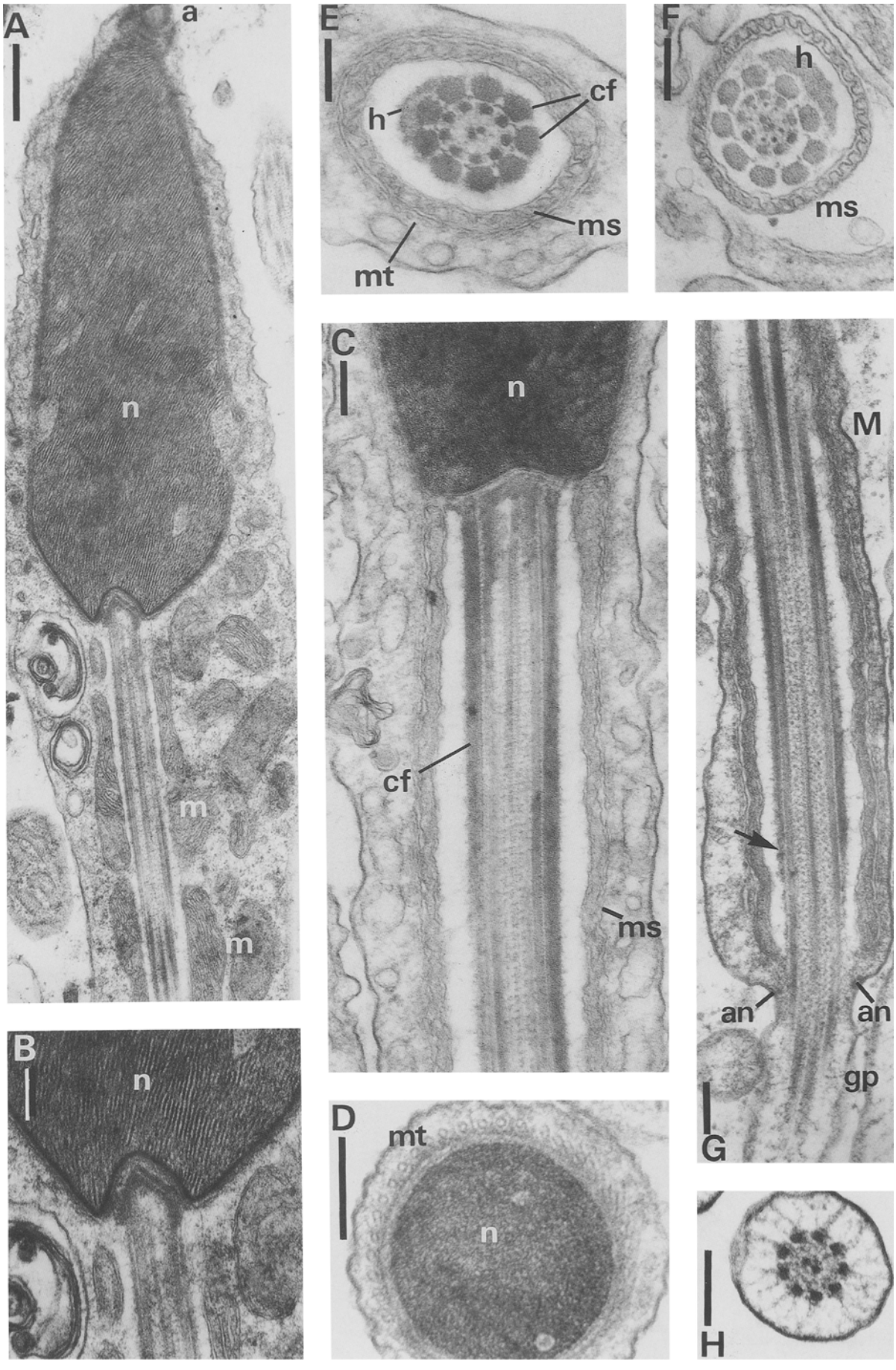

Fig. 4(A)–(H)

One of the most intriguing aspects of midpiece development in *Heliacus variegatus* is the origin of the nine coarse fibres and banded periaxonemal helix. In the case of the banded helix, a Golgian source seems unlikely given that the helix first appears after formation of the mitochondrial sheath has been completed (Fig. 4C, E). No evidence of a mitochondrial origin for the helix could be found, although it is interesting to note that a cylindrical extension of the helix accompanied by the mitochondrial sheath enclose the posterior half of the mature nucleus. The coarse fibres, later followed by the helix, appear to develop gradually with no evident input from mitochondrial or Golgian sources. It therefore seems likely that the helix and coarse fibres develop by accretion or perhaps even polymerization of cytoplasmic materials around the axoneme. The banded substructure of coarse fibres and the helix (reminiscent of banded centriolar rootlets) suggests close participation of the centriolar region in this process.

Annulus, glycogen piece

Development of the annulus and glycogen piece was not traced in this study. An annulus is present in late spermatids, in which deposition of the putative glycogen granules around the axoneme has yet to occur (Fig. 4G, H). It remains to be shown how these granules pass through the constriction caused by the annulus in late spermatids. Visible within the immature glycogen piece are forked fibres connecting axonemal doublets to the plasma membrane (Fig. 4H). Presumably these fibres act as scaffolding for the glycogen deposits of mature spermatozoa.

Mature testicular spermatozoa

Testicular spermatozoa measure 120 to 130 μm in length (from light microscopic observations) and comprise an acrosomal complex, helical nucleus, elongate midpiece, annulus (at junction of midpiece and glycogen piece), glycogen piece and an end piece.

Fig. 4. *Heliacus variegatus.* (A) Spermatid, showing acrosome (a), lamellar stage nucleus (n), axoneme (attached to nucleus) and unfused periaxonemal mitochondria (m). (B) Detail of nuclear invagination in (A), showing centriolar derivative lodged within invagination. (C) In late spermatids the nuclear invagination becomes reduced to a shallow depression; also visible, mitochondrial sheath (ms) and coarse fibres (cf) surrounding axoneme; periaxonemal helix not yet formed. (D) Transverse section (TS) of almost mature sperm nuclei, surrounded by microtubules (mt). (E), (F) TS through midpiece region of late spermatids, showing incompletely formed periaxonemal helix (h), mitochondrial sheath, coarse fibres, axoneme and cytoplasmic microtubules. (G) Junction of midpiece (M) and glycogen piece (gp) in almost mature spermatozoon; note annulus (an) and termination of coarse fibres (arrow). (H) TS glycogen piece of immature sperm; forked connectives attach plasma membrane to axonemal doublets. Scale bar in (A) = 0.5 μm; all others = 0.2 μm

Acrosome

The acrosomal vesicle is ovoid, 0.2 μm in diameter, with finely granular contents and a dense periphery (Fig. 5A, B). The basal portion of the vesicle exhibits parallel bands, the curved shape of which is mirrored by a thin layer of subacrosomal material and the apical depression of the nucleus – the latter forming the attachment site for the acrosomal complex. The nuclear membrane on which the acrosomal complex rests is lined with dense material and lifted slightly above the nuclear surface (Fig. 5B).

Nucleus

Mature nuclei are helical, 10 μm long (Fig. 5A), and circular in transverse section. Shallow invaginations occur anteriorly for acrosomal attachment and posteriorly for attachment of the centriole, axoneme and coarse fibres. Longitudinal sections reveal that the posterior 6 μm of the nucleus is enveloped by a cylindrical sheath, the anterior extremity of which is defined by a nuclear ridge (Fig. 5A, C). The sheath is composed of two layers – the inner one a cylindrical extension of the periaxonemal helix, the outer one an extension of the mitochondrial sheath (Fig. 5D). These two layers are separated by a space of approx 60 to 80 nm (Fig. 5C).

Midpiece

The midpiece is composed of a 9+2 axoneme, nine coarse fibres (one associated with each axonemal doublet) and a periodically banded helix all enclosed by the mitochondrial sheath (Fig. 5D, E, G). As noted above, anterior extensions of the helix and mitochondrial sheath enclose the posterior region of the mature nucleus (Fig. 5A, C). Intra-axonemal granules, arranged as longitudinal rows, occur between the central pair of axonemal microtubules and the doublets (Fig. 5G, H). The coarse fibres are approximately 70 nm thick anteriorly and periodically banded (periodicity 20 to 30 nm) (Fig. 5D, E, G). A short arm (? dynein arm) projects from each of the coarse fibres toward the β portion of the succeeding doublet (Fig. 5G). These arms appear to link with the outer dynein arms of each doublet in the same way that connectives occur between the inner dynein arms and intra-axonemal granules (Fig. 5G). The banded helix lies between the coarse fibres and the mitochondrial sheath (Fig. 5D–G). It is crescentic in transverse section and composed of dense bands (periodicity 25 to 30 nm) set in a granular matrix. The helix has a wavelength of 1.4 to 2.3 μm from crest to crest (Fig. 5D). Longitudinal sections reveal that some dense bands of the helix actually connect with the adjacent coarse fibres (Fig. 5E), although these are not readily apparent in transverse sections. Coarse fibres terminate approximately 0.5 μm from the distal edge of the mitochondrial sheath (Fig. 5H).

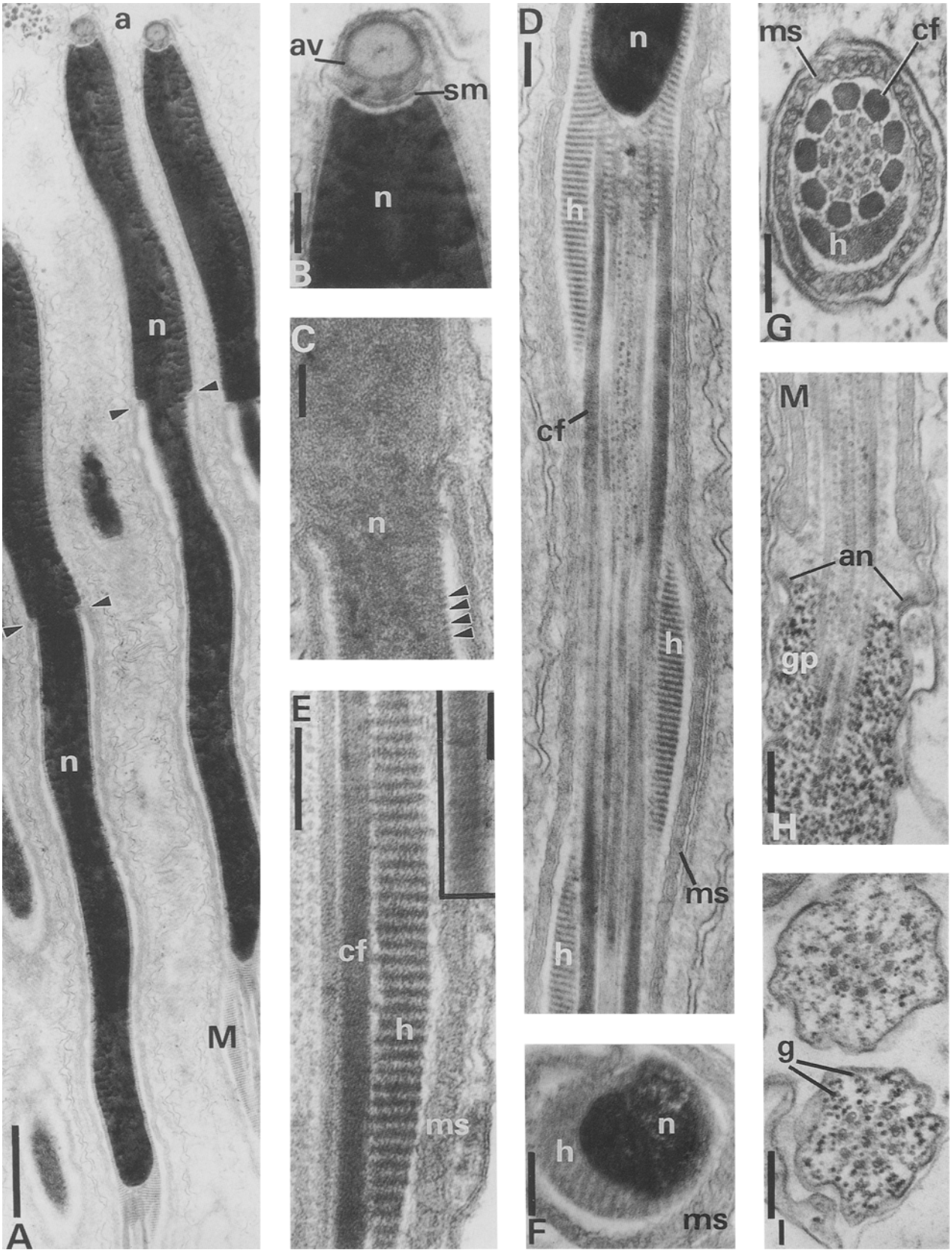

Fig. 5. *Heliacus variegatus*. (A) LS showing acrosome (a), nucleus (n) and proximal portion of midpiece (M) of testicular sperm; arrowheads indicate level to which nuclei are enveloped by mitochondrial sheath and cylindrical extension of banded helix. (B) Acrosomal vesicle (av), subacrosomal material (sm) and nuclear apex of almost mature sperm. (C) Middle portion of nucleus, showing limit of enclosure by midpiece components (arrowheads indicate banded substructure). (D) LS of junction of nucleus and midpiece , showing progression anteriorly of mitochondrial sheath (ms) and cylindrical extension of banded helix (h) cf: coarse fibre. (E) LS showing banded helix, mitochondrial sheath and coarse fibre; inset shows faint banding of coarse fibre. (F) TS showing base of nucleus with mitochondrial sheath and banded helix. (G) TS of midpiece, showing axoneme, coarse fibres, banded helix and mitochondrial sheath; note also arms projecting from coarse fibres towards doublets. (H) Junction of midpiece and glycogen piece (gp), showing annulus (an). (I) TS of glycogen pieces; note organization of granules (g) as periaxonemal tracts. Scale bar in (A) = 1.0 μm; all others = 0.2 μm

Annular complex

The annulus occurs at the junction of the midpiece and glycogen piece. It consists of a circular membrane attached to the inner surface of the plasma membrane and embedded in granular material (Fig. 5H). The annulus is separated from the posterior extremity of the mitochondrial sheath by a 0.15 μm gap (filled by flocculent material), but abuts the anterior extremity of the glycogen piece.

Glycogen piece

Immediately posterior to the annulus, the 9+2 axoneme is sheathed by nine loosely-defined tracts of putative glycogen granules (Fig. 5H, I). Intra-axonemal granules continue into this region of the spermatozoon.

Discussion

In most aspects, the pattern of spermiogenesis in *Heliacus variegatus* follows that observed in other investigated architectonicids (*Philippia* (*Psilaxis*) *oxytropis*, Healy 1982a; *Architectonica perspectiva*, Healy 1991 and unpublished data). However, unlike *H. variegatus*, spermatids of *P. oxytropis* and *A. perspectiva* do not produce a banded helix, nor does the mitochondrial sheath enclose the lower half of the nucleus. In *A. perspectiva*, an elongate, banded column forms late in spermiogenesis between the centriolar region and base of the nucleus (Healy 1991). Thus, there are morphological and developmental grounds for regarding this structure as homologous with the periaxonemal helix of *H. variegatus* (see Healy 1991).

General features such as the phases of nuclear condensation (granular, fibro-reticular, fibrous, lamellar), Golgi participation in acrosomal development and occurrence of a cytoplasmic manchette (associated with nuclear and midpiece development) are commonly reported during sperm development in many other gastropods (caenogastropods and heterobranchs: Walker and MacGregor 1968, Giusti and Mazzini 1973, Buckland-Nicks and Chia 1976, Eckelbarger and Eyster 1981, Healy 1982a, b, 1988b, Dohmen 1983, Maxwell 1983, Kohnert and Storch 1984, Koike 1985, Medina et al. 1985, 1986, 1988) and in many other animal taxa (Horstmann 1970, Fawcett et al. 1971, Baccetti and Afzelius 1976, Franzén 1987, Jamieson 1987). Nevertheless, specific features of spermiogenesis, namely the morphology of the spermatid acrosome, presence of anterior and posterior nuclear plaques (and their substructure), mode of mitochondrial fusion, development of banded coarse fibres, are characteristic of heterobranchs rather than prosobranchs (for comparison see Takaichi and Dan 1977, Takaichi 1978, Dan and Takaichi 1979, Eckelbarger and Eyster 1981, Medina et al. 1985, 1986, Healy 1988b). Results of the present study and the preceding paper (Healy 1991) permit clarification of the morphology of the developing and mature architectonicid acrosome earlier reconstructed by Healy (1982a). Our work shows that the structure earlier designated by Healy (1982a) as an acrosomal pedestal in sperm of architectonicids is, in fact, the differentiated basal region of the acrosomal vesicle (see also Healy 1991 for micrographs of *Architectonica perspectiva*). The subacrosomal layer, however, is confirmed as a true extravesicular feature, homologous with the acrosomal pedestal of other heterobranchs and the basal plate underlying the conical acrosomal vesicles in euspermatozoa of caenogastropods (see Healy 1988a for comparative figures, review and further references).

Despite the fact that marked changes in mitochondrial substructure occur during midpiece formation in architectonicids (Healy 1982a and present study, Healy unpublished data), a glycogen helix and paracrystalline layers – both features of opisthobranch and pulmonate sperm (Healy 1988a) – are not produced. Taxonomically this is significant, for it indicates that the Architectonicidae, and probably the unstudied Mathildidae, are distinguishable from the bulk of the Heterobranchia including other allogastropod groups (see Healy 1991 for further discussion).

One of the most notable events of spermiogenesis in *Heliacus variegatus* is the development of a banded helix within the midpiece of late spermatids. We have also observed this structure in sperm and late spermatids of another architectonicid, *Granosolarium* sp. (Healy and Jamieson unpublished data). Superficially similar helical structures occur in sperm of insects (Bawa and Kanwar 1975, Jamieson 1987) and certain bryozoans (Franzén 1976), but these are mitochondrial in origin. The precise origin of the helix in *H. variegatus* is still uncertain. We could find no evidence of mitochondrial or Golgi participation in helix development; instead, the helix and nine coarse fibres appear to form through accretion of cytoplasmic materials around the axoneme – the fibres appearing first, followed very late in spermiogenesis by the helix. Considering that centrioles are well known for their ability to generate banded rootlets during spermiogenesis [Baccetti and Afzelius 1976, Carré 1984, Franzén 1987; helical, periaxonemal rootlets within the midpiece of some fish sperm (van Deurs 1973)], it seems possible that formation of the helix and coarse fibres in *H. variegatus* is initiated or in some way controlled by the centriolar complex. This view is also supported by the formation, late in spermiogenesis of *Architectonica perspectiva*, of a long, banded column between the base of the nucleus and the centriolar region (Healy 1991). Like the periaxonemal helix of *H. variegatus*, the banded column of *A. perspectiva* is continuous with the banded coarse fibres, centriole and axoneme (Healy 1991). Franzén (1975) demonstrated the presence of a helical, periaxonemal structure in the midpiece region of late spermatids of the polychaete *Fabricia sabella*, but did not comment on its developmental origins. The helix of *F. sabella* lacks banded substructure, and at regular intervals connects with the mitochondrial derivative (see Franzén 1975, his Figs. 10–14). In contrast, the helix of *H. variegatus* is attached via extensions of some of the bands to adjacent coarse fibres, which in turn are attached by discrete arms to axonemal doublets. These facts suggest some role in sperm motility for the banded helix. Transverse banding of molluscan sperm

components is comparatively rare, but has been observed in the acrosomal complex of some opisthobranchs and pulmonates (Healy and Willan 1984, Medina et al. 1985, Healy 1986), patellid prosobranchs (Hodgson and Bernard 1988, and *Octopus* (Galangau and Tuzet 1968, Longo and Anderson 1970, Healy 1989), and in the coarse fibres of almost all heterobranch gastropods (Anderson and Personne 1967, Maxwell 1976, Dan and Takaichi 1979, Healy and Willan 1984, Healy 1988 a, b).

Thus, our study and that of Healy (1982a) demonstrate that spermiogenesis in architectonicids essentially follows a heterobranch (euthyneuran) pattern. The family differs from other heterobranchs in the later stages of midpiece development, notably in the formation of unusual banded structures (probably centriolar in origin). Paracrystalline layers and glycogen helices – so widely recorded in spermatozoa of heterobranchs – are absent from all investigated architectonicid species. Future work in this laboratory will be directed toward comparative sperm and spermatid morphology of the Architectonicoidea, in particular the unstudied and primitive deep-sea family Mathildidae.

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