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Descriptions of the Mature Spermatozoa of the Lizards *Crotaphytus bicinctores*, *Gambelia wislizenii* (Crotaphytidae), and *Anolis carolinensis* (Polychrotidae) (Reptilia, Squamata, Iguania)

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ABSTRACT The spermatozoa of *Crotaphytus bicinctores* and *Gambelia wislizenii* (Crotaphytidae), and *Anolis carolinensis* (Polychrotidae) exhibit the squamate autapomorphies of a single perforatorium extending anteriorly from the apical tip of the paracrystalline subacrosomal cone, the presence of an epinuclear electron-lucent region, and extension of the fibrous sheath into the midpiece. Crotaphytid sperm differ from those of polychrotids in several respects, including: the structure of the perforatorium, the size of the epinuclear electron-lucent region, aspects of the acrosome complex, the arrangement and structure of intermitochondrial dense bodies, and in the distance the fibrous sheath extends into the midpiece. The sperm of *C. bicinctores*, *G. wislizenii*, and *A. carolinensis* are most similar to those of the agamids and phrynosomatids examined to date. No spermatozoal autapomorphies for Crotaphytidae or Polychrotidae were found. The condition of having the intermitochondrial dense bodies arranged in regular incomplete rings is tentatively defined as a synapomorphy of Iguania (although modified in Chamaeleonidae). Spermatozoal ultrastructure offers no characters that justify the separation of Iguanidae (sensu lato) into several separate families. J. Morphol. 247:160–171, 2001.

KEY WORDS: *Crotaphytus; Gambelia;* Crotaphytidae; *Anolis;* Polychrotidae; Iguania; spermatozoa; phylogeny

Sperm ultrastructure has been shown to provide valuable characters for phylogenetic studies (Jamieson, 1995, 1999; Oliver et al., 1996; Teixeira et al., 1999b). As part of a larger study using sperm morphology in phylogenetic reconstructions of squamate reptiles, we provide the first description of the mature spermatozoon of three species of iguanian lizards representing two major clades: Crotaphytidae (*Crotaphytus* and *Gambelia*) and Polychrotidae (*Anolis*). Despite the lack of consensus and resolution among the major clades of iguanian lizards (Frost and Etheridge, 1989; Macey et al., 1997; Schulte et al., 1998), monophyly of North American collared and leopard lizards (Crotaphytidae) has never been questioned (McGuire, 1996). Currently, Crotaphytidae contains two genera, *Crotaphytus* and *Gambelia,* that have nine and three extant species, respectively (McGuire, 1996). While many aspects of the reproductive biology of crotaphytid lizards have been well studied (reviewed in McGuire, 1996), the morphology of crotaphytid sperm has not been previously described. Here we provide detailed descriptions of the sperm of *Crotaphytus bicinctores* and *Gambelia wislizenii*.

Within Polychrotidae, mature spermatozoa have been previously described in *Pristidactylus scapulatus* (Furieri, 1974), although of questionable maturity, and in *Polychrus acutirostris* (Teixeira et al., 1999a). Spermiogenesis has been examined in *Anolis carolinensis* by Clark (1967). Here we describe the mature spermatozoon of *A. carolinensis* and compare it with that of other polychrotids, in particular that of *P. acutirostris*.

MATERIALS AND METHODS

Two male *Crotaphytus bicinctores* (UNR 6194-95), were collected from Washoe Co., Nevada, USA, on 29 June 1998, and two male *Gambelia wislizenii* (UNR 7186-87) were taken from Mineral Co., Nevada, USA, on 7 June 1999. Two male *Anolis carolinensis* were collected from Augusta, Georgia, USA, on 7 November 1998. All lizards were in a reproductive state and were killed shortly after capture with a lethal injection of anesthetic. The testis and ducts were removed and fixed for TEM in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) at

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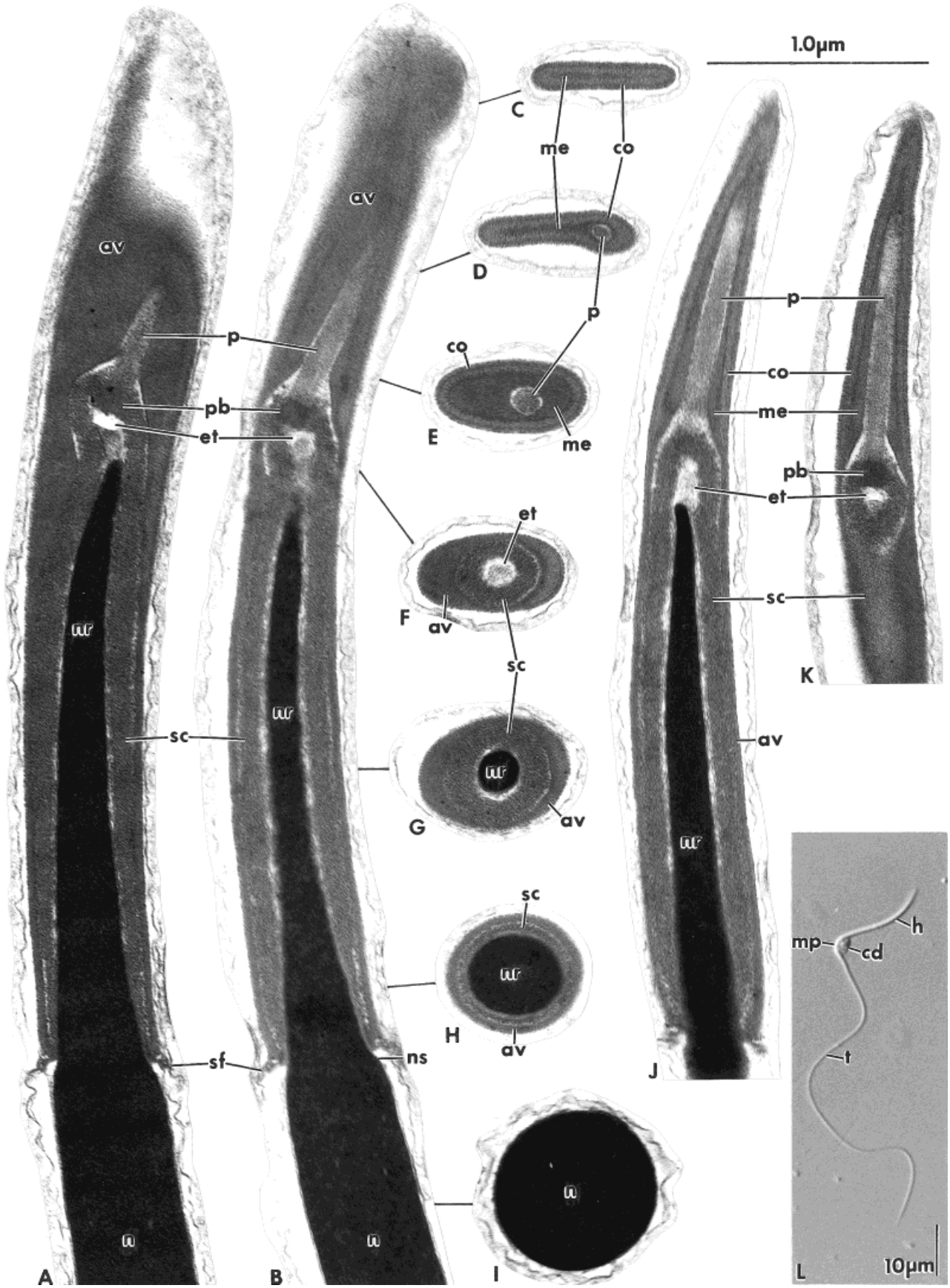

Figure 1

TABLE 1. Dimensions of spermatozoa taken from light and transmission electron microscopy

Dimensions	*Crotaphytus bicinctores*	*Gambelia wislizenii*	*Anolis carolinensis*
Total length	85.5μm (n = 32, SD = 11.6)	98.8μm (n = 19, SD = 4.0)	83.2μm (n = 32, SD = 2.3)
Head (acrosome and nucleus)	20.0μm (n = 33, SD = 0.06)	20.2μm (n = 35, SD = 0.9)	16.5μm (n = 6, SD = 0.2)
Acrosome complex	4.73μm (n = 8, SD = 0.18)	4.74μm (n = 3, SD = 0.12)	5.76μm (n = 6, SD = 0.07)
Nuclear rostrum	2.78μm (n = 7, SD = 0.11)	2.6μm (n = 6, SD = 0.13)	3.27μm (n = 2, SD = 0.09)
Midpiece	3.99μm (n = 9, SD = 0.21)	4.97μm (n = 8, SD = 0.2)	4.61μm (n = 15, SD = 0.24)
Tail	57.2μm (n = 18, SD = 11.8)	76.8μm (n = 14, SD = 4.6)	63.3μm (n = 8, SD = 2.6)
Nuclear diameter (shoulders)	0.49μm (n = 7, SD = 0.03)	0.53μm (n = 4, SD = 0.02)	0.5μm (n = 3, SD = 0.03)
Nuclear diameter (posterior)	0.67μm (n = 9, SD = 0.03)	0.68μm (n = 6, SD = 0.03)	0.65μm (n = 9, SD = 0.05)

4°C for at least 2 h before being transported at ambient temperature to Brisbane, Australia, for processing and sectioning. The material was then rinsed in 0.1 M phosphate buffer, postfixed for 80 min in similarly buffered 1% osmium tetroxide, rinsed in buffer, dehydrated through an ascending ethanol series (20–100%), and infiltrated and embedded in Spurr's epoxy resin.

Sections were cut with diamond knives on a LKB 2128 UM IV microtome. Thin sections, 50–80 nm thick, were collected on carbon-stabilized, colloidin-coated, 200 μm mesh copper grids, stained for 30 sec in Reynolds' lead citrate, rinsed in distilled water, then placed in 6% aqueous uranyl acetate for 4 min, rinsed in distilled water, and stained for a further 2 min in lead citrate before final rinsing. Electron micrographs were taken on a Hitachi 300 electron microscope at 75 kV. Light microscopic observations and photographs of spermatozoa, from glutaraldehyde-fixed tissue squashes, were made using an Olympus BH2 microscope with Nomarski interference contrast and an attached OM-2 camera. All spermatozoa described and shown herein are from duct material.

RESULTS

The general structure of the spermatozoa of *Crotaphytus bicinctores*, *Gambelia wislizenii*, and *Anolis carolinensis* are sufficiently similar to be described together, while noting the few observed differences. The spermatozoa are filiform (Figs. 1L, 3J, 5M). Dimensions of the sperm are provided in Table 1.

Acrosome Complex

The head (acrosome complex and nucleus) is long and curved. In longitudinal section the acrosome complex appears sharply attenuated in one plane (Figs. 1J,K, 5J,K) but flattened and spatulate in the plane at right angles to this (Figs. 1A,B, 3A,I). Posteriorly, the acrosome vesicle forms a progressively narrowing sheath around the subacrosomal cone (Figs. 1B,J, 3A,I, 5K,L) and anteriorly can be divided into a central medulla and surrounding cortex on the basis of differences in electron density (Figs. 1C–E, 3C, 5B–D,J). Cross striations are seen where the cortex and medulla join (Figs. 1E, 3C, 5B–D). Within the acrosomal medulla, the subacrosomal space contains a perforatorium in the form of a very narrow elongate cone with a pointed tip in *Crotaphytus bicinctores* and *Gambelia wislizenii* (Figs. 1J,K, 3A,I), or a rounded tip in *Anolis carolinensis* (Fig. 5J,K). When viewed in longitudinal section the perforatorium of *A. carolinensis* has distinct regular transverse striations (Fig. 5J,K), whereas in *C. bicinctores* and *G. wislizenii* indistinct longitudinal fibers are observed (Figs. 1A,B,J,K, 3A,I). The perforatorium of all three species is in the same longitudinal axis as the nuclear rostrum and at the apical tip of the subacrosomal cone has a slightly widened basal modification, which is identifiable as a stopper-like perforatorial base plate (Figs. 1A,B,K, 3A,I, 5J,K).

The material of the subacrosomal cone is paracrystalline. Within the anterior portion of the cone, anterior to the nuclear rostrum, an epinuclear electron-lucent region is present in all three species. In *Crotaphytus bicinctores* and *Gambelia wislizenii* this region is large and well developed (Figs. 1A,B,F,J, 3A,I), whereas in *Anolis carolinensis* it is small but still distinct (Fig. 5E,J).

At the base of the nuclear rostrum the acrosome complex is circular in transverse section (Figs. 1H, 3G). In the direction of the tip of the acrosome vesicle the acrosome complex becomes progressively depressed (Figs. 1C–G, 3B–F, 5B–H). The acrosome complex of *Anolis carolinensis* differs from that of *Crotaphytus bicinctores* and *Gambelia wislizenii* in

Fig. 1 (Overleaf.) *Crotaphytus bicinctores* spermatozoon. **A,B:** Longitudinal sections (L.Ss) through the nuclear rostrum and acrosome. Note the stopper-like perforatorial base plate. **C–H:** A series of transverse sections (T.Ss) through the acrosome and nucleus. Note that anteriorly in **C–F** the acrosome is laterally compressed in transverse section, while further posteriorly, in **G**, it is unilaterally ridged, and at its posterior limit, in **H,** it is circular. **I:** T.S. through the nucleus. **J,K:** L.Ss through the acrosome showing the concentric zonation of the acrosome vesicle and the epinuclear electron-lucent region. **L:** Whole spermatozoon (Nomarski contrast light microscopy). **A–K** to the same scale as indicated. av, acrosome vesicle; cd, cytoplasmic droplet; co, cortex of acrosome vesicle; et, epinuclear electron-lucent region; h, sperm head (acrosome and nucleus); me, medulla of acrosome vesicle; mp, midpiece; n, nucleus; nr, nuclear rostrum; ns, nuclear shoulders; p, perforatorium; pb, perforatorial base plate; sc, subacrosomal cone; sf, flange of subacrosomal material; t, tail.

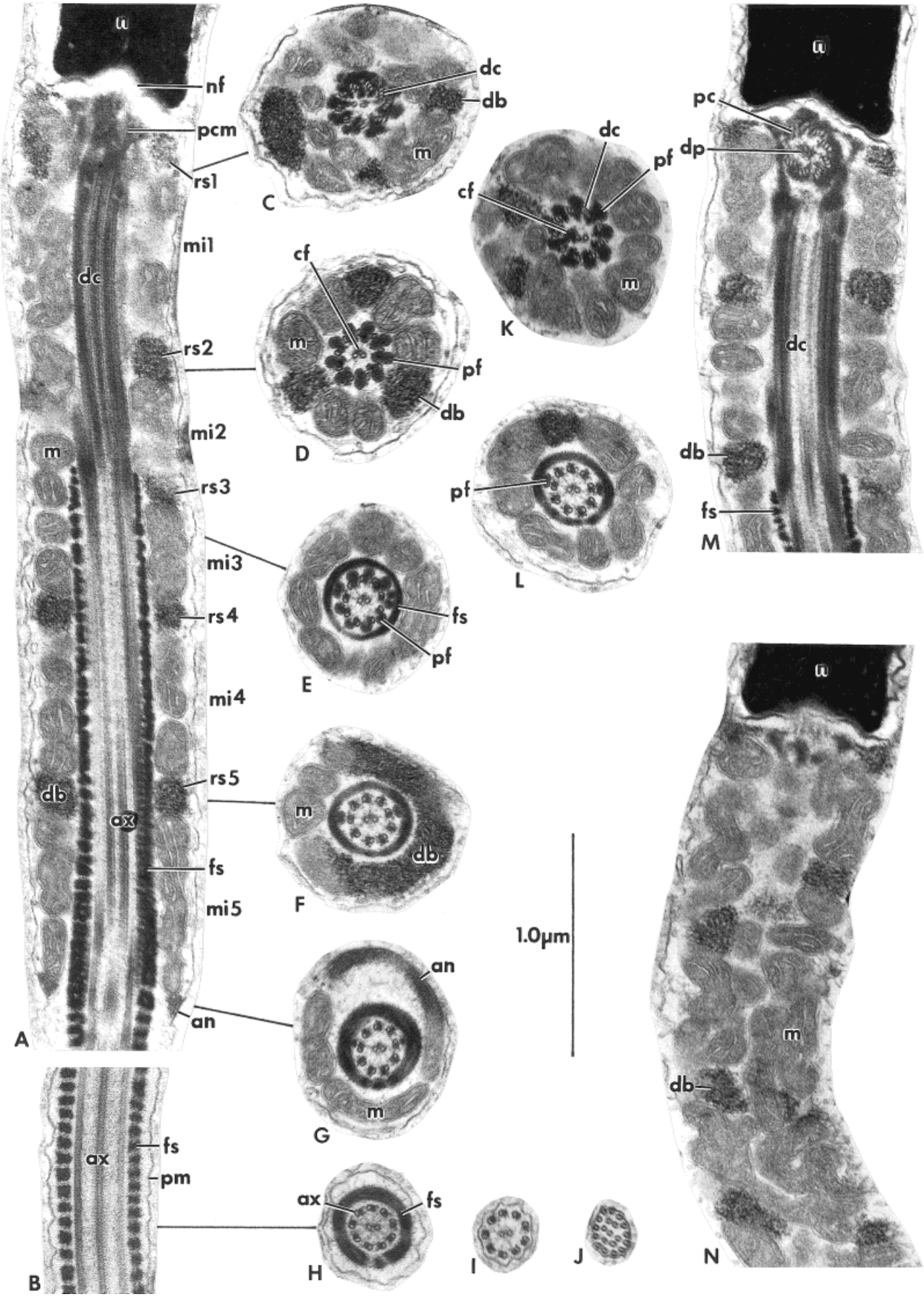
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Figure 2

two ways. First, within the apical portion of the acrosome vesicle two distinct lateral projections of the cortex material are present in *A. carolinensis* (Fig. 5C,D). Second, while the acrosome vesicle of *A. carolinensis* has a unilateral ridge in the region of the epinuclear electron-lucent zone, posteriorly the acrosome vesicle, together with the subacrosomal cone, is again depressed (Fig. 5G,H). In contrast, in *C. bicinctores* and *G. wislizenii* the acrosome vesicle retains its unilateral ridge until it becomes circular at its base. The subacrosomal cone of *C. bicinctores* and *G. wislizenii* is circular in transverse section throughout its entire length. A flange of the subacrosomal cone projects posterolaterally behind the base of the acrosomal vesicle (Figs. 1A,B, 3A,I, 5A,L).

Nucleus

In all three species the nucleus consists of highly condensed, electron-dense chromatin without lacunae. The nuclear rostrum forms a slender cone demarcated from the cylindrical main part of the nucleus by smooth, rounded shoulders (Figs. 1A,B, 3A,I, 5A,L). The nucleus is elongate and circular in transverse section (Figs. 1I, 3H, 5I). Dimensions of the diameter of the nucleus immediately posterior to the nuclear shoulders and at its posterior end are given in Table 1. Basally the nucleus terminates with a shallow nuclear fossa which appears asymmetrical in one plane (Figs. 2A,M, 4H, 6J). At the level of the nuclear fossa the nucleus slightly flares out laterally (Fig. 2M).

Neck Region

The neck region includes the proximal and distal centrioles and associated densities, including the first of the dense bodies of the midpiece. The nuclear fossa contains the anterior half of the proximal centriole and invests dense material (pericentriolar material) which extends bilaterally as an insignificant laminar structure (Figs. 2A,M, 4H, 6A). The proximal centriole is composed of nine short triplets of microtubules and is tilted at approximately 80° relative to the distal centriole (Figs. 4H, 6J). The distal centriole does not project into the fibrous sheath (Figs. 2A,M, 4A,H, 6A). Within the center of the proximal centriole a short solid cylinder of electron-dense material is present for approximately half of the centriole's length (Figs. 2M, 4I, 6J). Dense pericentriolar material surrounds the proximal centriole and extends posteriorly around the distal centriole and possibly continues as the peripheral dense fibers (Figs. 2A,M, 4H, 6A).

Midpiece

The midpiece includes the neck region at its anterior end. The entire short midpiece of *Crotaphytus bicinctores*, *Gambelia wislizenii*, and *Anolis carolinensis* is shown in longitudinal section in Figures 2A, 4A, and 6A, respectively. Each midpiece begins with the first "ring" (an incomplete circle) of dense bodies and ends posteriorly at a small annulus. In the three species examined, the dense bodies form discontinuous or incomplete rings interrupted by mitochondria (Figs. 2D,N, 4C, 6C,E,H). The dense bodies are separated longitudinally by one or more tiers of short sinuous mitochondria with distinct lamellate cristae (Figs. 2N, 4J, 6H). In transverse section a maximum of eight or nine mitochondria are usually seen (Figs. 2E, 4D, 6D). There are five of the incomplete rings of irregularly ovoid dense bodies in *C. bicinctores* and *G. wislizenii* (Figs. 2A, 4A), whereas only four such rings are seen in *A. carolinensis* (Fig. 6A). In all species the first ring abuts onto the base of the nucleus and the last ring is separated from the annulus by mitochondria (Figs. 2A,M,N, 4A,H–J, 6A,H,I). In transverse sections a ring is composed of up to three distinct, irregularly spaced dense bodies in *C. bicinctores* and *G. wislizenii* (Fig. 2D), whereas those of *A. carolinensis* contain up to seven distinct, irregularly spaced dense bodies (Fig. 6E). Although the dense bodies of each ring never form a completely solid/continuous ring, they do occasionally encircle a large percentage of the fibrous sheath and axoneme (Figs. 2F, 4E), with as much as 75% in *C. bicinctores* and 90% in *G. wislizenii* being encircled. The dense bodies of the more posterior ring structures are larger and encircle a greater proportion of the fibrous sheath. The dense bodies of *C. bicinctores* and *G. wislizenii* are moderately electron-dense and granular in appearance (Figs. 2A,D,F, 4A), whereas those of *A. carolinensis* are compact and strongly electron-dense (Fig. 6A,C,E). The distance between each ring of dense bodies remains relatively constant at approximately 0.66 μm in *C. bicinctores*, 0.83 μm in *G. wislizenii*, and 0.93 μm in *A. carolinensis*.

The distal centriole is comprised of nine short triplets of microtubules and forms the basal body of

Fig. 2 (Overleaf.) *Crotaphytus bicinctores* spermatozoon. **A:** Longitudinal section (L.S.) through the entire midpiece showing the five alternating rings of dense bodies (incomplete rings) and mitochondria, and the annulus. **B:** L.S. through the principal piece of the tail. **C–J:** A series of transverse sections (T.Ss) through the distal centriole (**C**), a ring of dense bodies (**D**), a mitochondrial ring of the midpiece near the level of the beginning of the fibrous sheath (**E**), another ring structure, showing that the dense body may surround a large portion of the axoneme as a single body (**F**), annulus (**G**), principal piece (**H**), and endpiece (**I,J**). **K,L:** T.Ss through the midpiece. **M:** L.S. through the anterior region of the midpiece. **N:** Oblique L.S. of the midpiece showing the short sinuous mitochondria. All to the same scale as indicated. an, annulus; ax, axoneme; cf, central singlets fiber; db, dense body (mitochondrial transformation); dc, distal centriole; dp, density within the center of the proximal centriole; fs, fibrous sheath; m, mitochondria; mi, mitochondrial ring; n, nucleus; nf, nuclear fossa; pc, proximal centriole; pcm, pericentriolar material; pf, peripheral dense fiber; pm, plasma membrane; rs, ring structure.

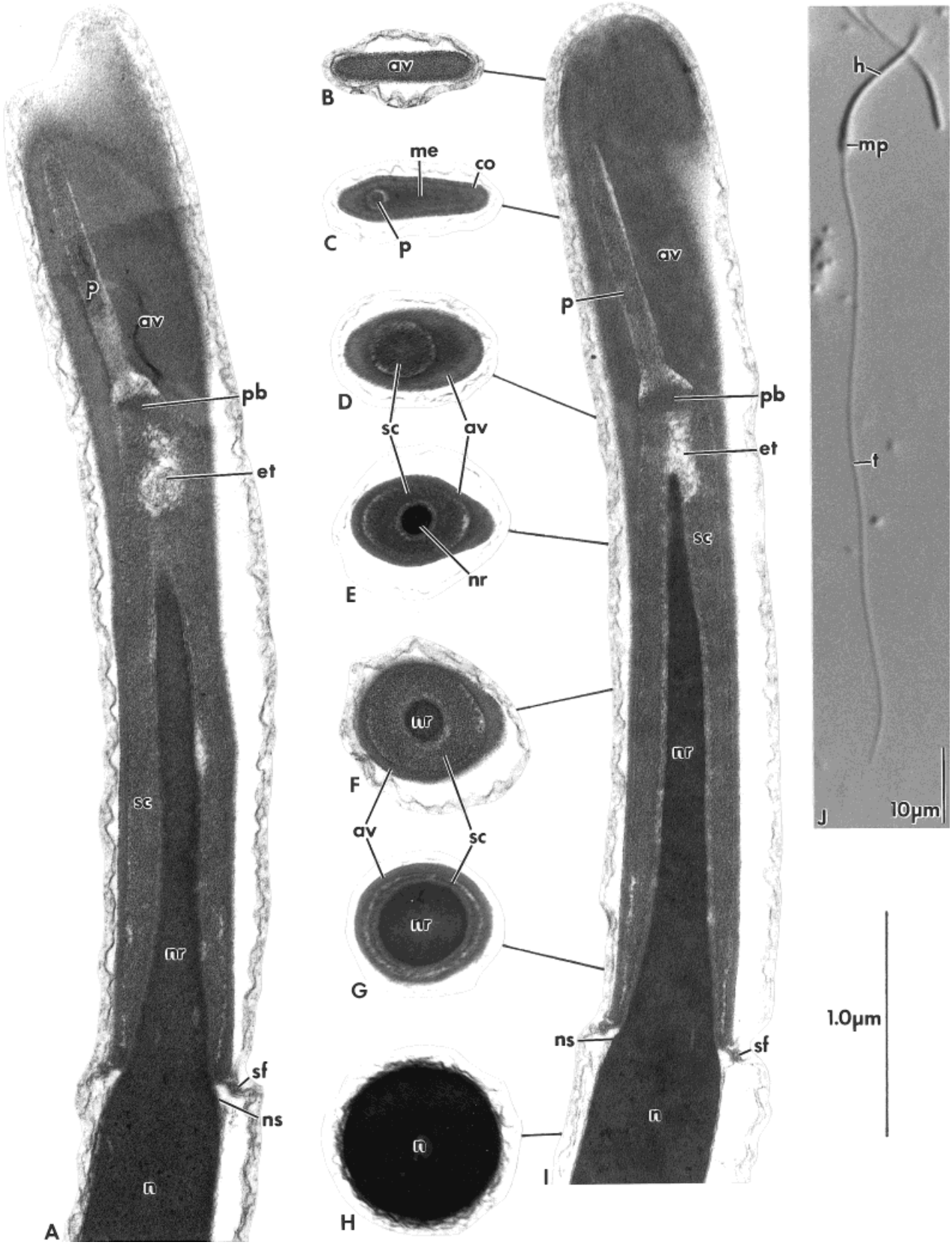

Fig. 3. *Gambelia wislizenii* spermatozoon. **A:** Longitudinal section (L.S.) through the nuclear rostrum and acrosome. **B–G:** A series of transverse sections (T.Ss) through the acrosome. Note that anteriorly, in **B–D**, the acrosome is laterally compressed in transverse sections, while further posteriorly, in **E,F**, it is unilaterally ridged, and at its posterior limit, in **G**, it is circular. **H:** T.S. through the nucleus. **I**: L.S. through the nuclear rostrum and acrosome. **J:** Whole spermatozoon (Nomarski contrast light microscopy). **A–I** to the same scale as indicated. av, acrosome vesicle; co, cortex of acrosome vesicle; et, epinuclear electron-lucent region; h, sperm head (acrosome and nucleus); me, medulla of acrosome vesicle; mp, midpiece; n, nucleus; nr, nuclear rostrum; ns, nuclear shoulders; p, perforatorium; pb, perforatorial base plate; sc, subacrosomal cone; sf, flange of subacrosomal material; t, tail.

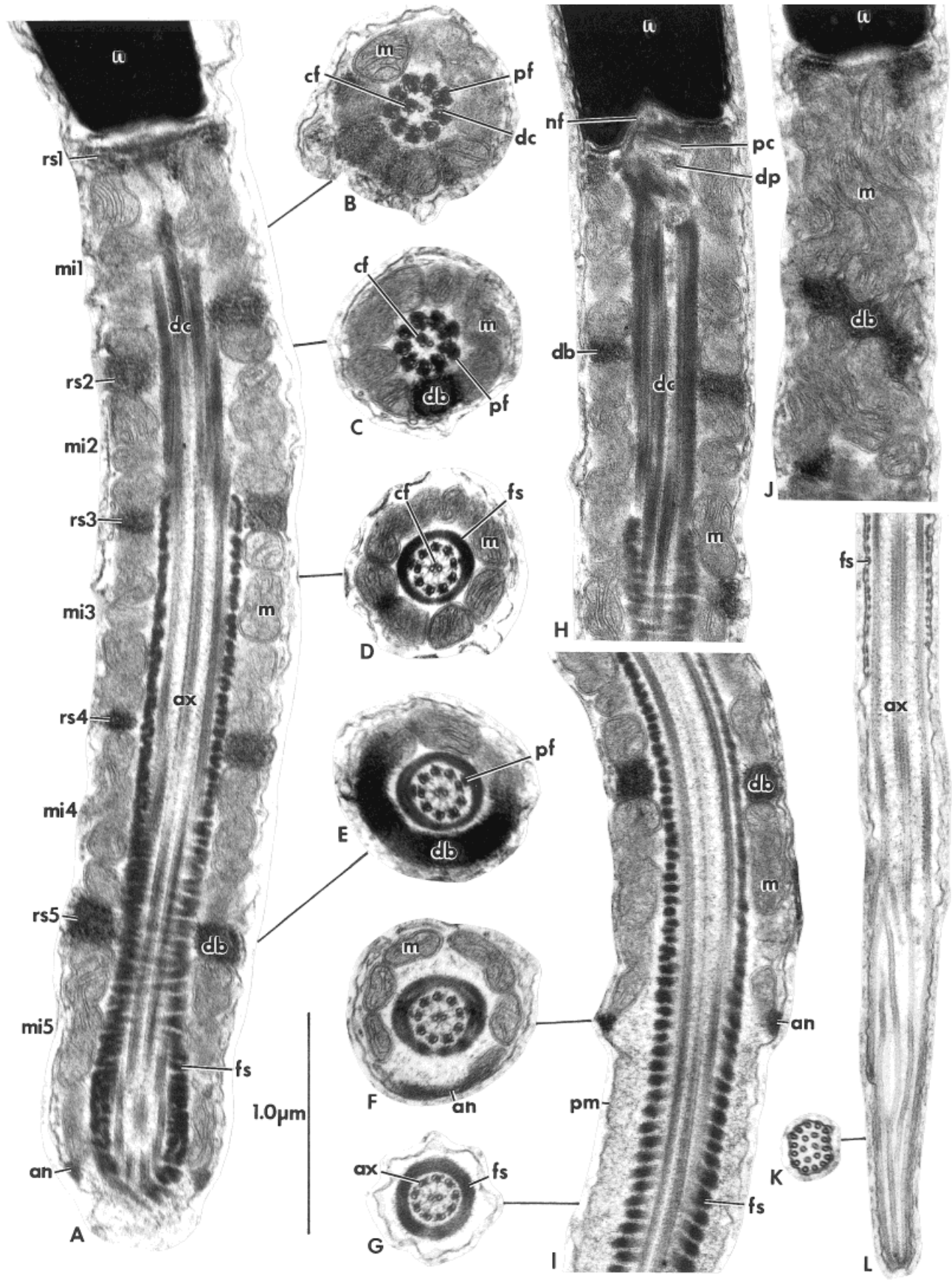
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Figure 4

the axoneme. The two central microtubules (singlets) of the axoneme extend anteriorly throughout the length of the distal centriole (Figs. 2C,K, 4B,C, 6B). Associated with the two singlets is a central fiber which is anteriorly located closer to triplet 9, posteriorly it decreases in size and is positioned centrally between the singlets of the axoneme (Figs. 2D–F,K,L, 4B–E, 6B–E). The central fiber is vestigial at the level of the annulus (Figs. 2G, 4F, 6F).

Nine peripheral dense fibers encircle the distal centriole (Figs. 2C,E,K, 4B,C, 6B) and extend posteriorly, though much narrower, around the axoneme, into the midpiece. One of these peripheral fibers is attached externally to each triplet or doublet. Initially the peripheral fibers at doublets 3 and 8 are not distinctly enlarged relative to the other fibers. More posteriorly, at an undetermined level, all but the peripheral fibers associated with doublets 3 and 8 become greatly reduced in size (Figs. 2F,L, 4D,E, 6C–E). Posteriorly, the peripheral fibers at 3 and 8 form double fiber structures, separate from their doublets, and become closely associated with the fibrous sheath. At the level of the annulus, all nine dense fibers are vestigial or absent (Figs. 2G, 4F, 6F).

The fibrous sheath encloses the axoneme and associated peripheral fibers and has an annulated structure (Figs. 2A,B,E–L, 4A,C–I,L, 6A,C–G,I,L). It extends anteriorly into the midpiece to the level of ring structure 3 (rs3) in *Crotaphytus bicinctores* (Fig. 2A,M) and *Gambelia wislizenii* (Fig. 4A,H), and to the level of rs2 in *Anolis carolinensis* (Fig. 6A). The distance that the fibrous sheath extends anteriorly into the midpiece is constant within each species but differs among species. It extends 2.41 μm, 3.2 μm, and 3.68 μm into the midpiece of *C. bicinctores*, *G. wislizenii*, and *A. carolinensis*, respectively, which equates to 60.4, 64.4, and 79.8% of the total midpiece length, respectively. The midpiece terminates at a well-developed annulus which appears triangular in longitudinal section (Figs. 2A, 4A,I, 6A,I).

Fig. 4 (Overleaf.) *Gambelia wislizenii* spermatozoon. **A:** Longitudinal section (L.S.) through the entire midpiece showing the five alternating rings of dense bodies (incomplete rings) and mitochondria, and the annulus. **B–G:** A series of transverse sections (T.Ss) through the distal centriole (**B,C**), a mitochondrial ring of the midpiece (**D**), a ring structure, showing that the dense body may surround a large portion of the axoneme as a single body (**E**), annulus (**F**) and principal piece (**G**). **H:** L.S. through the neck region showing the density extending for approximately half of the proximal centriole. **I:** L.S. through the annulus. **J:** Oblique L.S. of the midpiece showing the short sinuous mitochondria. **K:** T.S. through the endpiece. **L:** L.S. through the endpiece. All to the same scale as indicated. an, annulus; ax, axoneme; cf, central singlets fiber; db, dense body (mitochondrial transformation); dc, distal centriole; dp, density within the center of the proximal centriole; fs, fibrous sheath; m, mitochondria; mi, mitochondrial ring; n, nucleus; nf, nuclear fossa; pc, proximal centriole; pcm, pericentriolar material; pf, peripheral dense fiber; pm, plasma membrane; rs, ring structure.

Principal Piece

The fibrous sheath continues to surround the axoneme into the principal piece, which, with the absence of mitochondria, it defines. Posterior to the annulus (for some distance), the plasma membrane is widely separated from the fibrous sheath by granular cytoplasm (Figs. 4I, 6I). All peripheral dense fibers and the central fiber are absent from the principal piece (Figs. 2H, 4G, 6G).

Endpiece

A short length of axoneme extends beyond the posterior limit of the fibrous sheath as the endpiece (Figs. 4L, 6L). Posteriorly within the endpiece, the normal 9+2 pattern of microtubules becomes increasingly disrupted (Figs. 2I,J, 4K, 6K).

DISCUSSION

Similarities in Spermatozoon Ultrastructure of Major Iguanian Lineages

The spermatozoa of *Crotaphytus bicinctores*, *Gambelia wislizenii*, and *Anolis carolinensis* exhibit the squamate autapomorphies of a single perforatorium extending anteriorly from the apical tip of the paracrystalline subacrosomal cone, the presence of an epinuclear electron-lucent region, and the fibrous sheath extending into the midpiece. Spermatozoal characters shared with, but not restricted to, other iguanians are as follows. The acrosome vesicle is flattened and concentrically zoned apically and basally it overlies a subacrosomal cone which invests the nuclear rostrum. A stopper-like perforatorial base plate, rounded nuclear shoulders, and a basal nuclear fossa are present. The proximal centriole contains a density within its center for approximately one half its length and lies at approximately 80° to the distal centriole. The two central singlets of the axoneme extend throughout the short distal centriole. A peripheral dense fiber is associated with each of the nine triplets of the distal centriole and extends posteriorly with each of the nine doublets of the axoneme. A central fiber is associated with the two central singlets. All fibers are absent or vestigial at the level of the annulus. Mitochondria are short sinuous, with a maximum of eight or nine observed in transverse section. Intermitochondrial dense bodies are arranged in regular incomplete rings.

The spermatozoa of the Crotaphytidae and Polychrotidae examined here share many similarities with those of other iguanian lizards. Because a detailed comparison of iguanian spermatozoal characters, both within Iguania and with that of other squamates, has recently been presented (Scheltinga et al., 2000), it will not be reiterated here. We here limit the discussion to a comparison of crotaphytid, polychrotid, and phrynosomatid spermatozoa.

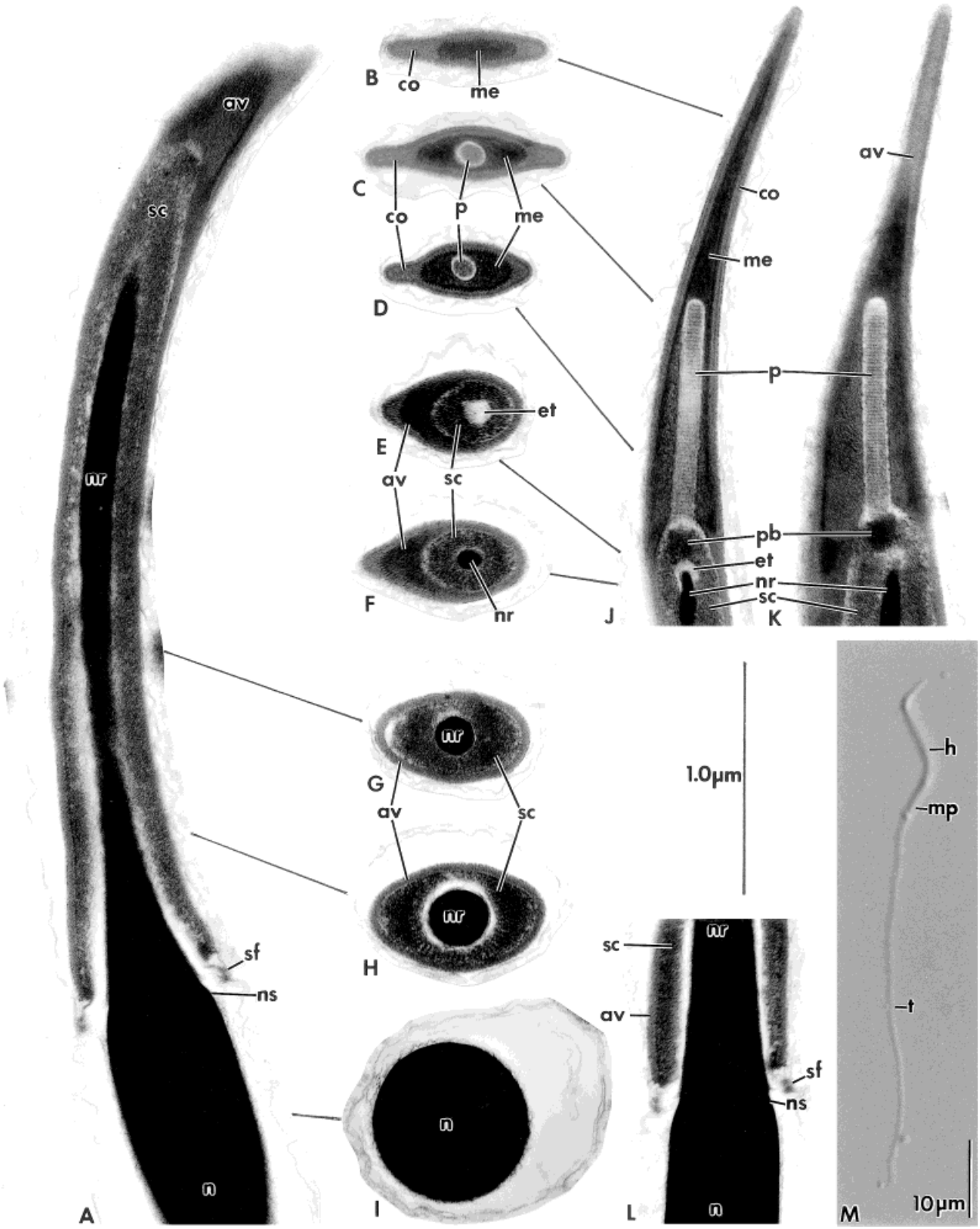

Fig. 5. *Anolis carolinensis* spermatozoon. **A:** Longitudinal section (L.S.) through the nuclear rostrum and acrosome. **B–H:** A series of transverse sections (T.Ss) through the acrosome. Note that anteriorly, in **B,C**, the acrosome is laterally compressed in transverse sections, while further posteriorly, in **E,F**, it is unilaterally ridged, and at its posterior limit, in **G,H**, it again appears laterally compressed. **I:** T.S. through the nucleus. **J,K:** L.Ss through the apical region of the acrosome vesicle. Note the transverse striations of the perforatorium and the stopper-like perforatorial base plate. **L:** L.S. at the level of the nuclear shoulders. **M:** Whole spermatozoon (Note that part of the tail is broken off) (Nomarski contrast light microscopy). **A–L** to the same scale as indicated. av, acrosome vesicle; co, cortex of acrosome vesicle; et, epinuclear electron-lucent region; h, sperm head (acrosome and nucleus); me, medulla of acrosome vesicle; mp, midpiece; n, nucleus; nr, nuclear rostrum; ns, nuclear shoulders; p, perforatorium; pb, perforatorial base plate; sc, subacrosomal cone; sf, flange of subacrosomal material; t, tail.

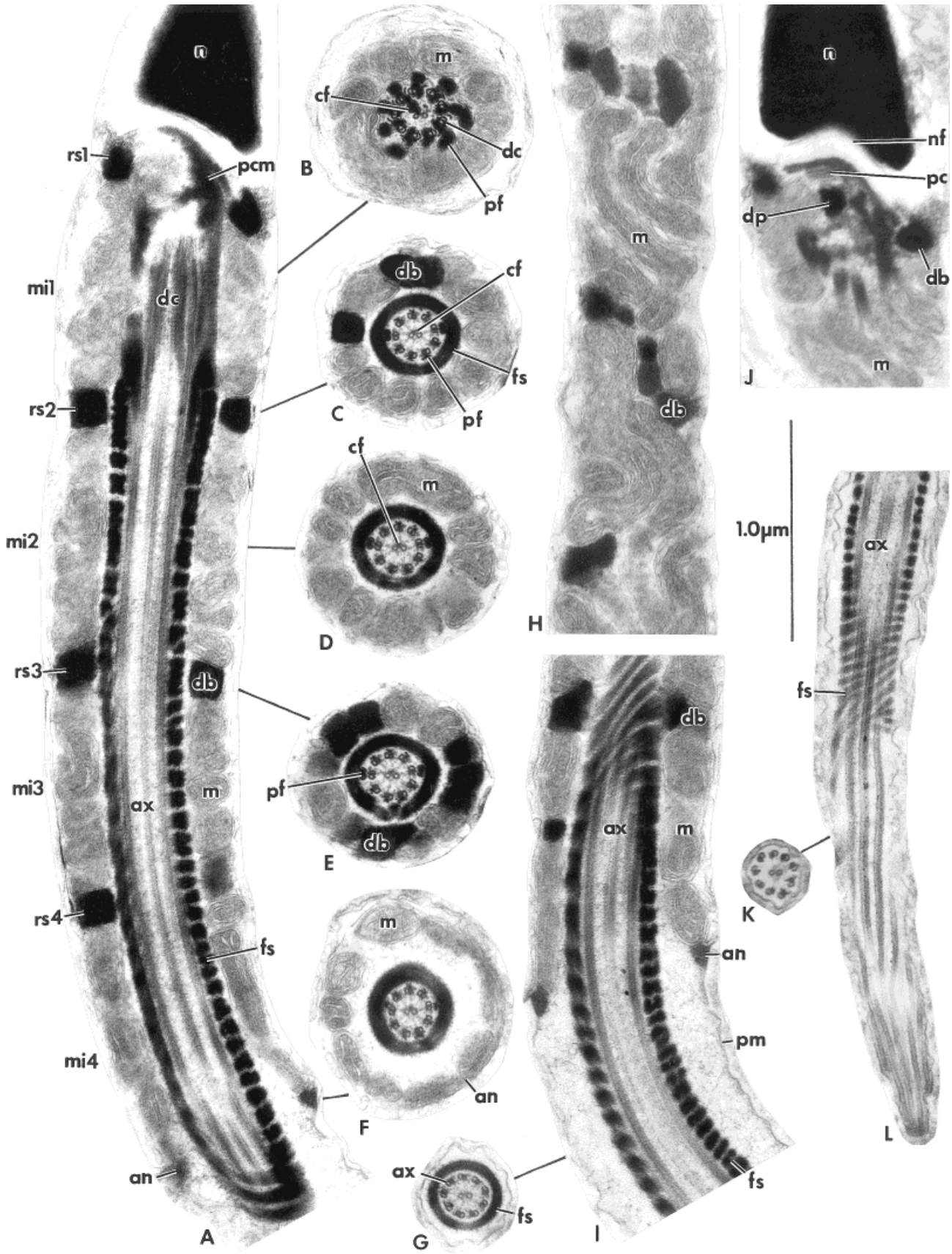
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Figure 6

The spermatozoa of *Crotaphytus bicinctores*, *Gambelia wislizenii*, and *Anolis carolinensis* are similar to those of the agamids and phrynosomatids examined to date (see Oliver et al., 1996; Scheltinga et al., 2000). As with the phrynosomatids, no spermatozoal autapomorphies for Crotaphytidae or Polychrotidae were found.

Comparisons of Crotaphytidae Spermatozoon with that of other Iguanians

The spermatozoon of the two Crotaphytids examined herein is notably similar to the phrynosomatid *Uta stansburiana* (Scheltinga et al., 2000). They differ only in the number of dense bodies occurring within each ring structure (with a maximum of three in the crotaphytids and four in *Uta*) and in the large size of some of the posterior rings in the crotaphytids. The spermatozoon of *Anolis carolinensis* is similar to that of the phrynosomatid *Urosaurus ornatus* in having a transversely striated perforatorium and the midpiece having four ring structures. However, the two differ in that *A. carolinensis* has a flattened subacrosomal cone, a rounded perforatorial tip, and the fibrous sheath enters the midpiece to the level of the second ring structure (rs2) (Scheltinga et al., 2000). As with the phrynosomatids examined to date, crotaphytid and polychrotid sperm exhibit no autapomorphic characters that could be used to distinguish their respective lineages.

Crotaphytid sperm differ from those of polychrotids examined to date in several well-defined characters: 1) The perforatorium of *Crotaphytus bicinctores* and *Gambelia wislizenii* has indistinct longitudinal fibers and a pointed tip, whereas *Anolis carolinensis* has distinct regular transverse striations and a rounded tip. 2) In *C. bicinctores* and *G. wislizenii* the epinuclear electron-lucent region is large and well developed, whereas in *A. carolinensis* it is small yet distinct. 3) The acrosome complex of *A. carolinensis* has distinct lateral projections of cortex material and the subacrosomal cone is depressed. 4) The arrangement of intermitochondrial dense bodies differs in several aspects. In *C. bicinctores* and *G. wislizenii* five incomplete rings of dense bodies are present in the midpiece, whereas *A. carolinensis* has only four such rings. In transverse section, a ring of *C. bicinctores* and *G. wislizenii* is composed of up to three distinct, irregularly spaced, dense bodies which have a granular, moderately electron-dense appearance, whereas those of *A. carolinensis* contain up to seven distinct, irregularly spaced dense bodies which are compact and strongly electron dense. Posterior ring structures may be as much as 75% complete in *C. bicinctores* and 90% complete in *G. wislizenii*. 5) The fibrous sheath extends into the midpiece to the level of rs3 in *C. bicinctores* and *G. wislizenii*, while only reaching the level of rs2 in *A. carolinensis*.

Comparison of *Anolis carolinensis* Spermatozoon with Other Polychrotidae and Iguanians

The sperm of *Anolis carolinensis* differs from that of the polychrotid *Polychrus acutirostris* (see Teixeira et al., 1999a) in possessing an acrosome vesicle divided into cortex and medulla, a perforatorium with transverse striations and rounded tip, lacunae absent from the nucleus, and a stopper-like perforatorial base plate. Teixeira et al. (1999a) stated that the dense bodies form regular incomplete rings in *P. acutirostris*, but did not specify the number of rings. Although there are only two rings illustrated in their figure 1, there are four questionably visible in their figure 17, the latter in agreement with the number in *A. carolinensis*. At least four incomplete rings are observed in the polychrotid *Pristidactylus scapulatus* (Furieri, 1974; see Scheltinga et al., 2000). The midpiece in iguanians is plesiomorphically short. In *A. carolinensis*, the midpiece is 4.61 μm long and considerably shorter than the 7.5 μm length reported for *P. acutirostris* (Teixeira et al., 1999a). However, from the scale in their figure 17, which we find consistent with an axoneme width of 0.18 μm, it appears that the midpiece of *P. acutirostris* is actually only 3.93 μm long, a length similar to *A. carolinensis* and other iguanians.

Another questionable difference between *Anolis carolinensis* and *Polychrus acutirostris* is the initial location of the central fiber of the midpiece. In *A. carolinensis* the fiber is initially located between the central singlets and triplet nine. In *P. acutirostris* it is reported to connect the central singlets with triplet three (Teixeira et al., 1999a). However, it appears from their figure 16 that it connects triplet eight and/or nine to the central singlets, as in *A. carolinensis* and other squamates (Scheltinga et al., 2000).

The presence of well-developed lacunae in the nucleus of *Polychrus acutirostris* has not been observed in any other polychrotids examined to date (Teixeira,

Fig. 6 (Overleaf.) *Anolis carolinensis* spermatozoon. **A:** Longitudinal section (L.S.) through the entire midpiece showing the four alternating rings of dense bodies (incomplete rings) and mitochondria, and the annulus. **B–G:** A series of transverse sections (T.Ss) through the distal centriole (**B**), a ring structure of the midpiece (**C**), a mitochondrial ring of the midpiece (**D**), another ring structure (**E**), annulus (**F**), and principal piece (**G**). **H:** Oblique L.S. of the midpiece showing the short sinuous mitochondria. **I:** L.S. through the annulus. **J:** L.S. through the neck region showing the density extending for approximately half of the proximal centriole. **K:** T.S. through the endpiece. **L:** L.S. through the endpiece. All to same scale as indicated. an, annulus; ax, axoneme; cf, central singlets fiber; db, dense body (mitochondrial transformation); dc, distal centriole; dp, density within the center of the proximal centriole; fs, fibrous sheath; m, mitochondria; mi, mitochondrial ring; n, nucleus; nf, nuclear fossa; pc, proximal centriole; pcm, pericentriolar material; pf, peripheral dense fiber; pm, plasma membrane; rs, ring structure.

1999a). It has, however, been previously reported in the tropidurids *Tropidurus semitaeniatus* and *T. torquatus* (Teixeira, 1999c), in *Eugongylus* subgroup skinks (Jamieson et al., 1996), and in the gymnophthalmid *Micrablepharus maximiliani* (Teixeira, 1999b), and is possibly a vestige of the endonuclear canal seen in basal tetrapods. The mitochondria in *P. acutirostris* are described as elongate, sinuous and spirally arranged by Teixeira et al. (1999a) but they appear to zigzag and not spiral around the midpiece in their figure 20, a condition similar to that seen in *Anolis carolinensis*, which we have termed short sinuous.

Phylogenetic Inferences of Spermatozoon Ultrastructure

Description of mature spermatozoa of representatives of the iguanian lineages Tropiduridae, Phrynosomatidae, Polychrotidae, Crotaphytidae, Agamidae, and Chamaeleonidae have now been accomplished. Although four families await spermatozoal description, the condition of having the intermitochondrial dense bodies arranged in regular incomplete rings, as is seen in species belonging to each of these lineages (although modified in the Chamaeleonidae), is therefore tentatively considered a synapomorphy of the Iguania. However, currently no spermatozoal autapomorphies are known that distinguish any of the nine iguanian families recognized by Frost and Etheridge (1989). As shown above, there are several distinct differences in the sperm structure of crotaphytids and polychrotids. However, within Iguania intrafamilial differences are as great as interfamilial, for example, the phrynosomatids discussed above. Thus sperm characters support recognition of a single family, the Iguanidae (sensu lato; see also Schulte et al., 1998). Variation seen in the sperm structure of the different taxa examined may prove useful in elucidating generic and subfamilial relationships within iguanian lizards.

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