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Ultrastructural study of spermatozoa of the neotropical lizards, *Tropidurus semitaeniatus* and *Tropidurus torquatus* (Squamata, Tropiduridae)

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Abstract

A detailed description of the sperm ultrastructure of *Tropidurus semitaeniatus* and *T. torquatus* is provided. Mature spermatozoa of *T. semitaeniatus* and *T. torquatus* are filiform and characterized by: apical portion of acrosome depressed; perforatorium single; epinuclear lucent zone well developed; midpiece short; mitochondria columnar; midpiece with three sets of alternating ring structures and mitochondria (rs1/m1, rs2/m2, rs3/m3); nuclear shoulders rounded; nucleus elongate; fibres 3 and 8 enlarged; and fibrous sheath in midpiece. Spermatozoa of *Tropidurus* are unusual in possessing a unilateral electron-lucent ridge at the surface of the acrosome and an epinuclear electron-lucent zone. The two species are very similar, differing in details such as degree of acrosome flattening, presence of bulging at nuclear base, and arrangement of microtubules in the endpiece. Comparisons between Tropiduridae and other families of iguanian lizards are made.

Keywords: ultrastructure, spermatozoon, Reptilia, Squamata, Tropiduridae, lizard

Introduction

Studies on the ultrastructure of spermatozoa have fostered knowledge on the development and functional significance of many spermatozoal organelles (Stanley, 1971), elucidating the relationship among these components and reproduction itself (Cruz-Landim & Cruz-Hofling, 1977). Detailed accounts on the ultrastructure of spermatozoa also supply an independent source of characters for phylogenetic analyses, which are especially useful when other character sets are not enlightening (Jamieson, 1995a; 1995b; Teixeira et al., 1999a). Moreover, phylogenetic studies may provide a framework for reconstructing the features of ancestral spermatozoa and the course of spermatozoal evolution, establishing the relationship between sperm morphology and systematic position.

Iguania is a monophyletic assemblage of nine families of squamate reptiles whose phylogenetic relationships are poorly understood (Frost & Etheridge, 1989). Studies on the sperm ultrastructure have been carried out in five families of iguanians: Chamaeleonidae, Iguanidae, Phrynosomatidae, Polychrotidae, and Tropiduridae. In Chamaeleonidae, the mature spermatozoon has been described in detail for *Bradypodion karrooicum* (Jamieson, 1995b) and

Pogona barbata (Oliver et al., 1996), whereas the spermiogenesis has been examined in *Agama stellio* (Al-Hajj et al., 1987), *A. adramitana* (Dehlawi et al., 1992; Ismail et al., 1995), and *Uromastix philbyi* (Dehlawi & Ismail, 1990; Dehlawi et al., 1990). A description of the spermiogenesis is available for *Iguana iguana* (Iguanidae), misidentified as *I. delicatissima*, by Saita et al. (1988). A few details of the spermiogenesis are known for a member of Phrynosomatidae, *Phrynosoma coronatum* (Clark, 1967). In Polychrotidae, Teixeira et al. (submitted) gave a detailed account on the ultrastructure of mature spermatozoa of *Polychrus acutirostris*, Furieri (1974) described the mature spermatozoon of *Pristidactylus* (=Cupriganus) *scapulatus*, and Clark (1967) described the spermiogenesis of *Anolis carolinensis*. Among Tropicuridae, the mature spermatozoon of *Liolaemus austromendocinus* and *Phymaturus flagellifer* (=palluma) have been described (Furieri, 1974), and an account on the spermiogenesis of *Tropidurus cf. torquatus* is available (Cruz-Landim & Cruz-Höfling, 1977; Cruz-Höfling & Cruz-Landim, 1978).

Herein we provide, for the first time, a detailed description of the ultrastructure of mature spermatozoa of Tropicuridae. We also make comparisons between tropicurids and other iguanian families. Finally, by examining two congeneric species, we attempt to ascertain the degree of intrafamilial variability in sperm ultrastructure characters, a problem that may plague phylogenetic analysis when supraspecific taxa are used, but has received little attention (Wiens, 1998).

Materials and methods

We obtained epididymal, mature spermatozoa from two adult specimens of *Tropidurus semitaeniatus* and two adult specimens of *T. torquatus*, collected at Lençóis, Bahia, Brazil and Brasília, Distrito Federal, Brazil, respectively. Specimens were deposited at the Coleção Herpetológica da Universidade de Brasília (*T. semitaeniatus* CHUNB 00571, 00572; *T. torquatus* CHUNB 09676, 10087).

We killed the lizards with Tiopental, removed the epididymides by dissection, placed them in a Petri dish with phosphate buffered saline (PBS) pH 7.2, and cut them into small pieces. We fixed epididymal tissues overnight at 4°C in a solution containing 2.5% glutaraldehyde, 2% paraformaldehyde, and 3% sucrose in 0.1 M sodium cacodylate buffer pH 7.2. Subsequently, we washed specimens in 0.1 M sodium cacodylate buffer, pH 7.2, with 3% sucrose, and postfixed them for 1 h in 1% osmium tetroxide, 0.8% potassium ferricyanide, and 5 mM CaCl₂ in 0.1 M sodium cacodylate buffer, pH 7.2. We dehydrated the material in a series of ascending acetone (30%–100%) and embedded it in Spurr's epoxy resin. We stained

ultrathin sections with uranyl acetate and lead citrate, and made observations in a Jeol 100 C transmission electron microscope.

Results

Spermatozoa of *Tropidurus semitaeniatus* and *T. torquatus* are filiform, consisting of a head region containing the nucleus and the acrosomal structures, a midpiece, and a tail region subdivided into principal piece and endpiece. A generalized spermatozoon based on both species is represented diagrammatically in Figure 1.

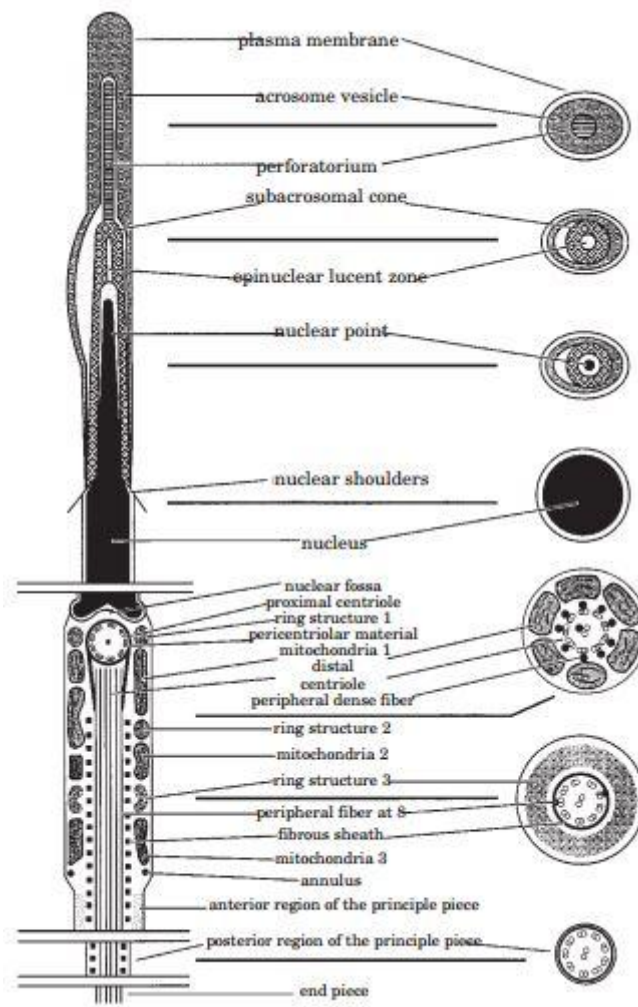


Fig. 1 Generalized diagram of the spermatozoon of *Tropidurus semitaeniatus* and *T. torquatus*, in longitudinal section and corresponding transverse section. Scales of various components are only approximate. Scale bar 0.5 μ m

Acrosome complex

In the acrosome, an external and an internal cap, the acrosome vesicle and the subacrosomal cone, respectively, are recognized (Figs 2A–B, H & 4A–B, H). The acrosome

vesicle has a homogeneous and moderately opaque matrix, bounded both outside and inside by a membrane (Figs 2A–F, H & 4D–F, H). The acrosome vesicle is thicker in its apical half, whereas the basal half is hollowed to surround the subacrosomal cone. The apical portion of the acrosome vesicle is not completely solid, having a narrow central canal 1.2 μm long, the perforatorium (Figs 2A, C, H & 4A–C). This structure is a slender rod with a pointed tip, which extends anteriorly from the subacrosomal cone. The subacrosomal cone fits into the basal half of the acrosome vesicle, being shorter and thicker than the latter, and lacks a membrane of its own (Figs 2A–B, D–F, H & 4D–F, H). It consists of a material having an unevenly aspect, which tapers the anterior end of the nucleus. The subacrosomal cone surface is closely associated with the inner membrane of the acrosome vesicle, except at its apical and anterolateral surface, where an electron-lucent layer separates these two caps, forming a unilateral ridge (Figs 2B & 4H). At its base, the acrosome complex rests on a widening region of the nucleus with a distinct shoulder-like shape, lying on a postero-lateral membranous flange (Figs 2H & 4H). The acrosome of the two species is circular at its base, develops an unilateral electron-lucent ridge anteriorly, and becomes increasingly depressed in transverse section near the apical tip (Figs 2C–F & 4C–F). Apically, the acrosome vesicle of *T. torquatus* is more depressed than that of *T. semitaeniatus* (Figs 4C & 2C).

Nucleus

The nucleus is elongate, slightly curved, and circular in cross-section. At its anterior extremity, it forms a point deeply wedged in the acrosome (Figs 2H & 4H). The length of this nuclear projection, which is surrounded by the subacrosomal cone, is approximately 2.5 μm . Anterior to the nuclear tip and within the subacrosomal cone there is an elongate, narrow chamber with a membrane of its own, the epinuclear lucent-zone, filled with an electron-lucent substance (Figs 2D, H & 4D, H). Small rounded shoulders (Figs 2B, H & 4H) mark the transition from the tapered apical portion to the cylindrical region. The posterior pole of the nucleus is marked by a shallow conical depression, the nuclear fossa, for receiving the centriolar apparatus (Figs 3A, H, I & 5A, I). The main body of the nucleus appear homogeneous and completely electron-dense, but in some instances there are small, electron-lucent channels penetrating it, the lacunae (Figs 2G & 4G). Along the nuclear point the boundary between the nuclear contents and the nuclear envelope is irregular, containing evenly dispersed, fine granular material (Figs 2B, E–F, H & 4B, E–F, H). In *T. semitaeniatus*, the nuclear base abruptly increases in width, producing a conspicuous, rounded bulge (Fig. 3A), which is absent in *T. torquatus* (Figs 4H & 5I).

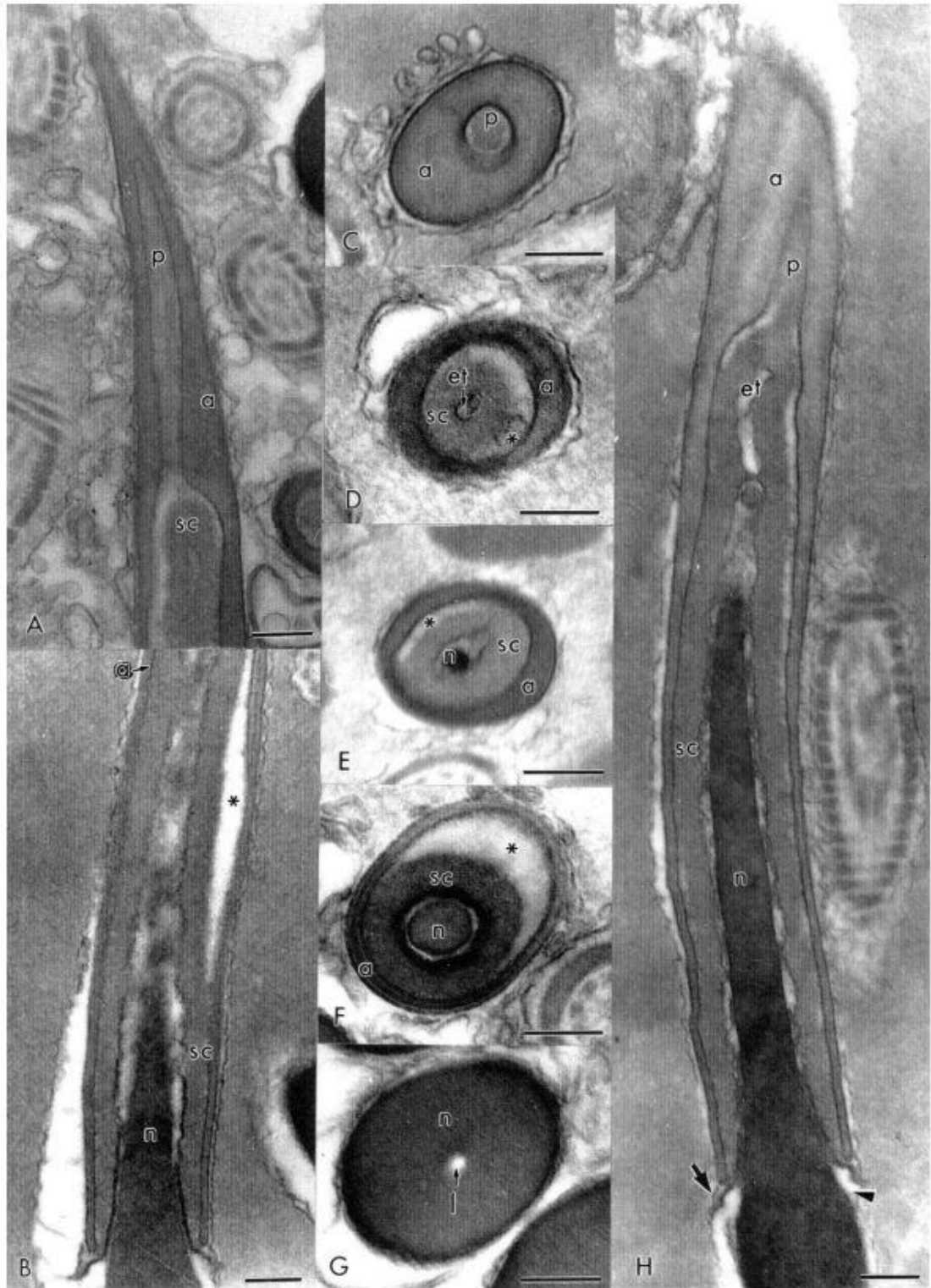


Fig. 2 *Tropicurus semitaeniatus*. (A) Longitudinal section through the apical end of the acrosome showing the perforatorium. (B) Longitudinal section through the basal portion of the acrosome showing the unilateral ridge, formed by an electron-lucent layer (*) between the acrosome vesicle and the subacrosomal cone. (C–F) Successive transverse sections through the acrosome. Note that anteriorly, in C, the acrosome appears depressed, while posteriorly, in D–F, it is more circular. An electron-lucent layer (*) between the acrosome vesicle and the subacrosome cone may be observed in D–F. (G) Transverse section through the nucleus showing lacuna. (H) Longitudinal section through the acrosome showing its flattened apical region and the well-developed epinuclear lucent zone. Note the space between the condensed chromatin and the nuclear membrane, occupied by a granular material. An

arrowhead and an arrow indicate the nuclear shoulders and the membranous flange, respectively. a: acrosome vesicle; et: epinuclear lucent zone; 1, nuclear lacuna; n: nucleus; p: perforatorium; sc: subacrosomal cone. Scale bar 0.2 μm .

Neck region

The neck region connects the tail with the sperm head. This region consists of two centrioles, an extensive deposit of pericentriolar material, and the first ring of dense bodies (Figs 3A, H–I & 5A, I). The proximal centriole is positioned centrally, parallel to the base of the nucleus, and presents a central, electron-dense structure in its interior (Figs 3H & 5A, I). The distal centriole constitutes the basal body of the flagellum. It lies perpendicular to the proximal centriole and extends caudally through approximately two-thirds of the midpiece, continuing with the axoneme. The distal centriole consists of nine triplets of microtubules. A peripheral dense fibre (coarse fibre) is associated with each of the nine triplets of the distal centriole (Figs 3C & 5C). In both species, a pair of central microtubules extends into the transitional region between the distal centriole and the axoneme, and a dense structure, presumably a fibre, appears connecting triplet 3 with one of the central microtubules. Both centrioles are enclosed within a homogeneously dense material, the pericentriolar material that conforms in shape to the nuclear fossa. This material extends posteriorly between the two centrioles and contacts the anterior portion of the distal centriole, continuing as the dense peripheral fibers longitudinally (Figs 3A, H & 5A, I).

Midpiece

The midpiece is approximately 2.8 μm long and is much shorter than the head. It consists of a single layer of mitochondria, interposed between rings of dense bodies, which encircles the anterior portion of the flagellum, including the neck region. The flagellum is formed by the axoneme, extending throughout the remaining length of the spermatozoon. It is organized in the usual 9+2 microtubules pattern, surrounded by nine peripheral fibres. These peripheral fibres rapidly decrease in diameter along the axoneme, with the exception of fibres at doublets 3 and 8, which are thicker than the others, double, and detached from their doublets (Figs 3D–F & 5D–F). A fibrous sheath, which surrounds the axoneme, extends into the midpiece to just posterior of the distal centriole (Figs. 3A; 5A, J). In longitudinal section it appears as regularly spaced, approximately square dense blocks connected by the longitudinal peripheral fibers at doublets 3 and 8. The mitochondria are elongate columnar structures, with longitudinal cristae, and surround the distal centriole and fibrous sheath (Figs 3A & 5A, J). In cross section, they appear irregularly shaped, and usually 5 to 6 are seen around the axoneme

(Figs 3D & 5D–E). In the two species, the mitochondrial sheath is interspersed by dense bodies, which are not limited by a membrane, and are composed of a granular dense material identical to the pericentriolar material. However, they appear less electron-dense than the mitochondrial sheath (Figs 3A, C, E, I & 5A, E, J). Dense bodies are arranged in three ring structures, separated from each other by portions of mitochondrial columns (Figs 3A, I & 5A, J) symbolized as rs1/m1, rs2/m2, rs3/m3. The first ring of dense bodies adjoins the base of the nucleus. The second ring structure often appears only on one side in longitudinal section (Figs 3A, I & 5A, J) and is often interrupted by mitochondria in transverse section (Figs 3C & 5E). This suggests that this structure forms a semicircular ring. The third ring structure forms a closed ring (Figs 3E & 5F), appearing in longitudinal section as kidney-shaped structures on each side of the fibrous sheath (Figs 3A & 5A, J). Occasionally, mitochondria are present lateral to this ring structure (Fig. 3E). At the distal extremity of the midpiece, a small dense ring, the annulus, with an irregular, oval cross section, defines the terminus of the midpiece (Figs 3A–B & 5A–B, J).

Principal piece

The principal piece constitutes the tail of the spermatozoon. It extends posteriorly from the annulus and is formed by the axoneme surrounded by the fibrous sheath and the plasma membrane. In its anterior portion, there is a short region in which a thin zone of finely granular cytoplasm is observed between the fibrous sheath and the plasma membrane (Figs 3B, F & 5B, G). Posteriorly, the plasma membrane becomes closely attached to the fibrous sheath (Figs 3G & 5G).

Endpiece

The very slender tail of the spermatozoon, posterior to the termination of the fibrous sheath, is referred to as the endpiece. In *Tropidurus torquatus*, the 9+2 pattern in this region becomes disarrayed and the doublets break apart into singlets (Fig. 5H), whereas in *T. semitaeniatus* the pattern of microtubules is apparently maintained, although its diameter becomes reduced (Fig. 3G).

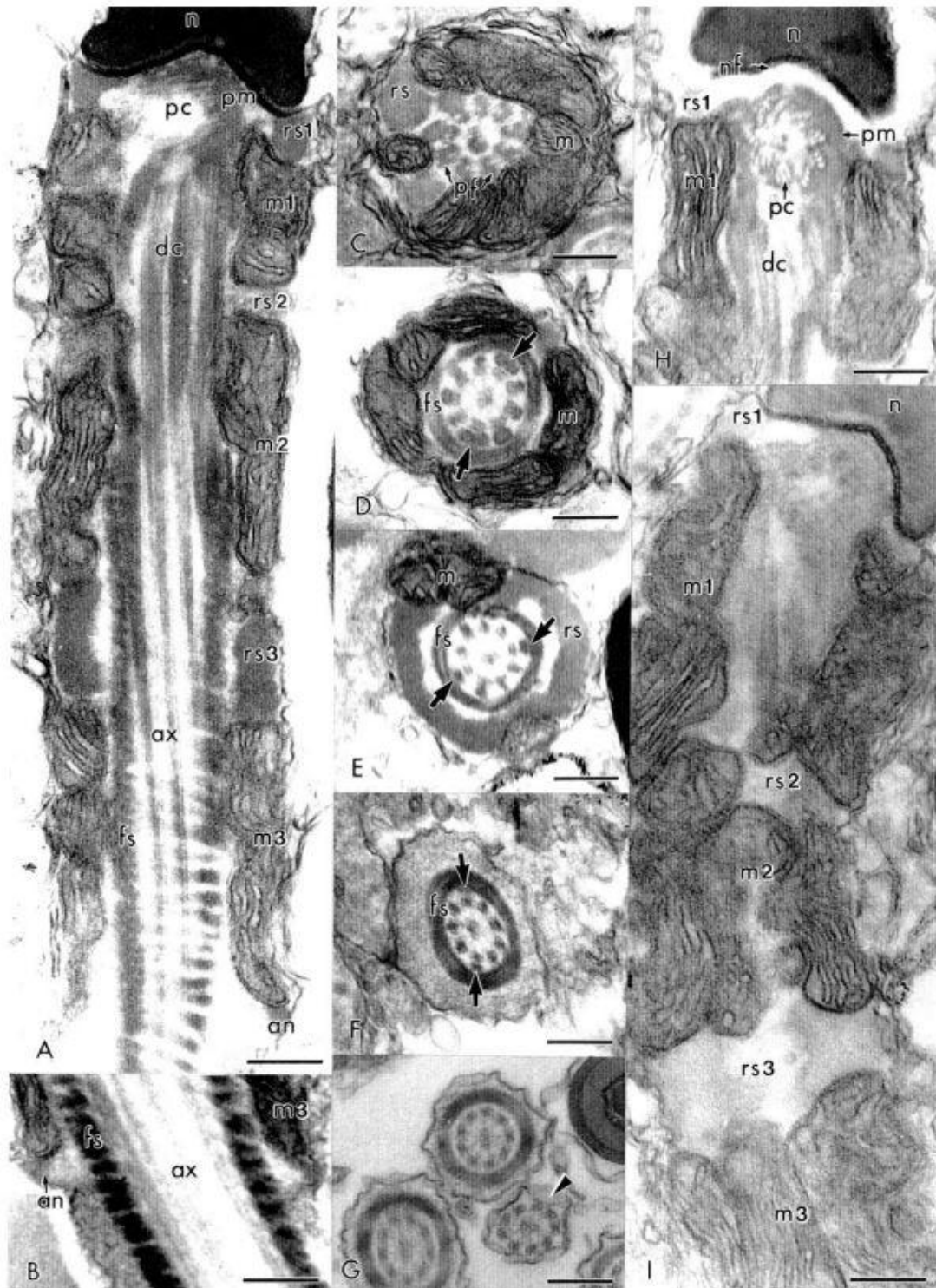


Fig. 3 *Tropicurus semitaeniatus*. (A). Longitudinal section of the posterior end of the nucleus and the midpiece. (B) Transition region between the midpiece and the principal piece. (C) Transverse section through the distal centriole showing the pair of microtubules and the dense material within it. (D) Transverse section through the axoneme showing the fibrous sheath and the nine peripheral fibres associated with the doublets. Note that the peripheral fibres at 3 and 8 (arrows) are double and detached from their doublets. (E) Transverse section through the third ring structure at the posterior portion of the midpiece showing the peripheral fibres at 3 and 8 (arrows) thicker than the others and detached from their doublets. (F) Transverse section through the anterior portion of the principal piece. (G) Transverse section of the posterior portion of the principal piece and the end piece (arrowhead). (H) Detail of the neck region. (I) Longitudinal section through the midpiece showing three ring structures

separated by three sets of columnar mitochondria. an: annulus; ax: axoneme; dc: distal centriole; fs: fibrous sheath; m: mitochondria; n: nucleus; nf: nuclear fossa; pc: proximal centriole; pf: peripheral fibres; pm: pericentriolar material; rs: ring structure. Scale bar 0.2 μm .

Discussion

In *Tropidurus semitaeniatus* and *T. torquatus*, the acrosome complex forms a tripartite pattern (acrosome vesicle, subacrosomal cone and the constricted nuclear tip), a plesiomorphic condition of tetrapods (Jamieson, 1995a). The following features, regarded as plesiomorphic in amniotes (Jamieson, 1995a), are also present in the two species: nucleus elongate; distal centriole extending through midpiece, penetrated by two central singlets from the axoneme; several mitochondria in cross-section of midpiece; annulus present; nine peripheral fibres associated with the nine doublets of the axoneme; peripheral fibres adjacent to doublets 3 and 8 enlarged, forming a double structure detached from their respective doublets. *Tropidurus semitaeniatus* and *T. torquatus* have a number of character-states considered synapomorphies of Squamata (Jamieson, 1995b): perforatorium single, wholly prenuclear; endonuclear canal absent; epinuclear lucent zone present; mitochondrial cristae linear; intermitochondrial dense bodies present; fibrous sheath extending into midpiece; and nuclear shoulders rounded.

A suite of character-states present in mature spermatozoa of tropidurids is also seen in the chamaeleonids *Bradypodion karrooicum* (Jamieson, 1995b) and *Pogona barbata* (Oliver et al., 1996): apical portion of acrosome in transverse section depressed; perforatorial base plate absent (questionably present in *P. barbata*); epinuclear lucent zone clearly defined and well developed; midpiece short (moderately long in *B. karrooicum*); dense bodies forming regular rings, regularly separated from each other by mitochondria (dense bodies more scattered in *B. karrooicum*). *Tropidurus semitaeniatus* and *T. torquatus* share with the polychrotid *Polychrus acutirostris* (Teixeira et al., 1996b) the following features: apical portion of acrosome in transverse section depressed; acrosome vesicle homogeneous and electron-dense; subacrosomal cone filled with an unevenly arranged matrix, instead of a paracrystalline structure; nucleus with interspersed lacunae; epinuclear lucent zone well developed; a finely granular material dispersed between the condensed chromatin and the nuclear envelope; midpiece short; dense bodies without their own membrane; mitochondrial matrix more electron-dense than dense bodies. The account by Furieri (1974) on the polychrotid *Pristidactylus* (*Cupriganus*) *scapulatus* permits only limited comparisons with our results: the midpiece is short as in tropidurids.

Likewise, only limited comparisons are possible with the tropidurids studied by Furiere (1974): in *Liolaemus austromendocinus* and *Phymaturus flagellifer* (**palluma*), the midpiece is short as in *Tropidurus*, and only *P. flagellifer* has the arrangement of ring structures and mitochondria like *Tropidurus*. This account reveals two unusual features of tropidurids relative to other iguanians: the presence of an electron-lucent, unilateral ridge at the acrosome surface, and an epinuclear lucent zone bounded by a membrane. The subacrosomal material is not closely attached to the acrosomal vesicle, being separated on one side by an electron-lucent layer. The lack of adherence can be explained by an uneven distribution of ionized groups on the surface of the inner membrane of the acrosome vesicle. This feature has been observed only in *Amphisbaena alba* (Teixeira et al., in press). The narrow chamber within the anterior limit of the subacrosomal cone, at the anterior end of the nucleus, is bounded by a membrane, suggesting that it can be a nuclear extension, with a small diameter. To the best of our knowledge, this feature has not been observed in any other squamate. Despite the presence of a membrane, we considered the chamber as an epinuclear lucent zone in this account.

The only published accounts on the sperm ultrastructure of *Tropidurus* are the papers by Cruz-Landim & Cruz-Höfling (1977) and Cruz-Höfling & Cruz-Landim (1978) on spermiogenesis. These works indicate that, in late spermatids of *T. cf. torquatus*, the fibrous sheath occurs posteriorly to the midpiece, the sperm head is not depressed, and dense bodies or ring structures are absent. In the mature spermatozoon of *T. semitaeniatus* and *T. torquatus*, however, the fibrous sheath penetrates into the midpiece to the level of m₂, the acrosome is depressed, and ring structures are present, separating sets of mitochondria. In 1987, the *T. torquatus* species complex was split into 11 taxa (Rodrigues, 1987) and, without precise locality data and/or the re-examination of specimens, it is impossible to determine the current taxonomic status of the material studied by Cruz-Landim & Cruz-Höfling (1977) and Cruz-Höfling & Cruz-Landim (1978). It is possible that the differences reported above between *T. semitaeniatus* and *T. torquatus* and the species studied by those authors reflect taxonomic differences, i.e. the *Tropidurus* studied by Cruz-Landim & Cruz-Höfling (1977) and Cruz-Höfling & Cruz-Landim (1978) was not *T. torquatus*, but a related species within the same species group. A phylogenetic analysis of *Tropidurus* based on osteology, squamation, color, and hemipenes suggests that *T. semitaeniatus* is a closer relative of *T. torquatus* than other members of the *T. torquatus* group (Frost, 1992).

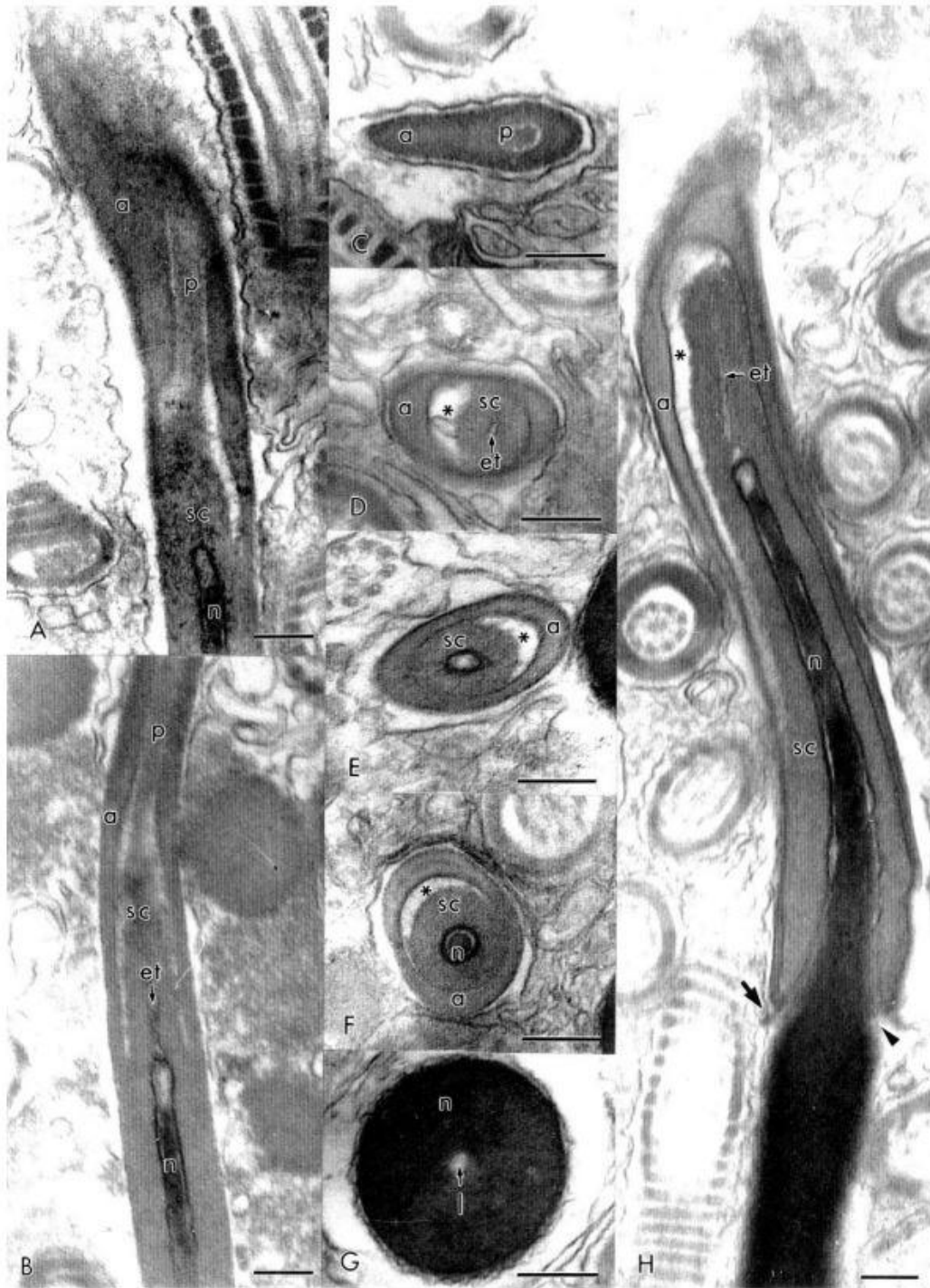


Fig. 4 *Tropidurus torquatus*. (A) Longitudinal section through the apical end of the acrosome showing its flattened portion and the perforatorium. (B) Longitudinal section through the acrosome showing the nuclear tip, the epinuclear lucent zone and the perforatorium. Note the space between the condensed chromatin and the nuclear membrane, occupied by a granular material, at the nuclear tip. (C–F) A series of transverse section through the acrosome. Note that anteriorly, in C–E, the acrosome appears very depressed, while posteriorly, in F, it is more circular. An electron-lucent layer (*) between the acrosome vesicle and the subacrosome cone may be observed in D–F. (G) Transverse section through the circular nucleus showing lacuna. (H) Longitudinal section through the basal region of the acrosome showing the electron-lucent ridge (*) between the acrosome vesicle and the subacrosomal cone. Note the nuclear shoulders (arrowhead) and the membranous flange (arrow). a: acrosome vesicle; et: epinuclear lucent zone; 1, nuclear lacuna; n: nucleus; p: perforatorium; sc: subacrosomal cone. Scale bar 0.2 μm .

Alternatively, the differences between *T. cf torquatus*, *T. torquatus* and *T. semitaeniatus* might reflect structural changes resulting from the maturation process during epididymal transit. Spermatozoa functionally immature undergo structural, biochemical, and functional changes during epididymal transit (Bedford & Nicander, 1971; Bedford, 1975; Orgebin-Crist, 1981; Carcupino et al., 1989), often completing maturation in the female reproductive tract as an adaptation to prevent premature acrosomal reactions during storage (Newton & Trauth, 1992). In this way, epididymides act not only as sperm storage organs, but also as sites of maturation (Healy & Jamieson, 1994). We strongly suggest that specimens should be deposited in accessible scientific collections and collection identification numbers should be provided in publications, enabling the reexamination of specimens in case of taxonomic changes. Further, mature, epididymal spermatozoa should preferentially be described.

The congeneric lizards *Tropidurus semitaeniatus* and *T. torquatus* are practically identical with respect to the ultrastructure of mature sperm cells. They differ in details such as degree of acrosome flattening, presence of bulging at nuclear base, and arrangement of microtubules in the endpiece. Differences in sperm ultrastructure characters of reptiles have been reported at the level of orders and families (Jamieson, 1995a; 1995b), suggesting that they can be useful in phylogenetic analyses. Nevertheless, the existence of major areas of disagreement between phylogenetic hypotheses derived from spermatozoa ultrastructural characters and gross morphological characters, led Teixeira et al. (1999a) to suggest that the intra-familial variability in these characters may be higher than currently thought. That seems to be the case within Chamaeleonidae (Jamieson, 1995b; Oliver et al., 1996), Scincidae (Jamieson & Scheltinga, 1994; Jamieson, 1995b; Jamieson et al., 1996), and Tropiduridae. Further studies investigating the degree of variability in sperm ultrastructure characters across taxonomic categories might aid in clarifying at which taxonomic level they can be most profitably used in phylogenetic analysis.

A variety of characters derived from the sperm ultrastructure of squamates can profitably be used in phylogenetic analyses, where other datasets are uninformative (Teixeira et al., 1999a). Additional work describing the sperm ultrastructure of the families Corytophanidae, Crotaphytidae, Hoplocercidae, Iguanidae, Opluridae, and Phrynosomatidae (Frost & Etheridge, 1989) are warranted and may cast light on the phylogenetic relationships of iguanians.

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