Ultrastructural study of spermiogenesis in the Jamaican Gray Anole, *Anolis lineatopus* (Reptilia: Polychrotidae)

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Abstract


As the number of spermatozoal characters being described in reptiles increases, it is important to detail the ontogeny of the features leading to the mature morphology of the spermatozoa which may give rise to more comprehensive data matrices for future phylogenetic analyses within the Reptilia. Therefore, spermiogenically active testes from *Anolis lineatopus* were investigated ultrastructurally to describe the intracellular changes that occur throughout spermiogenesis. The primary events of spermiogenesis (acrosome formation, nuclear condensation, and elongation) seen in *A. lineatopus* are similar to those previously described for other amniotes. Characters including a round perforatorium tip, stopper-like perforatorial base plate, open pits of nucleoplasm during condensation, and protein layers within the acrosome complex corroborate trends from previous studies in squamates. However, uniquely defined in *A. lineatopus* are the excessive amounts of endoplasmic reticulum and Golgi complexes that contribute to cellular secretions during mid elongation of the spermatids and the lack of a manchette. During acrosome formation, the acrosome granule is found in a basal rather than an apical position, which has been observed in previous studies. These similarities and differences observed during spermiogenesis may be helpful in elucidating the development of mature spermatozoal characters as well as aid in future phylogenetic analyses.

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Introduction

Multiple data sets exist detailing the morphological characteristics of the mature spermatozoa, and the number of studies on the ontogeny of sperm is increasing in the Reptilia. One unifying goal of these studies is to provide sufficient ultrastructural information to develop more complex phylogenetic matrices of nontraditional characters on which phylogenetic analyses can be performed (Jamieson 1991; Newton and Trauth 1992; Jamieson et al. 1995; Teixeira et al. 1999a,b; Tavares-Bastos et al. 2002; Vieira et al. 2004; Wiens 2004). Although data matrices have been previously attempted, the scope of sampling (characters, species and lineages) must increase to permit more reptilian taxa to be included in these evolutionary assessments (Teixeira et al. 1999a).

The past 10 years have led to a substantial increase in the number of sperm morphology studies in squamates, with many focusing on iguanid lizards (Scheltinga et al. 2001; Ferreira and Dolder 2002; Tavares-Bastos et al. 2002; Vieira et al. 2004, 2005). Because sister group comparisons in phylogenetic inferences have been previously reported as essential (Barraclough et al. 1998), this increase in the amount of sperm morphology characters for iguanid lizards may lead to Iguania being utilized as an excellent sister taxon to the Scleroglossids. Thus, additional information on the ultrastructure of either the spermatozoa or events of spermiogenesis within other iguanid species will add needed data to this possible sister taxon designation for Iguania.

Although studies on specific events of spermiogenesis (acrosome development, nuclear elongation/DNA
condensation, and flagellar development) exist (Clark 1967; Butler and Gabri 1984; Hondo et al. 1994; Jamieson et al. 1996; Al-Dokhi 2004, 2006; Al-Dokhi et al. 2004), a complete description of the ultrastructural changes during spermiogenesis in lepidosaurs is thus far limited to the tuatara, *Sphenodon punctatus* (Healy and Jamieson 1994), Green Iguana, *Iguana iguana* (Ferreira and Dolder 2002), Ground Skink, *Scincella lateralis* (Gribbins et al. 2007), and the Cottonmouth, *Akhistrodon piscivorus* (Gribbins et al. in press). Some aspects of spermiogenesis, however, have been reported in *Anolis carolinensis* (Clark 1967), and many iguanid lizards have mature spermatozoal characters previously described in an evolutionary context (Vieira et al. 2004, 2007).

Multiple characters in mature sperm morphology have been reported as possible synapomorphies for many squamate clades (Vieira et al. 2004). For example, a round tipped perforatorium has been recognized as a possible synapomorphy for *Anolis* lizards and a single perforatorium as a synapomorphy for squamates. The morphological changes that occur during spermiogenesis should lead to the morphological characters observed within the mature sperm (Gribbins et al. 2007; in press). Furthermore, many of the characters previously used in phylogenetic analyses using sperm morphology are recognized during the events of spermiogenesis and, therefore, could be combined in a phylogenetic matrix. The combination of these characters could lead to a larger phylogenetic matrix and an even more robust phylogenetic analyses of iguanid lizards overall.

Thus, the purpose of this study is to describe the morphological changes that occur during spermiogenesis within the Jamaican Gray Anole, *Anolis lineatopus*. This neotropical lizard occurs island-wide on Jamaica (Underwood and Williams 1959) and exhibits continuous spermatogenesis (Gribbins et al. 2009). The results from this study will be compared to the spermiogenic data of *Anolis carolinensis* (Clark 1967), *Sphenodon punctatus* (Healy and Jamieson 1994), *Iguana iguana* (Ferreira and Dolder 2002), *Scincella lateralis* (Gribbins et al. 2007), and *Akhistrodon piscivorus* (Gribbins et al. in press). These results will also be compared with the mature spermatozoal data of *Anolis carolinensis* (Scheltinga et al. 2001) and other squamates.

**Materials and Methods**

Sexually mature male Jamaican Gray Anoles, *A. lineatopus*, were collected from southern Jamaica from October 2004 to September 2005. Individuals were killed by decapitation, and the testes were removed through gross dissection. The testes were cut into transverse sections, fixed in Trump’s fixative (EMS, Hatfield, PA, USA), and stored at 4 °C.

Testicular tissues from predetermined spermiogenically active months (Gribbins et al. 2009) were cut into small cubes (2–3 mm), washed twice with cacodylate buffer (pH 7.0) for 20 min each, post-fixed in 2% osmium tetroxide for 2 h, and washed again three times with cacodylate buffer (pH 7.0) for 20 min each. Tissues were then dehydrated in a series of ethanol solutions (70%, 85%, 95% X2, 100% X2), and cleared twice with propylene oxide for 10 min each. Tissues were then gradually introduced into epoxy resin (Embed 812; EMS) with 2 : 1 and 1 : 1 solutions of 100% ethanol: epoxy resin before being placed in pure epoxy resin for 24 h on a rotary system. Fresh resin was prepared and tissues were embedded in small beam capsules and cured for 48 h at 70 °C in a Fisher isotemperature vacuum oven (Fisher Scientific, Pittsburgh, PA, USA). Resin blocks were sectioned (90 nm) using a diamond knife (DDK, Wilmington, DE, USA) on a LKB automated ultramicrotome (LKB Produkter AB, Bromma, Sweden), and sections were placed on 300 mesh copper grids. The tissues were then stained with uranyl acetate and lead citrate.

Tissue sections were viewed using a JEOL JEM-1200 EX II transmission electron microscope (Jeol Inc., USA) and photographs were taken of representative germ cells and cellular components of spermiogenesis with a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale, CA, USA). Micrographs were analyzed and composite plates were constructed using Adobe Photoshop CS (Adobe Systems, San Jose, CA, USA).

**Results**

During the early stages of spermiogenesis the seminiferous epithelium of *A. lineatopus* is dominated by round spermatids that contain multiple cytoplasmic bridges (Fig. 1A; black arrowheads) between one another, which allow them to develop as a synchronized cohort. The acrosome vesicle (Fig. 1A,B; Ac) begins to develop from fusing vesicles (Fig. 1C, inset; white arrowhead) originating from the proximal cisterna of the Golgi complex (Fig. 1C, inset; D; Gb) and this enlarged vesicle creates an indentation in the apex of the spermatid nucleus (Fig. 1A–C; Nu). The cytoplasm contains scattered mitochondria (Fig. 1B; black arrow) and Sertoli cell processes can be seen adjacent to the developing spermatids (Fig. 1B; SL). The latter stages of round spermatid development are subject to the acrosome (Fig. 1C; Ac) increasing further in size because of the continuous fusing of vesicles from the Golgi complex (Fig. 1C,D; Gb). As these transport vesicles fuse with the growing acrosome, they release flocculent material into the matrix of the acrosome vesicle (Fig. 1D; black arrow). There is also an abundance of smooth endoplasmic reticulum associated with the outer acrosome membrane during the round spermatid stage (Fig. 1D; white arrows).

During the late round spermatid stage and the early stages of elongation, the cytoplasm rearranges and the developing spermatid occupies the most apical portion of the cytoplasm. At the same time, the majority of the spermatids become oriented with their acrosomes facing the basement membrane of the seminiferous epithelium. The nucleus consists primarily of heterochromatin with some darker staining euchromatin occupying the areas near the nuclear membrane. An acrosome granule (Fig. 2A; black arrowhead) becomes evident in a
basal position near the nuclear membrane within the acrosome (Fig. 2A, B; Ac) and the subacrosome space (Fig. 2A; Sas) becomes visible. There are two prominent protein layers observed in the subacrosome space of the late round/early elongate spermatids (Fig. 2A; PI1 and PI2). The posterior portion of the spermatid then begins to elongate, creating a tapered end caudally. The proximal and distal centrioles (Fig. 2A; Pc and Dc) can be seen in sagittal sections of the nuclear fossa (Fig. 2A; Nf), and elongating flagella (principle and endpieces) can be observed in the luminal space in juxtaposition to the elongating spermatids (Fig. 2B, C). A prominent Golgi complex (Fig. 2A; Gb) is still seen within the cytoplasm of the early elongating spermatids and multiple vesicles budding from the Golgi cisternae fuse with the plasma membrane (Fig. 2B, C).
membrane, emptying vesicular contents into the lumen of the seminiferous tubules (Fig. 2A; V). During early elongation, the acrosome vesicle (Ac) ceases to increase in size, flattens, and begins to envelop the anterior portion of the nucleus (Fig. 2C; Nu). The acrosome granule, if seen (Fig. 2C; black arrowhead), also begins to flatten and occupies a basal position with the acrosome vesicle. Only a single thin dark-staining protein layer (Fig. 2D; Pl) lies within the subacrosome space (Fig. 2D; Sas).

As elongation continues the chromatin continues to condense and open pits of nucleoplasm (Fig. 3A; white arrowhead) develop within the nucleus (Fig. 3A; Nu). The acrosome (Fig. 3A; Ac) and subacrosome space (Fig. 3A; Sas) continue to migrate caudally along the lateral surface of

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**Fig. 2**—Late developing round spermatids and early elongating spermatids within the seminiferous epithelium of *Anolis lineatopus*. — **A.** Scale = 2 μm. A late round spermatid occupies the apical portion of the cytoplasm. The acrosome vesicle (Ac) sits within the indentation of the nucleus (Nu) and a prominent acrosome granule (black arrowhead) can be visualized within the acrosome vesicle. The subacrosome space (Sas) has two prominent protein layers (Pl1 and Pl2). The proximal and distal centrioles (Pc and Dc) are sitting in the nuclear fossa (Nf). Vesicles (V) with an electron dense coating from the Golgi complex (Gb) are seen fusing with the cellular membrane. — **B.** Scale = 1 μm. The very beginning of acrosomal vesicle (Ac) flattening at the apex of the nucleus (Nu) is occurring in a late round spermatid. A small acrosome granule (black arrowhead) can still be seen in the acrosome vesicle. Fl: Principle piece of the flagellum. — **C.** Scale = 2 μm. The nucleus (Nu) has started to elongate and the acrosome vesicle (Ac) and its acrosome granule (black arrowhead) continues to flatten and move laterally along the nucleus. Principle pieces (Pp) and endpieces (Ep) of the flagellum can be observed in the lumen of the seminiferous tubule. — **D.** Scale = 1 μm. The chromatin of the nucleus (Nu) begins to condense and the acrosome vesicle (Ac) and subacrosome space (Sas) continue to migrate laterally along the nucleus. Within the Sas is a think dark protein layer associated with the inner acrosome membrane (Pl). The acrosome shoulders (white arrow) are in close association with the Sertoli cell processes (Sc) that support the early elongating spermatids.
the nucleus. Elongates in transverse section reveal lipids and dense bodies within the cytoplasm, which will be organized into cytoplasmic droplets (Fig. 3B; Ld and Db). Also, note that early and late elongating spermatids are found in close association with one another (Fig. 3B; S5 and S7). Prominent caudal nuclear shoulders (Fig. 3C; black arrow) are seen just rostral to the nuclear fossa (Fig. 3C; Nf) in middle elongates. At the end of the middle elongation stage, the acrosome has completed its migration caudally and produces a subacrosomal flange at its most lateral termination point (Fig. 3D; black arrow).

Fig. 3—Middle stage elongating spermatids within the seminiferous epithelium of *Anolis lineatopus*. — A. Scale = 1 μm. The acrosome vesicle (Ac), subacrosomal space (Sas), and acrosome shoulders (white arrow) continue to move laterally along the nucleus (Nu). Pits of open nucleoplasm (white arrowhead) can be seen within the nucleus and chromatin condensation is occurring. — B. Scale = 2 μm. Cross-sectional views of middle and late stage elongates reveal the nuclei (Nu, S5) surrounded by the subacrosomal space (Sas) and acrosome vesicle (Ac). An acrosome surrounds the Sas of the S7 spermatid. Also, note the lipid droplets (Ld) and dense bodies (Db) within the S5 cytoplasm, which will become part of cytoplasmic droplets later in elongation. — C. Scale = 2 μm. The nucleus (Nu) continues to elongate and the acrosome vesicle (Ac) and subacrosomal space (Sas) are seen on the apex of the spermatid nucleus. The caudal end of the nucleus has prominent nuclear shoulders (black arrow) just rostral to the nuclear fossa (Nf). — D. Scale = 1 μm. The spermatid nucleus (Nu) continues elongation and the acrosome vesicle (Ac), subacrosomal space, and acrosome shoulders (black arrowheads) have migrated to a lateral position on the cranial nucleus. A new protein layer (Pl3) has developed just under the outer acrosome membrane and multiple layers of Sertoli cell membrane (Sc) surround the exterior of the outer acrosome membrane. Several cisternae of smooth ER are still associated with the shoulders of the acrosome (white arrowheads).
arrowheads). The thin dark-staining protein layer remains between the apex of the nucleus and the acrosome within the subacrosome space (Fig. 3D; Sas). A third protein layer (Fig. 3D; Pl3) forms just inside the outer acrosome membrane and multiple Sertoli cell membranous laminae (Fig. 3D; Sc) are found exterior to the outer acrosome membrane and run along the entire length of the acrosome in sagittal section. There are several smooth endoplasmic reticulum cisternae in transverse section in close association to the lateral acrosome shoulders at the termination of acrosome migration (Fig. 3D; white arrowheads).

During the climax of elongation the nucleus begins to stain uniformly and multiple dense bodies (Fig. 4A,B; Db) occupy the cytoplasm. The cytoplasm continues to decrease and excessive amounts of material are packaged into cytoplasmic droplets (Fig. 4B; Cd) and lipid droplets (Fig. 4B; Ld). The acrosome complex continues to compartmentalize as the apical portion of the nucleus (Fig. 4C; Nu) extends into the

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**Fig. 4**—Late elongate spermatids within the seminiferous epithelium of *Anolis lineatopus*. — **A.** Scale = 2 μm. The acrosome complex (Ac) can still be observed on the apex of the elongating spermatid. The cytoplasm has shrunk greatly and contains multiple dense bodies (Db). — **B.** Scale = 2 μm. The nucleus (Nu, S5) and cytoplasm in cross-section show large cytoplasmic droplets (Cd), lipid droplets (Ld), and dense bodies (Db). The late elongates are still in close association with earlier stages of spermiogenesis as a step 5 spermatid (S5) is seen in cross-section. — **C.** Scale = 1 μm. The acrosome complex in sagittal section shows high compartmentalization with the rostrum of the nucleus (Nu) extending into the subacrosome space. The subacrosome space is divided by a translucent layer (*) into two distinguishable proteins layers (Pl1, Pl2). An epinuclear lucent zone (Ep) is seen sitting on top of the apex of the nuclear rostrum within Pl1. The perforatorium (Pe) originates from a dark staining basal plate (Ba) and resides within Pl2. The acrosome (Ac) completely envelopes the subacrosomal space and is surrounded by many layers of Sertoli cell membranes (Sc). Smooth ER in transverse section is still associated with the acrosome shoulders (black arrowheads). — **D.** Scale = 1 μm. The caudal end of the spermatid is seen with minimal cytoplasmic material and the proximal centriole sits in the nuclear fossa (Nf). The distal principle piece (black arrow) is surrounded by a fibrous sheath as seen in sagittal and transverse views.
subacrosome space. An electron lucent zone (epinuclear space) (Fig. 4C; Ep) and a well-developed perforatorium (Fig. 4C; Pe) sitting on a stopper-like base plate (Fig. 4C; Ba) are found within the subacrosome space. The electron dense protein layers of the subacrosomal space (Fig. 4C; Pl1 and Pl2) are separated by a translucent layer (Fig. 4C; *) and multiple Sertoli cell membrane layers surround the acrosome (Fig. 4C; Sc and Ac). Numerous cisternae of the smooth ER are associated with the lateral acrosomal shoulders (Fig. 4C; black arrowheads). During late elongation, the flagellum (Fig. 4D; black arrows) extends from the nuclear fossa (Fig. 4D; Nf) and the distal portion of the principle piece axoneme surrounded by a fibrous sheath.

The final stage of elongation demonstrates many of the mature structures observed in the typical mature spermatozoa of squamates. The acrosome complex remains highly compartmentalized with the subacrosomal space (Fig. 5A; Sas) organized into two prominent protein layers (Fig. 5A–C; Pl1, Pl2). The perforatorium (Fig. 5B; black arrowhead) extends into protein layer 2 (Fig. 5B; Pl2) of the subacrosomal space. The apical portion of the nucleus (Fig. 5C; Nu) extends into protein layer 1 of the subacrosomal space (Fig. 5C; Pl1). Both the inner and outer acrosome membranes exhibit multiple membranous layers that appear continuous circumcylindrically in cross-section. The exterior membrane laminae are Sertoli cell membrane extensions (Fig. 5C; Sc) and their origin can be seen in transverse section (Fig. 5B; white arrow). Sertoli cell processes wrap around the elongating spermatids and contain slivers of cytoplasm with multiple mitochondria and smooth endoplasmic reticulum (Fig. 5C; Mi and Er). The chromatin is fully condensed and stains uniformly (Fig. 5D). The perforatorium extends into the nuclear fossa (Fig. 5, sagittal section; black arrow). The elongating midpiece axoneme (Fig. 5E) displays the conserved 9 + 2 microtubule arrangement and is surrounded by mitochondria (Fig. 5E; Mi). The proximal principle piece and the midpiece are surrounded by a fibrous sheath (Fig. 5E;F; Fs), whereas the endpiece lacks this sheath (Fig. 5F; black arrow). The axoneme of the midpiece also has dense peripheral fibers (Fig. 5E; Df) associated with microtubule doublets 3 and 8.

Discussion
Throughout spermiogenesis in vertebrates multiple nuclear and cytoplasmic changes can be observed and allow the steps of spermiogenesis to be staged based on a synonymous nomenclature (Healy and Jamieson 1994; Ferreira and Dolder 2002; Gribbins et al. 2007). These cytologically visible steps include acrosome formation, nuclear elongation/DNA condensation, and flagellar development. Many of cellular changes that occur during these three main stages are conserved within many vertebrate taxa and others are unique to certain phylogenetic lineages. The development of a compartmentalized acrosome complex provides numerous ultrastructure characters, many of which are unique to Squamata (Newton and Trauth 1992; Jamieson et al. 1995; Ferreira and Dolder 2002; Vieira et al. 2004, 2005; Cunha et al. 2008). A single perforatorium within the subacrosomal space has been described as unique for squamates (Teixeira et al. 1999a,c; Tavares-Bastos et al. 2007; Tourmente et al. 2008). This feature is also noted during spermiogenesis within A. lineatopus and it is in a similar location in the mature spermatozoa of Anolis carolinensis (Scheltinga et al. 2001). The compartmentalization of the acrosome has been suggested to aid in the release of hydrolytic enzymes during fertilization (Talbot 1991). The protein layer stratification found within the subacrosome of A. lineatopus has only been described in one other species of snake, Agkistrodon piscivorus (Gribbins et al. in press). The function of this subacrosomal feature is unknown; we suggest that it further compartmentalizes the acrosome complex and may play an additional role in facilitating the release of enzymes from the acrosome during syngamy.

Early formation of the acrosome complex in A. lineatopus resembles that of other anniotes. Budding transport vesicles from the Golgi complex fuse near the apical portion of the spermatid nucleus to create a large acrosome vesicle. In the Green Iguana, Iguana iguana, Ferreira and Dolder (2002) suggested that the endoplasmic reticulum may also play a role in acrosome formation. The endoplasmic reticulum was not observed participating in the initial acrosome vesicle formation in the Jamaican Gray Anole, which is consistent with other squamates (Ground Skink, Gribbins et al. 2007; Cottonmouth, Gribbins et al. in press). However, the smooth endoplasmic reticulum is found in close association with the acrosome during spermiogenesis and may be linked to the development of the multiple inner membrane layers of the acrosome seen late in elongation. These extra membrane layers of the inner acrosome membrane have not been described previously during spermiogenesis or within the mature spermatozoa of any other reptile studied to date. Their absence during spermiogenesis and within the mature sperm of Anolis carolinensis suggests that this character may be an autapomorphy for A. lineatopus. The location of the inner membranes suggests that an intracellular organelle of the spermatid contributes to these structures and not the Sertoli cell, which is implicated in the development of the outer laminar layer surrounding the acrosome. The close association of the smooth ER to the acrosome during its formation and migration suggests that it may add cellular membrane components to the inner acrosome membrane, producing a multiple layered appearance of the final inner acrosome membrane morphology.

The acrosome granule is first seen in A. lineatopus in a basal position after the contact of the acrosome vesicle and nucleus. This location is similar to that in other lizard species (Clark 1967; Da Cruz-Landim and Da Cruz-Hofling 1977; Butler
Fig. 5—Scale = 2 μm. Sagittal section of a mature elongate spermatid right before spermiation within the seminiferous epithelium of *Anolis lineatopus*. Cross bars and letters represent approximate locations where cross-sections A–F were taken. The nuclear fossa and proximal centriole (black arrow) are easily seen in the sagittal section. — A–F. Subsequent cross-sectional views. Scale = .2 μm. — A. A transverse section just above the perforatorium. The acrosome (Ac) surrounds the PI2 layer of the subacrosome space (Sas) which both contain membranous structures. There are multiple membrane layers associated with the inner and outer acrosome membranes (Sc and Im). Multiple dense bodies (Db) surround the acrosome complex. — B. Cross-section through the perforatorium (black arrowhead), which is found in PI2. PI1 of the subacrosome space is also visible in the oblique cross-section of the acrosome complex. The acrosome (Ac) surrounds the subacrosome space and still contains the multiple membrane layers (Sc, Im) associated with the inner and outer acrosome membranes. Thin Sertoli cell processes (black arrow) radiating off the Sertoli cell proper (SL) surround these late elongating spermatids and reveal the origin (white arrow) of the Sertoli cell membrane layers that surround the outer acrosome membrane. — C. Cross-section through the nuclear rostrum within PI1. The acrosome vesicle (Ac) and its multiple membrane layers (Sc and Im) surround PI2. Sertoli cell processes (*) with thin slivers of cytoplasm containing mitochondria (Mi) and smooth ER (Er) surround the acrosome complex of late elongating spermatids. — D. The apical body of the nucleus (Nu) in cross-section showing its homogeneity and dense bodies (Db) surrounding it. — E. Cross-section through the midpiece of a late elongating spermatid disclosing a ring of mitochondria (Mi) surrounding the axoneme, which has a thick fibrous sheath (Fs) and dense peripheral fibers (Df) associated with microtubule doublets 3 and 8. — F. The distal principle piece is surrounded by the fibrous sheath (Fs) and lacks dense peripheral fibers associated with microtubule doublets 3 and 8. The endpiece (black arrow) seen in cross-section lacks the fibrous sheath of the midpiece and principal piece.
and Gabri 1984; Dehlawi et al. 1992; Ferreira and Dolder 2002), but differs from Scincella lateralis (Gribbins et al. 2007) and Agkistrodon piscivorus (Gribbins et al. in press); in these two squamates the granule is actually visualized before the vesicle contacts the nucleus. As elongation commences in the latter stages of spermiogenesis in A. lineatopus, the acrosome granule flattens, occupies a basal position, and eventually becomes diffuse and concentrated just under the outer acrosome membrane.

Upon the completion of acrosome vesicle formation, the vesicle flattens and begins to migrate over the apex of the spermatid nucleus, as is observed in other squamate species. During this stage of development, the cytoplasm rearranges and the spermatid occupies the most apical portion of the cytoplasm. This event has also been observed in other lizard species (Da Cruz-Landim and Da Cruz-Hofling 1977; Butler and Gabri 1984; Dehlawi et al. 1992; Gribbins et al. 2007). The cytoplasmic rearrangement has been suggested in the rearrangement of organelles, such as mitochondria, which become associated with the eventual midpiece of the spermatid (Sprando and Russell 1988; Lin and Jones 1993; Lin and Jones 2000; Soley 1997; Vieira et al. 2001; Gribbins et al. 2007). Multiple cytoplasmic bridges are seen throughout development of Jamaican Gray Anole spermatids and may function in cellular communication, such as haploid gene product sharing (Ventela et al. 2003). Recent reports on the germ cell development strategy in reptiles, including A. lineatopus (Gribbins et al. 2006, 2009; Rheubert et al. 2009), suggest that germ cells develop as a single population through spermatogenesis and thus the cytoplasmic bridges may also aid in the synchronizing of this temporal germ cell development strategy.

Nuclear elongation in A. lineatopus consists of the nucleus stretching in a dorso-ventral plane and the condensation of DNA. This, again, is consistent with what has been described in other lizard species (Jamieson et al. 1995; Teixeira et al. 1999c, 2002; Colli et al. 2007). However, in Iguana iguana (Ferreira and Dolder 2002) and Agkistrodon piscivorus (Gribbins et al. in press), the chromatins condenses in a spiral fashion creating open pits of nucleoplasm. In A. lineatopus, open pits of nucleoplasm were observed but spiral condensation of the chromatins was not, suggesting that spiraling of the chromatin does not necessarily create the open pits of nucleoplasm. The most conspicuous observation during elongation in A. lineatopus is the lack of a functional manchette. It has been suggested that the manchette aids in the stretching of the nucleus during spermiogenesis in most amniotes (Russell et al. 1990; Soley 1997). This unique observation in A. lineatopus suggests that other factors (such as cytoplasmic stretching, constraint during cytoplasmic elimination, and/or the cytoskeletal network of the Sertoli cell) may actually play a role in nuclear stretching during elongation.

During the climax of elongation, the apical portion of the nucleus, historically called the nuclear rostrum (Clark 1967), extends into the acrosome complex in A. lineatopus. In addition, an epinuclear clear zone next to the nuclear rostrum forms within the first protein layer of the subacrosome space in A. lineatopus. The origin of this zone has been described to be either at the apex of the nucleus (Tavares-Bastos et al. 2002) or within the body of the nucleus (Teixeira et al. 1999a,c). In A. lineatopus, no origin in the body of the nucleus was observed; thus, the epinuclear electron-lucent zone terminology is used for the Jamaican Gray Anole. The function of this space is unknown, but its association just below the perforatorium suggests that it aids the perforatorium during enzyme release from the acrosome upon meeting the external layers of the ovum.

Jamaican Gray Anole flagellar development is first observed during the early stages of spermatid development. The proximal centriole can be observed within the nuclear fossa at approximately 60° to the distal centriole. The flagellum lengthens from the distal centriole throughout spermatid development. At the termination of spermatid elongation, the mitochondrial tiers reside very close to the proximal centriole; thus, only a very short neck region exists at this point in development in A. lineatopus. The midpiece axoneme consists of the typical 9 + 2 microtubule arrangement and the beginning of the fibrous sheath extends into the distal portion of the principle piece. The midpiece can be easily distinguished from the principle piece because of the mitochondrial sheath surrounding the midpiece axoneme and the large peripheral fibers associated with microtubule doublets 3 and 8, which are absent in the principle piece axoneme. The observation of peripheral fibers associated with the 3 and 8 microtubule doublets within the Jamaican Gray Anole midpiece axoneme corroborates this proposed synapomorphy for lepidosaurs (Vieira et al. 2005, 2007).

Many of structures associated with spermiogenesis in A. lineatopus resemble that of other amniotes and squamates, whereas some ultrastructural features appear unique to A. lineatopus. The presence of the manchette has been reported in all squamates to date including Anolis carolinensis (Clark 1967) and Polycherus (Teixeira et al. 1999b). The absence of this structure within A. lineatopus may imply that this feature is an autapomorphy for the Jamaican Gray Anole, as are the previously mentioned multiple membranes associated with the inner acrosome membrane. Another synapomorphy suggested for Polychrotidae is the beginning of the fibrous sheath within a very short midpiece (Vieira et al. 2004), which is also observed within the Jamaican Gray Anole elongating spermatids. The Anolis synapomorphy, a round perforatorium tip (Vieira et al. 2004), was also observed in A. lineatopus, giving further support to its usage as a shared derived character for this genus. However, since data for spermiogenesis are limited (only two species of Anolis), these evolutionary trends remain speculative.

This is the first complete study of spermiogenesis within A. lineatopus, the second for the genus Anolis, and the third for Polychrotidae. These data add to the nontraditional characters previously gathered in lizards and mapped onto squamate
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