The Ultrastructure of the Spermatozoa of Squamata—I. Scincidae, Gekkonidae and Pygopodidae (Reptilia)

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(Accepted for publication 9 January 1995)

Abstract

Squamate autapomorphies seen in sperm of the Scincidae (e.g. Ctenotus robustus, Carlia pectoralis, Cryptoblepharus virgatus, and Lampropholis delicata) are penetration of the fibrous sheath of the axoneme into the midpiece, and the paracrystalline subacrosomal cone. Sphenomorphous group spermatozoa (e.g. Ctenotus) and the Egernia group (Tiliqua) differ from the more derived Eugongylus group (C. virgatus, L. delicata and C. pectoralis) in that the acrosome is elongate and apically depressed; the perforatorium is strongly oblique; the midpiece is relatively short, with four dense ring structures in longitudinal succession; mitochondria are columnar; and enlarged peripheral fibres 3 and 8 do not show the gross anterior enlargement seen in Carlia and Lampropholis. Heteronota binoei (Gekkonidae) sperm have no epinuclear electron-lucent region; nuclear shoulders are smooth, as in sphenomorph but not Eugongylus group skinks; mitochondria are columnar; unlike skinks, the median surfaces of the mitochondria are indented by triangular, sometimes longitudinally, interconnected dense bodies. In Lialis burtonis (Pygopodidae) sperm, the perforatorium extends virtually to the tip of the fore-shortened apically domed acrosome; nuclear shoulders are absent; the mitochondria alternate singly or in groups with one or more dense bodies which also form an interrupted collar around the distal centriole. Spermatozoal ultrastructure suggests that a common ancestry of snakes and pygopods deserves consideration.

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Materials and Methods

The following taxa are examined: Scincidae—Ctenosurus robustus (Storr, 1970) (Samford, Queensland); Cryptoblepharus virgatus (Garman, 1901) (Manly West, Queensland); Lampropholis delicatula (De Vis, 1888) (Manly West, Queensland); and Carlia pectoralis (De Vis, 1885) (Harvey Bay, Queensland); Gekkonidae—Heteronotia binoei (Gray, 1845) (Northern Territory); Pygopodidae—Lialis burtonis (Gray, 1835) (Thargomindah, Queensland).

With the exception of Lialis burtonis, testes of which were removed from two preserved specimens from the collection of the Queensland Museum, the testis and ducts were dissected from a single euthanased specimen of each species and processed as follows. The tissues were diced into 1–2 mm³ portions, and fixed for transmission electron microscopy (TEM), in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), at 4°C for 2 h, being agitated for the first hour. In the case of Lialis burtonis, the testicular material was rehydrated in a descending ethanol series, and placed in phosphate buffer for 1 or 2 days before being subjected to the same processing. The material was then rinsed in 0.1 M phosphate buffer, post-fixed for 80 min in similarly buffered 1% osmium tetroxide; rinsed in buffer; dehydrated through an ascending ethanol series; and infiltrated and embedded in Spur's epoxy resin. Sections were cut with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 50–80 nm thick, were collected on carbon stabilized, collodion-coated, 200-μm mesh copper grids, stained for 30 s in Reynolds's lead citrate, then in 6% aqueous uranyl acetate for 1–4 min and for a further 1–2 min in lead citrate, rinsing in distilled water after each stage. Electron micrographs were taken on a Hitachi 300 electron microscope at 75 kV and a JEOL 100-s electron microscope at 60 kV. Light microscopic observations of spermatozoa, from glutaraldehyde-fixed tissue squashes, were made under Nomarski contrast using an Olympus BH2 microscope. Measurements throughout this paper are indicative only, being based on small numbers of transmission electron micrographs. They were made from the original negatives, or 1.3 magnification proofs, with an accurate steel ruler under a 10 × magnifier. Statistical analysis is not attempted, but the variation in dimensions was established as being small.

Results

(a) Sperm of the Scincidae

Ctenosaurus robustus (Sphenomorphus group). A generalized spermatozoon based on those of the Sphenomorphus group species and Tiliqua scincoides is shown diagrammatically in Fig. 1. This diagram is also applicable to the sperm of Nangua spinosa, Ctenosaurus taeniolatus, Anomalopus verreauxii (Sphenomorphus group) and Tiliqua scincoides scincoides (Egernia group).

Spermatozoa of Ctenosaurus robustus are filiform. The total length by light microscopy is 67–73 μm (n = 2). The head (acrosome and nucleus), and often the midpiece and flagellum, are slightly curved (Figs 2A–C). As a result of this curvature, it has not been possible to obtain a complete longitudinal section through the head. The sperm is circular in cross-section, with the exception of the acrosome. Although the acrosome is circular at its base (Fig. 2J), anterior to this, it develops a unilateral ridge, and anterior to the tip of the subacrosomal cone, it becomes increasingly depressed and elliptical in transverse section (Figs 2F–I). Flattening is deduced to be in the plane of curvature of the nucleus. It has not been determined whether this plane coincides with the ‘dorsoventral’ plane of the spermatozoon which is arbitrarily defined as the plane which places doublet 1 of the axoneme in the dorsalmost position.

Acrosome complex. The length of the acrosome complex by transmission electron microscopy is 5.2 μm. The complex (Figs 2A–C, E, F–J) consists of an acrosome vesicle in the form of an elongate hollow cone, an underlying subacrosomal cone and, axial within the acrosome vesicle, a slender rod, the putative perforatorium. The anterior end of the acrosome vesicle, extending over slightly less than half its total length, forms a thick-walled hollow cone with a narrow lumen housing the perforatorium (Figs 2A, C,G). The longer, posterior region of the vesicle is a thin-walled continuation of this hollow cone, the vesicle here being no more than a sleeve-like investment (acrosome sleeve) of the subacrosomal material, as seen in a longitudinal (Figs 2A–C) or transverse section (Figs 2H–J). The underlying subacrosomal material forms a thick-walled hollow cone (subacrosomal cone), the material of which is paracrystalline, its matrix having fine, obliquely longitudinal and less distinct transverse striations, indicating that it forms a fine lattice (Fig. 2E). For most of its length, from its posterior end anteriorly, the subacrosomal cone invests the tapered anterior end of the nucleus (nuclear attenuation or nuclear point) (Fig. 2B) and fills the attenuated bell-shaped space within the acrosomal sleeve (Figs 2A, C). The nuclear point terminates within the anterior limit of the subacrosomal material at an epinuclear electron-lucent region (Fig. 2A) which is, however, much less clearly defined in Ctenosaurus robustus than in the sperm of Nangua.

The perforatorium is a slender, moderately electron-dense rod, with some signs of internal longitudinal fibrils. It extends anteriorly from the subacrosomal material, lying in a narrow lumen internal to the inner acrosome membrane (Figs 2A,C). It is 1.2 μm long and extends to within approximately 0.4 μm of the tip of the acrosome (Fig. 2A).
subacrosomal material. No basal plate is recognizable. Even allowing for the pronounced curvature of the acrosome, the longitudinal axis of the perforatorium appears to be slightly oblique relative to that of the acrosome vesicle (Figs 2A,C).

In transverse sections of the acrosome vesicle through the perforatorium (Fig. 2G), the vesicle is seen to have a concentric zonation; possibly representing cortex and medulla, which, in sequence from the perforatorium outwards, is: a narrow space around the perforatorium; a wide, dense, homogeneous zone; a narrow zone with radial striations; and a thin, dense, homogeneous layer apposed to the plasma membrane.

**Nucleus.** The nucleus is curved and tapers to a point within the subacrosomal cone in the basal region of the acrosome (acrosome sleeve). The transition from the tapered region (nuclear point) to the much longer cylindroid region is well defined but in *Ctenotus*, as in other examined members of the Sphenomorphus group and in *Tiliqua scincoides*, the abrupt 'shoulders' seen in the sperm of many other reptiles, including skinks of the Eugongylus group, are represented only by a gentle curvature on each side (Fig. 2B). The nuclear point is surrounded by the subacrosomal cone (Figs 2B, C, H–J). The total length of the nucleus is 7.6 μm, of the nuclear point, 2.4 μm and of the nucleus posterior to the acrosome, 5.2–5.3 μm. The nucleus is almost parallel-sided, showing only a slight increase in width posterior, from 0.6 μm, immediately behind the nuclear shoulders, to its greatest width of 0.8 μm, shortly before its posterior end. The cross-section of the nucleus is circular throughout (Figs 2H–L). The chromatin is condensed and strongly electron-dense. Basally, the nucleus has a compact conical fossa which houses dense material extending from the proximal centriole (Figs 2D, L,T).

**Neck region.** The neck region (Figs 2D,T) is the region where the nucleus joins the midpiece and is recognized, as in all other squamates, by virtue of its internal components, although the anterior end of the midpiece directly abuts the posterior end of the nucleus. The neck region includes the proximal and distal centrioles and associated densities, including the first of the ring structures (mitochondrial transformations) of the midpiece. Each centriole consists of nine triplets. The proximal centriole lies immediately anterior to the distal centriole and with its long axis at slightly less than a right angle to it (Fig. 2T). The long axis of the distal centriole, which forms the basal body of the flagellum, is in the long axis of the axoneme. The centrioles do not lie in the basal nuclear fossa, but the proximal centriole, immediately behind this is surmounted by a hollow conical density (dense cone) which conforms in shape with the nuclear fossa which it occupies (Fig. 2D). An electronlucent space separates it from the wall of the fossa. Compact

![Diagram of Squamate Spermatozoon](image-url)
Fig. 2. *Ctenotus robustus* (Scincidae, Sphenomorphus group). Transmission electron micrographs of spermatozoa.—A–C. Acrosome and anterior nuclear region.—D. Longitudinal section (LS) of the midpiece.—E. Detail of the subacrosomal cone showing its paracrystalline structure.—F–Q. Transverse sections (TS) at the levels indicated by corresponding lettered arrows in the previous longitudinal sections.—R and S. TS of the principal piece.—T. LS of the posterior region of the nucleus and of the anterior region of the midpiece.—LS of the principal piece. Scale as the bottom right scale bar, except where indicated in D, E and R.
dense material extends from the base of the dense cone to cover the more axial end of the proximal centriole, and insinuates itself between the proximal and distal centrioles (Figs 2D, T). The two central singlets of the axoneme extend anteriad at least into the region of transition between the distal centriole and the axoneme (Fig. 2M). No transverse sections of the distal centriole (recognized by the presence of triplet microtubules) have been obtained in which two central singlets are not present. One of the central centriolar singlets is connected by a density to triplet 3 and its peripheral dense fibre (Fig. 2M).

A conspicuous stratified laminar structure forms a wing-like projection on each side of the proximal centriole, near its anterior limit and is continuous around its axial pole (Figs 2D, T). It is deduced, as in Nagura spinosa, other sphenomorphs and Tiliqua scincoides, that the lamina forms a thick disc around the proximal centriole but that the disc is interrupted at the peripheral end of the proximal centriole. The outer edges of the laminar structure make contact with the first of the dense ‘ring structures’ of the midpiece, described below (Figs 2D, T).

**Midpiece.** The midpiece, 5.5 μm long, includes the neck, described above. It consists of mitochondria, ring structures, and the contained axoneme with its fibrous sheath and ends posteriorly with the annulus (Fig. 2D).

There are four ring structures (rs 1–4) in longitudinal succession, posterior to which lies the much smaller annulus (an). The ring structures, with the annulus, are separated by mitochondrial regions (mi1–4) giving a formula rs1/mi1, rs2/mi2, rs3/mi3, rs4/mi4, which pertains to Ctenotus robustus, all other examined sphenomorphs and Tiliqua scincoides. Each ring structure appears in longitudinal section as an irregular-trapezoidal to kidney-shaped density on each side of the fibrous sheath of the axoneme but that the disc is interrupted at the peripheral end of the proximal centriole. The outer edges of the laminar structure make contact with the first of the dense ‘ring structures’ of the midpiece, described below (Figs 2D, T).

The mitochondria mostly form elongate, columnar structures of irregular longitudinal section though appearing circular or oval in transverse section, with numerous predominantly longitudinal cristae. Each mitochondrion usually extends from one ring structure to the next (Figs 2D, T). There are in the order of 10 or slightly more mitochondria (an). The ring structures, with the annulus, are separated by mitochondrial regions (mi1–4) giving a formula rs1/mi1, rs2/mi2, rs3/mi3, rs4/mi4, which pertains to Ctenotus robustus, all other examined sphenomorphs and Tiliqua scincoides. Each ring structure appears in longitudinal section as an irregular-trapezoidal to kidney-shaped density on each side of the fibrous sheath of the axoneme (Figs 2D, T). The profile on one side may be staggered relative to that on the other, particularly when the axoneme is bent, though always overlapping it, but in transverse section (Fig. 2M) the ring is complete.

The mitochondria mostly form elongate, columnar structures of irregular longitudinal section though appearing circular or oval in transverse section, with numerous predominantly longitudinal cristae. Each mitochondrion usually extends from one ring structure to the next (Figs 2D, T). There are in the order of 10 or slightly more mitochondria around the axoneme as seen in transverse section (Figs 2N, O) but there is some tendency to transverse fusion. A few small mitochondrial profiles are sometimes present lateral to the ring structures, the outer surface of which may be scalloped by them; this is particularly the case for rs1 which is scalloped by as many as 11 small mitochondria (Fig. 2M).

The axoneme has the usual 9+2 pattern. Each doublet has two dynein arms. The majority, at least of the A subtubules, are occluded by dense material. Around the axoneme almost as far anteriorly as its junction with the distal centriole, there is a fibrous sheath. In longitudinal section (Figs 2D, T, U), the fibrous sheath exhibits rather regularly arranged, approximately square or oblong dense blocks which, from glancing longitudinal sections (Figs 2D) and transverse sections (Figs 2O–S) are shown to form rings around the axoneme.

Nine large peripheral dense fibres, initially of different sizes, are associated with the transition between the distal centriole and the axoneme (Fig. 2M) and continue posteriorly, though much narrower, along the axoneme into the midpiece. One is attached externally to each triplet or doublet, though initially those of adjacent triplets may be fused. More posteriorly within the midpiece, at an undetermined level, all but two of the peripheral fibres become greatly reduced in size (Fig. 2O). At the annulus, only peripheral fibres adjacent to doublets 3 and 8 remain conspicuous, each as a double structure nearer the fibrous sheath than it is to its doublet (Fig. 2P). At the beginning of the principal piece (the region posterior to the annulus), all nine dense fibres are already vestigial or absent (Fig. 2Q). They are absent from the remainder of the principal piece (Figs 2R, S).

The annulus (Figs 2D, P) is a small dense ring with an irregular cross-section, approximately triangular in Ctenotus robustus. It is closely applied to the inner surface of the plasma membrane.

**Principal piece.** The principal piece, the longest part of the spermatozoon, consists of the continuation, behind the midpiece, of the axoneme with its surrounding fibrous sheath and plasma membrane. It begins, immediately behind the annulus, with a short region in which a wide zone of cytoplasm intervenes between the fibrous sheath and the plasma membrane (Fig. 2Q). Posterior to this, the plasma membrane is closely approximated to the fibrous sheath (Figs 2R, S).

**Endpiece.** The axoneme projects behind the fibrous sheath as an endpiece of undetermined length.

Cryptoblepharus virgatus (Eugongylus subgroup), Lampropholis delicata and Carlia pectoralis (Lampropholis subgroup)—Eugongylus group. A generalized spermatozoon based on those of the Eugongylus group species is shown diagrammatically in Fig. 3. It is based on the spermatozoon of Carlia pectoralis. As micrographs of the sperm of this species are given in Jamieson and Scheltinga (1994), only Cryptoblepharus virgatus and Lampropholis delicata are illustrated by micrographs in the present work.

The sperm of the three Eugongylus group species are closely similar and are here described together. They differ markedly from the sperm of the Sphenomorphus—Tiliqua assemblage in the proportions of the chief components. Thus, in the three Eugongylus group species, the midpiece is approximately twice as long as it is in the Sphenomorphus—Tiliqua assemblage, and the nucleus is much shorter relative to the midpiece than it is in that assemblage (see dimensions below).

**Acrosome complex.** The proportions of the various components of the acrosome do not differ markedly in the three species. The length of the acrosome vesicle is 2.5 μm in Cryptoblepharus virgatus, 2.0–2.7 μm in Lampropholis delicata, and 2.0–2.2 μm (n = 3) in Carlia pectoralis.

The subacrosomal cone (Figs 4B–D, 5A) is paracrystalline and has a blunt apex. At the region of the nuclear shoulders, the subacrosomal cone projects posterolaterally as a short flange behind the base of the sleeve-like posterior region of the acrosome vesicle. The acrosome vesicle is much shorter than that of sphenomorph and Tiliqua sperm, and correspondingly, the perforatorium is shorter (Figs 4B–
A general spermatozoon (diagrammatic) of the Eugongylus group species of the Scincidae, in longitudinal and corresponding transverse sections. It is drawn from the sperm of *Carlia pectoralis* but is applicable also to *Cryptoblepharus virgatus* (Eugongylus subgroup) and *Lampropholis delicata* (with *Carlia*, in the Lampropholis subgroup). Scales of various components are only approximate. Regional zonation of the acrosome vesicle, though present, is not indicated.

Nucleus. The length of the nucleus is 5.3 μm in *Cryptoblepharus virgatus*, 3.5 μm in *Lampropholis delicata*, and 2.4 μm in *Carlia pectoralis*. The length of the nuclear attenuation (nuclear point), from the tip to the base of the nuclear shoulders, is 1.5 μm in *Cryptoblepharus virgatus*. 1.0–1.2 in *L. delicata*, and 0.7 μm in *C. pectoralis*. In contrast with species of the Sphenomorphus group and *Tiliqua scincoides*, in the Eugongylus group, sharp nuclear shoulders mark the transition, at the base of the acrosome vesicle and subacrosomal cone, from the nuclear point to the main part of the nucleus (Figs 4A–D, 5A–C). The nuclear point often contains a lacuna about half way along its length (Figs 4A, C, D, 5A), possibly a vestige of the amniote endonuclear canal. The nuclear fossa is dome-shaped to rounded conical in *Cryptoblepharus virgatus* (Fig 4A, B) and *Carlia pectoralis* and *Lampropholis delicata* (Fig. 5D), but does not show the pointed conical form seen in sphenomorphs.

Neck region. The neck region conforms, in most respects, with that described for sphenomorphs. Only in *Lampropholis delicata* is there an extension of dense material into the fossa (Fig. 5D) comparable with the conical extension in sphenomorphs; the anterior portion of the proximal centriole is situated in the wide and shallow nuclear fossa and is closely apposed to the nucleus (Figs 4E, 5S, T). The laminar structure which extends laterally from the proximal centriole in sphenomorphs is absent but in *L. delicata* (Fig. 5S) and in *Carlia pectoralis* there is, lateral to the proximal centriole on at least one side, a large striated density resembling a mammalian striated column. The arrangement of mitochondria and mitochondrial transformations differs profoundly from that in sphenomorphs. The proximal centriole has a large dense central element in *L. delicata* (Fig. 5S).

Fig. 3. A generalized spermatozoon (diagrammatic) of the Eugongylus group species of the Scincidae, in longitudinal and corresponding transverse sections. It is drawn from the sperm of *Carlia pectoralis* but is applicable also to *Cryptoblepharus virgatus* (Eugongylus subgroup) and *Lampropholis delicata* (with *Carlia*, in the Lampropholis subgroup). Scales of various components are only approximate. Regional zonation of the acrosome vesicle, though present, is not indicated.
Fig. 4. Cryptoblepharus virgurus (Scincidae, Eugongylus group). Transmission electron micrographs of spermatozoa.—A. Acrosome, nucleus and mid-piece.—B–D. Acrosome and anterior nuclear region.—E. Neck and anterior region of midpiece.—F–J, K and L, M–O. P, transverse sections at the levels indicated by corresponding lettered arrows in the longitudinal sections shown in Figs D, E, R and S, respectively.—Q. Transverse section through the posterior end of the principal piece.—R. Longitudinal section (LS) of the posterior end of the midpiece and anterior principal piece.—S. Portion of LS of principal piece. Scale as the top left scale bar, except where indicated in A.
Midpiece. The length of the midpiece is 10.7 μm in Cryptoblepharus virgatus and 11.5 μm in Carla pectoralis (undetermined in Lampropholis delicata). The mitochondrial transformations of the Eugongylus group species are seen as scattered irregular dense bodies of varying sizes (Figs 4A, E, K, L, M, R, 5B, D, L–N, S–V). The mitochondria differ from the stout columnar structures regularly arranged around the axoneme seen in sphenomorphs. In C. virgatus, L. delicata and C. pectoralis, the mitochondria are elongate, tubular structures, with longitudinal cristae, which weave between the intermitochondrial bodies. In a transverse section of the midpiece, small scattered circumstantial mitochondrial profiles, numbering in the order of 20 or more, can usually be seen (Figs 4L, M, 5L–O). The origin of the dense bodies from mitochondria, validating their description as mitochondrial transformations, has been well demonstrated in Cryptoblepharus virgatus, and some evidence of this is seen in Fig. 4M. The mass formed by the mitochondria and their transformations is eccentrically placed relative to the fibrous sheath (Figs 4L, 5L–M).

As in other reptiles, nine large peripheral dense fibres are associated with the axonemal doublets. In sphenomorphs and Tiliqua scincoides, as in Carla pectoralis and apparently in Cryptoblepharus virgatus, only the peripheral fibres adjacent to doublets 3 and 8 remain conspicuous, and associated with the fibrous sheath, at the level of the annulus and in the principal piece. However, in Lampropholis delicata, the nine fibres have been observed to remain well developed in at least the anterior region of the principal piece, or to be absent (Figs 5P, Q), or those at doublets 3 and 8 sometimes persist as vestiges into the endpiece (Fig. 5R). At the anterior end of the fibrous sheath, near the distal centriole, in C. pectoralis and L. delicata (Fig. 5M), the peripheral fibres adjacent to doublets 3 and 8 are grossly enlarged, for a short distance, in contrast with the sphenomorph-Tiliqua assemblage. The condition in C. virgatus has not been determined from transverse sections, but from longitudinal sections (not illustrated) it appears that they also show this enlargement.

Principal piece. The structure of the principal piece (Figs 4R, S, 5V) conforms with that described for the sphenomorph-Tiliqua assemblage with the exception of some persistence of the nine fibres in Lampropholis delicata. The annuli of the fibrous sheath are interconnected by a longitudinal element (presumably on both sides) (Fig. 5W) as in mammals.

Endpiece. The axoneme projects behind the fibrous sheath (Fig. 5R) as an endpiece of undetermined length. In Cryptoblepharus virgatus, disruption of the arrangement of axonemal microtubules, typical of the spermatozoal endpiece in many animal groups, was observed at the posterior end of the principal piece before termination of the fibrous sheath (Fig. 4Q).

(b) Sperm of the Gekkonidae

Heteronotia binoei. The chief aspects of the ultrastructure of the spermatozoan of Heteronotia binoei are illustrated diagrammatically in Fig. 6.

Acrosome complex. The acrosome complex, 3.1 μm long, has the usual components. The acrosomal vesicle is divided into cortex and medulla by a narrow pale hiatus (Fig. 7A). The vesicle encloses a paracrystalline subacrosomal cone from which a basal flange projects posterolaterally behind the base of sleeve of the acrosomal vesicle (Fig. 7A). The subacrosomal cone is apically quadrangular rather than circular in cross-section (Fig. 7D). The acrosomal vesicle is not depressed anteriorly. The perforatorium extends for about four-fifths of the distance between the subacrosomal cone and the tip of the acrosomal vesicle. It is a slender and slightly tapering rod, with a length of 0.9 μm, or possibly slightly longer. Densification within the apex of the subacrosomal cone (Fig. 7A) appears to be a perforatorial base plate, as in Lygodactylus and Tarentola (see Discussion).

Nucleus. The nucleus is cylindrical and strongly electron-dense. The length of the nuclear point is 1.5 μm. The nucleus is circular in cross-section (Figs 7E–G) with a width of 0.4 μm immediately below the shoulders and 0.7 μm shortly before its posterior end. The nuclear shoulders are smooth, as in sphenomorph skinks. Basally, there is a shallow conical nuclear fossa (Figs 7B, M). A lacuna has been observed in the chromatin of the nuclear point (Fig. 7A). No definite epinuclear electron lucent region has been seen.

Neck region. The proximal centriole resides beneath the nuclear fossa. It has a conspicuous central element (Fig. 7M) and is tilted relative to the distal centriole (Fig. 7P). Dense material surmounting the centriole occupies the nuclear fossa and conforms to its conical shape (Fig. 7P). No definite laminar structure, comparable with that of sphenomorph skinks, has been observed. A dense staining matrix surrounding the proximal centriole also extends around the distal centriole and gives rise to the peripheral dense fibres of the axoneme. Mitochondria extend almost to the base of the nucleus from which they are separated by the first mitochondrial transformation (Figs 7B, M, P). In the distal centriole, one of the central singlets is connected by a dense structure to triplet 3 and the peripheral dense fibre associated with this (Fig. 7H).

Midpiece. By light microscopy, the midpiece is 11 μm long. Electron dense material encircles the peripheral fibres of the distal centriole and lies beneath the mitochondria (Figs 7H, M, P) and is here termed the pericentriolar collar. It forms a six- or seven-pointed star in cross-section (Fig. 7H).
Fig. 6. *Heteronotia binoei* (Gekkonidae). A diagrammatic representation of the spermatozoon, in longitudinal and corresponding transverse sections.

The collar does not persist intact into the fibrous sheath but breaks down into intermitochondrial protrusions (putative mitochondrial transformations, also termed dense bodies for brevity) which extend between adjacent mitochondria, or as crescentic rings, or as isolated star-points (Fig. 7J) between mitochondria. The protrusions have extensive longitudinal connections with each other which spiral around the fibrous sheath (Figs 7P, Q). The distal centriole does not extend into the fibrous sheath. The bilobed enlarged peripheral fibres, adjacent to doublets 3 and 8 as usual in squamates, persist for a greater distance into the fibrous sheath than do the other fibres. The latter are greatly reduced or absent at the level of the annulus (Fig. 7J).

The mitochondria are large and discrete, being flattened ovoids in shape in transverse sections of the midpiece (Figs 7H, I). They are arranged in a circle around the fibrous sheath (Figs 7H, I), with partly intervening mitochondrial transformations, and extend longitudinally as moderately slender columns, the median surfaces of which are indented at intervals by triangular, sometimes longitudinally interconnected dense bodies (Figs 7N, O–Q). A maximum of nine mitochondria have been observed in a single transverse section. Mitochondrial cristae are numerous and lamellate. The midpiece terminates with an annulus (Figs 7J, O) which is approximately circular (Fig. 7O) or is irregular in cross-section.

**Principal piece.** The principal piece, as usual, consists of the axoneme, surrounding fibrous sheath and plasma membrane in the absence of mitochondria (Figs 7K, R). In its posterior region, all nine peripheral dense fibres are absent (Fig. 7K) and the plasma membrane is closely applied to the fibrous sheath, but at, and immediately behind, the annulus, a wide band of granular cytoplasm separates the two (Figs 7J, O).

**Endpiece.** The fibrous sheath is absent and the pattern of microtubules are disrupted in the endpiece (Fig. 7L).

(c) Sperm of the Pygopodidae

*Lialis burtonis.* The spermatozoa of *Lialis burtonis*, obtained from a specimen preserved for museum collections, were inadequately fixed for ultrastructural studies, and only a partial description can be given. The head is slightly curved and is very short relative to the midpiece. Aspects of the ultrastructure are illustrated diagrammatically in Fig. 8.

**Acrosome complex.** The acrosome vesicle is blunt, with the appearance of a smooth elongate dome (Figs 9A, B); it is 3.3 μm long. Transverse sections (Figs 9D–H) reveal no departure from a circular form. The vesicle is differentiated (Figs 9A, B) into a large, saccular anterior enclave which extends posteriorly as far as the apical region of the subacrosomal cone and a more electron dense posterior region which includes the acrosome 'sleeve'. A parallel-sided rod-like perforatorium extends in the subacrosomal space from the apex of the subacrosomal cone almost to the apex of the acrosome vesicle (Figs 9A, B, D). This shows longitudinal and lateral striations, giving it the appearance of being composed of a cluster of small rods. The subacrosomal cone caps and invests the nuclear point (Figs 9A, B), but a large epinuclear electron-lucent space intervenes between the tip of the nuclear point and the cone (Figs 9A, B, E). The subacrosomal cone does not project as a flange beyond the basal termination of the acrosome vesicle. Fixation does not allow determination of whether the subacrosomal cone consists of a paracrystalline matrix (but see Discussion). The subacrosomal cone is denser apically than elsewhere, but that this density constitutes a stopper-like basal plate is questionable.
Fig. 7. *Heteronotia binoei* (Gekkonidae).—A. Longitudinal section (LS) through acrosome and anterior region of nucleus.—C–F, G–J, K. Transverse sections at the levels indicated by corresponding lettered arrows in the longitudinal sections shown in Figs A, B, O and K, respectively.—L. Transverse section of the endpiece.—M. LS posterior region of nucleus and of the neck region.—N, LS portion of the midpiece, showing dense bodies.—O, LS midpiece at the annulus.—P. LS posterior region of nucleus and of the neck region.—Q. LS portion of midpiece showing helical arrangement of dense bodies.—R. LS principal piece. Scale as the bottom right scale bar, except where indicated in Q.
Fig. 8. *Lialis burtonis* (Pygopodidae). A diagrammatic representation of the spermatozoon, in longitudinal and corresponding transverse sections.

**Nucleus.** The nucleus (Figs 9A–C, F–H) consists of electron dense chromatin. It is cylindrical in shape, with a width of 0.8 μm immediately below the acrosome and nuclear shoulders, and 1.1 μm near the base, and in transverse section is circular (Figs 9F–H). The nuclear point has the form of a blunt cone. Nuclear shoulders are absent, and there is a smooth transition from acrosome complex to nucleus. There is no evidence of lacunae in the chromatin. Basally, the nucleus has a compact conical nuclear fossa which accommodates the oblique proximal centriole (Fig. 9C).

**Neck.** On each side of the proximal centriole, which is oblique relative to the distal centriole, there is a column of stacked plates resembling a mammalian striated column (not illustrated). A small amount of dense material invests the proximal and distal centrioles. The distal centriole does not project into the fibrous sheath. Mitochondria and dense bodies (mitochondrial transformations) abut on the base of the nucleus where they form an interrupted collar (Fig. 9C).

**Midpiece.** The fibrous sheath extends anteriorly into the midpiece to a short distance below the base of the distal centriole (Fig. 9C). The fibrous sheath has an annulated and, in places, oblique conformation (Figs 9C, J–L). It encloses the acrosome, the doublets of the latter being associated, as usual, with nine peripheral dense fibres. Each fibre at doublet 3 and 8 becomes enlarged at some point along the midpiece and is concomitantly displaced to contact the fibrous sheath (Fig. 9I). It is not known whether the coarse and peripheral fibres extend into the principal piece, but longitudinal sections (Fig. 9K, L, right) show no evidence of this.

The mitochondria and dense bodies surround the fibrous sheath (Figs 9I–L). The mitochondria are small subspherical structures which number approximately four or five in the transverse section of the midpiece, interspersed with dense bodies (Fig. 9I). In longitudinal section, they are very numerous and lie in single file, alternating singly or in groups with one or more dense bodies. These bodies are similar in size to the mitochondria, but are often conjoined longitudinally (Figs 9J–L). Transition of mitochondria into dense bodies has been observed. The midpiece is narrower than the nucleus. It ends posteriorly at a small annulus with an irregular ovoid cross-section (Fig. 9R).

**Principal piece.** Posterior to the midpiece, the fibrous sheath extends into the principal piece and encloses the axoneme (Figs 9K, L, right). It lacks mitochondria and dense bodies.

**Discussion**

Spermatozoa of the scincids *Ctenotus robustus*, *Carlia pectoralis*, *Cryptoblepharus virgatus*, and *Lampropholis delicata* conform with other squamate sperm in the following respects: the sperm are filiform, the acrosome vesicle is in the form of a hollow, concentrically zoned cone which basally overlies a paracrystalline subacrosomal cone which invests the tapered anterior end of the nucleus; the perforatorium is a slender rod extending anteriorly from the subacrosomal material; the midpiece terminates with an annulus; peripheral dense fibres are associated with the nine triplets of the distal centriole and the doublets of the axoneme within the midpiece; the peripheral fibres adjacent to doublets 3 and 8 are enlarged, and each forms a double structure associated with the annulated fibrous sheath; usually all nine peripheral fibres are absent from the principal piece, though in *Lampropholis delicata*, they remain well developed in its anterior region, and fibres 3 and 8 sometimes extend into the endpiece; the fibrous sheath extends anteriorly into the midpiece (squamate autapomorphy).

The sperm of *Ctenotus* resemble those of other investigated species of the Sphenomorphus group and the Egernia group (*Tiliqua*) and differ from Eugongylus group species (*Cryptoblepharus virgatus*, *Lampropholis delicata* and *Car-
Fig. 9. *Lialis burtonis* (Pygodidae). Transmission electron micrographs of spermatozoa.—A and B. Longitudinal sections of acrosome and anterior region of nucleus.—C. Longitudinal section (LS) of posterior end of nucleus and of the neck and anterior midpiece.—D–H, I. Transverse sections at the levels indicated on Figs B and C, respectively.—J. LS portion of midpiece.—K. LS junction of midpiece, with annulus, and principal piece.—L. LS (left) portion of midpiece and (right) portion of principal piece. Scale as the bottom left scale bar, except where indicated in Figs D, F and I.

*Lialis pectoralis*, in the classification of Greer (1979), in the following features: (1) the acrosome is elongate (it is relatively short in Eugongylus group species); (2) the acrosome is depressed near its tip; (3) the perforatorium is strongly oblique (it is very slightly oblique in Eugongylus group species); (4) a conspicuous laminated structure is present on each side of the proximal centriole (it is absent, though possibly represented by striated column(s) in Eugongylus group species); (5) the midpiece is shorter absolutely and relative to the nucleus; (6) the midpiece has four dense ring structures in longitudinal succession (in Eugongylus group species, mitochondrial transformations are scattered irregular dense bodies of varying sizes); (7) mitochondria between the mitochondrial transformations form columnar structures in a circle around the fibrous sheath with numerous predominantly longitudinal cristae (in Eugongylus group species mitochondria are elongate, tubular structures, with indistinct cristae, and weave between the intermitochondrial bodies); and (8) enlargement of the peripheral fibres adjacent to doublets 3 and 8 occurs, as in all squamates, but not the gross enlargement which occurs in the anterior region of the axoneme in *Carlia* and *Lampropholis*.

In a valuable study using microcomplement fixation of albumin, Baverstock and Donnellan (1990) showed the Eugongylus group to be monophyletic as is here suggested by sperm ultrastructure. They represent it as the sister group
of the Egernia groups, including *Tiliqua*, estimating separation of the Egernia and Eugongylus groups as occurring about 60 million years ago. This cannot be taken as a confirmation of the sister-group relationship between the Eugongylus and Egernia groups, however, as no representative of the Sphenomorphus group or of other squamate groups was included in their study.

The sperm of the European scincid species investigated by Furieri (1970), *Chalcides ocellatus tiligugu*, conforms closely to the description given above for the Sphenomorphus group, particularly in having a longitudinal series of four dense rings alternating with columnar mitochondria. An annulus was not described, but its absence is doubtful in view of its presence in all squamates which have been examined by the authors. Differences of *Ch. ocellatus tiligugu* from the Sphenomorphus group are the composition of the dense rings which are shown diagrammatically as each being composed of large juxtaposed granules in single file and the circular, not depressed acrosomal cross-sections. Neither of these differences is substantiated by micrographs. Carcupinio et al. (1989) describe the ring structures as “four rings of electron-dense material” and do not mention a granular composition.

For the scincid *Eumeces laticeps*, Okia (1990) described a midpiece with nine mitochondrial columns around the axoneme, a condition reminiscent of that in sphenomorphs. These “columns lie segmented by partial or complete rings of dense material” but it is not clear whether their arrangement conforms to the sphenomorph pattern.

A suite of character states here described for the Sphenomorphus and Egernia groups of the Scincidae is also seen in the teiid lizard, *Cnemidophorus sexlineatus* (see Newton and Trauth 1992). This suite includes the following states: (1) anterior depression of the acrosome; (2) the conical nuclear fossa containing dense material projecting from the proximal centriole; (3) the presence of a laminar structure extending from the pericentriolar apparatus (possibly unilaterial in the teiid, and possibly homologous with the striated column(s) in Eungonylus group sperm); (4) presence of four intermitochondrial rings alternating with columnar mitochondria; (5) the absence of sharply defined nuclear shoulders; and (6) the wide separation of the plasma membrane from the fibrous sheath in the anterior region of the principal piece, although this has not been demonstrated with certainty for *Cnemidophorus*. Evaluation of the significance of these resemblances of the sperm of the Sphenomorphus–Egernia assemblage of skinks to those of teiids must await a comprehensive parsimony analysis when further squamate data are available. It does raise the possibility that these features are synapomorphies of a larger clade, including sphenomorphs, the Egernia group, and at least some teiids. Their presence in the sperm of the Sphenomorphus group and Egernia group may therefore be symplesiomorphies for these two groups and the distinctive features of the Eungonylus group, exemplified by *Carlia pectoralis*, need not indicate that this group is more distant phylogenetically from the Sphenomorphus and Egernia groups than each is from the other. On the other hand, sperm ultrastructure does not in the present state of our knowledge, demand a closer relationship of the Eungonylus group species to sphenomorphs than to some other squamate groups, and some similarities to snake sperm and to those of the pygopod *Lialis burtonis* are discussed below. Irrespective of what may be the true relationships of the Eugongylus group, the representatives studied here (*C. virgatus, L. delicata* and *C. pectoralis*) have a highly derived sperm relative to the sphenomorphs and *Tiliqua scincoides*.

The spermatozoa of the gekkonid, *Heteronota binoei*, share the basic features of scincid sperm outlined above. Among features which are variable in skink sperm, in *H. binoei*, no epinuclear electron-lucent region has been observed; nuclear shoulders are smooth; as in sphenomorph skinks; mitochondria are large and discrete, arranged in a circle around the fibrous sheath, with intervening mitochondrial transformations, and extend longitudinally as slender columns. A feature not known in skinks is the indentation of the median surfaces of the mitochondria at intervals by triangular, sometimes longitudinally interconnected dense bodies. The sperm of *Lydodactylus picturatus, Tarentola mauritanica* and *Hemidactylus frenatus* described by Furieri (1970) are generally similar to those of *H. binoei*, but the amount of intermitochondrial material (dense bodies or putative mitochondrial transformations of the present work) is greater in *Lydodactylus picturatus* and is present in decreasing amounts in *Tarentola mauritanica* and *Hemidactylus frenatus*, respectively. The stellate arrangement of dense bodies, seen in cross-section of the midpiece, in *T. mauritania* closely resembles that in *H. binoei*. A stopper-like electron dense perforatorial plate, seen in *H. binoei*, is illustrated for *T. mauritanica* and, less clearly, *L. picturatus*. The midpiece of *Spaerodactylus cinereus* illustrated in longitudinal sections by Phillips & Asa (1993) shows a longitudinal series of four columnar mitochondria on each side (said to total 20 mitochondria for the midpiece) alternating with dense bodies, of comparable length, and the small annulus. In the absence of transverse sections, it is difficult to compare this arrangement with that of other gekkonoids but the columnar form of the mitochondria is reminiscent of that in *Heteronota*.

The spermatozoa of the pygopod *Lialis burtonis* are again like those of scincids in their chief features, as noted by Harding (1994) in a preliminary report for the pygopods *Abrasia repens, Delma tincta, L. burtonis* and *Pygopus lepidopus*. However, it has been shown above that, in *L. burtonis*, the acrosome is fore-shortened and apically domed, and the perforatorium extends virtually to its tip; nuclear shoulders are absent; the mitochondria are small subshperoidal structures, four or five in a transverse section, and very numerous in longitudinal single file, alternating singly or in groups with one or more dense bodies; dense bodies also form an uninterrupted collar around the distal centriole. The *L. burtonis* sperm shares a suite of apparently apomorphic character states of varying distinctiveness with the three Eungonylus group species (*Cryptoblepharus virgatus, Carla pectoralis* and *Lamphropholis delicata*) and with the four snakes. They are as follows: The perforatorium is square-ended rather than pointed. This is a conspicuous resemblance between the Eungonylus group species and snakes, but in *Lialis burtonis* requires confirmation from properly fixed material. The midpiece is elongate, as in snakes (Jamieson & Koehler 1994; Oliver, Jamieson & Scheltinga, in press), that in the Eungonylus group species being less elongate but strikingly longer than that of sphenomorphs or other investigated squamates. In a transverse section of the midpiece, the mitochondria in *L.
delicata appear as small rounded profiles interspersed with dense bodies (mitochondrial transformations); although they form a single layer, there is not a circlet of large juxtaposed mitochondria, the latter condition being a pleiomorphic condition seen, for instance, in sphenomorphs (e.g. Ctenotus robustus) and in Sphenodon punctatus and Chelonia (see Healy & Jamieson 1992, 1994; Jamieson & Healy 1992). However, the mitochondria in L. burtonis, though forming a single layer as in snake sperm, are not as narrow, and do not appear to form the zigzagged tubes seen in snakes. They are not irregularly interspersed with the dense bodies as they are in the Eungongylus group skinks, and they show little of the great elongation which occurs in the mitochondria of the Eungongylus group and snakes. Nevertheless, an elongated zigzag configuration much as in snake sperm is illustrated in a superficial longitudinal section of the midpiece of Aprasia repens by Harding et al. (1994), and these authors have noted a further striking similarity to snakes in the existence of a multilaminar membrane around the midpiece in their material of L. burtonis, and around the flagellum in Delma tincta. These multilaminar membranes have previously been considered unique to snake sperm (Furieri 1970; Jamieson & Koehler 1994). The co-occurrence of elongate, tubular, zigzagged mitochondria and an, albeit transient, multilaminar cell membrane in the sperm of pygopods and snakes is considered here to warrant serious consideration that these two groups of legless squamates shared a common origin.

Sperm ultrastructure thus convincingly supports monophyly of Squamata. It presents a suite of character states which characterize sphenomorphs, Tiliqua and at least some teiids, although for how extensive a clade this suite is synapomorphic, and the extent of homoplasy, have yet to be determined. It also presents a highly apomorphic suite of character states distinguishing Eungongylus group species from these taxa. No unique synapomorphies are apparent between the sperm of Heteronotia and Lialis which would support a special relationship between gekkonids and pygopods suggested by Kluge (1967, 1983, 1986) which would support a special relationship between gekkonids and pygopods suggested by Kluge (1967, 1983, 1986) and around the flagellum in Delma tincta. These multilaminar membranes have previously been considered unique to snake sperm (Furieri 1970; Jamieson & Koehler 1994). The co-occurrence of elongate, tubular, zigzagged mitochondria and an, albeit transient, multilaminar cell membrane in the sperm of pygopods and snakes is considered here to warrant serious consideration that these two groups of legless squamates shared a common origin.

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Acknowledgements

We are grateful to Mrs Lina Daddow and Chris Tudge for excellent technical assistance and to Chris Tudge and Tom Gorringe for printing the micrographs. This work was partly supported by an Australian Research Council grant to BGJM.

Abbreviations used in the Figures

\begin{itemize}
\item \textit{a} acrosome vesicle
\item \textit{an} annulus
\item \textit{as} anterior saccular enclave in acrosome vesicle
\item \textit{co} cortex of acrosome vesicle
\item \textit{d} dense cone
\item \textit{db} dense body (mitochondrial transformation)
\item \textit{dc} distal centriole
\item \textit{el} electron lucent space
\item \textit{fs} fibrous sheath
\item \textit{l} nuclear lacuna
\item \textit{lax} laminar structure
\item \textit{me} medulla of acrosome vesicle
\item \textit{mi} mitochondria
\item \textit{n} nucleus
\item \textit{nf} nuclear fossa
\item \textit{p} paracrystalline material of subacrosomal cone
\item \textit{pc} proximal centriole
\item \textit{pf} peripheral dense fibre (coarse fibre)
\item \textit{pm} plasma membrane
\item \textit{rs} ring structure
\item \textit{sc} subacrosomal cone
\item \textit{st} striated column
\end{itemize}

References


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