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ORIGINAL ARTICLE

Ultrastructural differentiation of sperm tail region in *Diplometopon zarudnyi* (an amphisbaenian reptile)

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Abstract *Diplometopon zarudnyi*, a worm lizard belongs to amphisbaenia under *trogonophidae* family. This species exists in limited areas of the Arabian Peninsula and is an oscillating digger found in sub-surface soils. The present study aimed to investigate the sperm tail differentiation in *D. zarudnyi*. Ten male adults of *D. zarudnyi* were collected from Riyadh during April–May 2011. To study the sperm tail at the ultrastructural level the testes were fixed in 3% glutaraldehyde, than post fixed in 1% osmium tetraoxide followed by dehydration in ethanol grades; samples were cleared in propylene oxide and embedded in resin. Tail formation begins by the moving of centrioles and mitochondria towards the posterior pole of sperm head. Simultaneously many microtubules of the midpiece axoneme were enclosed by a thick layer of granular material. Mitochondria of mid-piece lie alongside the proximal centriole which forms a very short neck region and possess tubular cristae internally and concentric layers of cristae superficially. During this course a fibrous sheath surrounds the axoneme of mid and principal piece. At the end dissolution of longitudinal manchette takes place. The mitochondria then rearrange themselves around the proximal and distal centrioles to form a neck region. Later, the fibrous sheath surrounds the proximal portion of the flagella. This part along with sperm head of *D. zarudnyi* provides a classical model that could be used in future for evolutionary and phylogenetic purposes of class reptilia.

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1. Introduction

Diplometopon zarudnyi (*D. zarudnyi*), known as zarudny's worm lizard, is an amphisbaenian reptile in the family *trogonophidae* and it is a monotypic within the genus Diplometopon (Al-Sadoon, 1988). This species is generally found in selected areas of Arabian Peninsula such as west Iran, south Iraq, Kuwait, Oman, United Arab Emirates and north Saudi Arabia (Arnold, 1986; Al-Johany, 1999). *D. zarudnyi*

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is an oscillating digger, normally found burrowing in low sand surfaces in open terrain and in sub-surface soils of date palm farms. This amphisbaenian is a night hunter; sometimes it occupies the empty mounds of ants and termites (Al-Dokhi et al., 2013).

Continuos research in spermiogenesis over the past several years has provided persistent ultrastructural findings in the majority of reptiles. These findings have increased the knowledge of taxon sampling and have provided phylogenetic and evolutionary trends of reptile's gamete morphology (Vieira et al., 2004; Tourmente et al., 2006; Tavares-Bastos et al., 2008; Rheubert et al., 2010a,b). In recent years, several studies have been published on spermiogenesis of reptiles such as *Sceloporus bicanthalis* (Rheubert et al., 2012), *Agama adramitana* (Dehlawi et al., 1992; Ismail et al., 1995), *Mauremys caspica* (Al-Dokhi and Al-Wasel, 2001a,b), *Acanthodactylus boskinus* (Al-Dokhi, 2006, 2012), *Ptyodactylus hasselquistii* (Al-Dokhi, 2009), and *Anolis lineatopus* (Rheubert et al., 2010a,b). Recently Al-Dokhi et al. (2013) has published a paper on sperm head differentiation of *D. zarudnyi*. Spermiogenesis within multiple species of reptiles might increase structural data sets that could provide in-depth knowledge of reproductive biology in amphisbaenians. Only few studies shed complete morphological descriptions of spermiogenesis within the Squamata (Gribbins, 2011; Rheubert et al., 2012; Al-Dokhi, 2006). Furthermore, the sperm flagellum within the class Reptilian and other vertebrates are greatly different in morphology. Rheubert et al. (2010a,b) suggest flagellum morphology is most diverse region of spermatozoa within snakes and other limbed lizards. Therefore we aimed to investigate the sperm tail differentiation in *D. zarudnyi*. The outcome of this study provides a better understanding of the morphology of sperm tail region between this lizard and that of the other reptile spices and enables us to compare miniscule changes within the reptile family.

2. Materials and methods

2.1. Animal collections and housekeeping

A total of ten adult males of the *D. zarudnyi* were captured by hand at Riyadh (24°41′N, 46°42′E) in the central region of Saudi Arabia during April 2011–May 2011. The worm lizards were caged separately and maintained for short periods in Plexi glass boxes filled with 10 cm of clean sand. The sand was sprinkled periodically with water. Meal worms and water were available *ad libitum*. The laboratory temperature was 23 ± 1.5 °C (Al-Johany, 1999; Abdel-Baki and Al-Quraishy, 2011). All animals were euthanized according to the ethical guide lines approved by the King Saud University, Riyadh; Kingdom of Saudi Arabia on Animal Care guidelines.

2.2. Tissue preparation and ultra study

The animals were euthanized by chloroform and immediately dissected to remove the testis from lizards; tissues were sliced into small pieces (1 mm^3) and fixed in 3% buffered (0.1 M sodium cacodylate buffer; pH: 7.2) glutaraldehyde for 4 h at 4 °C. Tissue specimens were than post fixed in 1% osmium tetaroxide (OsO_4) for 1.30 h. Dehydration of the fixed tissue was performed using ascending grades of ethanol and then cleared in propylene oxide before embedding in pure resin (SPI, Toronto; Canada) (Reyenolds, 1963). Ultra-thin sections (60–70 nm) were cut on an ultra-microtome (Leica, UCT; Germany) with a diamond knife (Biel, Switzerland); sections were then placed on copper grids and stained with uranyl acetate (20 min) and lead citrate (5 min). The electron micrographs were produced using a transmission electron microscope (JEOL JEM-1011) operating at 80 kV and Gathan™ software at the Research Center, King Saud University, Riyadh, Saudi Arabia. The micrographs and electron micrographs were digitized and finalized using the Adobe Photoshop software.

3. Results

An ultrastructural analysis reveals the initiation of tail differentiation in sperm of *D. zarudnyi* by migration of centrioles towards the posterior pole of the spermatid (Fig. 1A, B). At the posterior side of the nucleus a depression called the implantation fossa (IF) was present (Fig. 2A, B); which displays the 9 triplet microtubule arrangement. Simultaneously at the posterior pole mitochondria migrate away from the nucleus and accumulate around the proximal centriole and distal centriole (Figs. 1 and 2A, B). The longitudinal arrangements of mitochondria in step 1 of the spermatids gradually become spherical and the whole sperm head turned into curve shape (Fig. 2A, B). Surrounding the mitochondrial core seven or eight concentric rings were formed. Numerous manchette microtubules aggregate along the peripheral region of the nucleus and then began to assemble the axoneme of the flagellum. In completing the condensation of the nucleus, the dense and coarse granules within nucleus were fused together (Fig. 2A, B).

The midpiece had large mitochondria that have tubular cristae internally and concentric layers externally (Fig. 3A, B) which surrounding the axoneme as shown in (Fig. 4B and C-i). The midpiece axoneme has a fibrous sheath (Fig. 4B and C-i); however, the microtubule doublets of the midpiece were surrounded by dark staining granular columns (Fig. 4C-ii). The principal piece beyond the annular ring does have a fibrous sheath that was seen in cross section (Fig. 4A and C-iii), and the end piece was easily distinguished from the principal piece, as it lacks a fibrous sheath around its axoneme (Fig. 4A and C-iv).

4. Discussion

The morphological and ultrastructural features of tail region of *D. zarudnyi* were similar to those described for other amphisbaenian reptiles. The flagellum of *D. zarudnyi* develops similarly to that described in other reptiles (Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2002; Rheubert et al., 2012; Gribbins et al., 2010, 2013). The proximal centriole rests within the nuclear fossa of *D. zarudnyi* spermatids. There was no prominent neck region and no peritubular dense material around this region was found. The mitochondria of the midpiece adjoining the proximal centriole resulted in a very short neck region, which was similar to what was seen in *Barisia imbricate* (Gribbins et al. 2013); *A. boskinus* (Al-Dokhi, 2012); *Sceloporus bicanthalis* (Rheubert et al., 2012) and chelonians (Healy and Jamieson, 1992). The microtubular

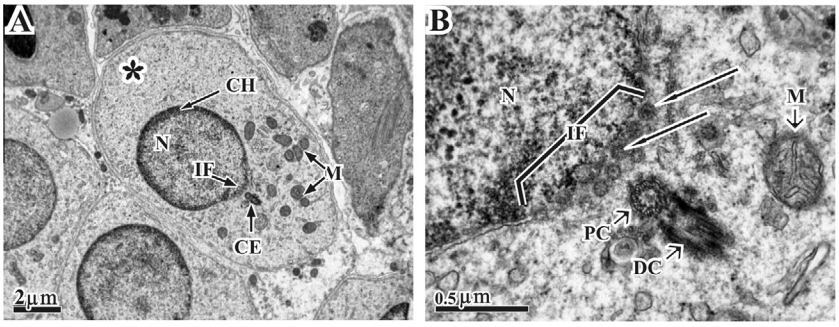

Figure 1 (A) Migration of centrioles (CE) towards the posterior pole of the spermatid. At the base of the nucleus (N) a depression called implantation fossa (IF) is present. At the posterior pole of the head, mitochondria (M) move backward and accumulate around the proximal centriole (PC) and distal centriole (DC). In the nucleus (N) the chromatin (CH) is distributed towards the nuclear membrane. The cell is filled with cytoplasm and other cell inclusions (*). (B) Enlargement of (A); focusing on implantation fossa (IF) which is present at the caudal end of the nucleus. At implantation fossa (IF) large number of mitochondria are seen as indicated by arrows. Below the implantation fossa rearrangement of proximal centriole (PC) and distal centriole (DC) is taking place.

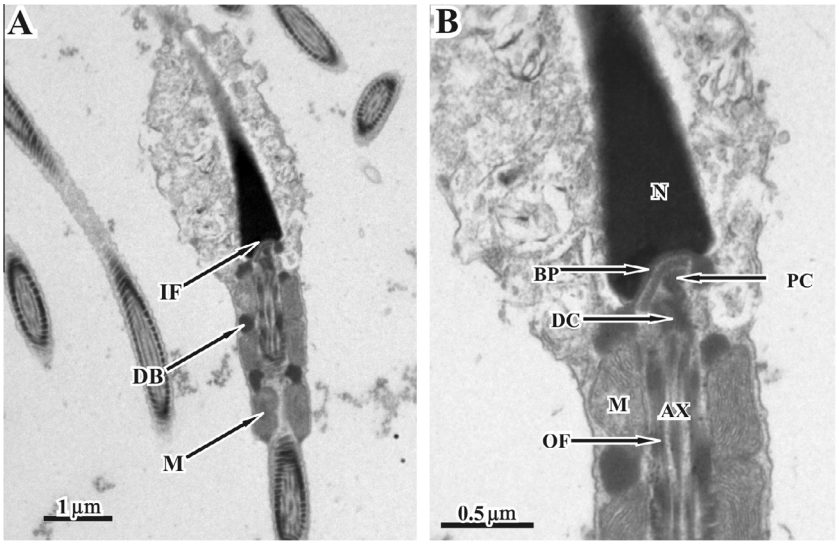

Figure 2 (A) A longitudinal section of spermatozoa showing implantation fossa (IF), dark bodies (DB) and at the mid piece region large number of mitochondria (M) are seen. (B) A whole spermatozoa is shown with a basal plate (BP), distinct proximal centriole (PC) and distal centriole (DC). At the mid piece large mitochondria (M) are seen. An axoneme is seen at the centre (AX) and outer fibre (OF) at the peripheral.

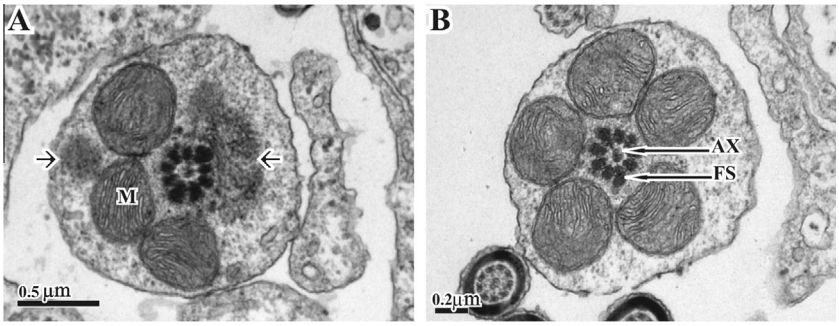

Figure 3 (A and B) Cross sections of tail region showing axoneme (AX), a fibrous sheath (FS) and large mitochondria with tubular cristae surrounded by concentric layers of cristae.

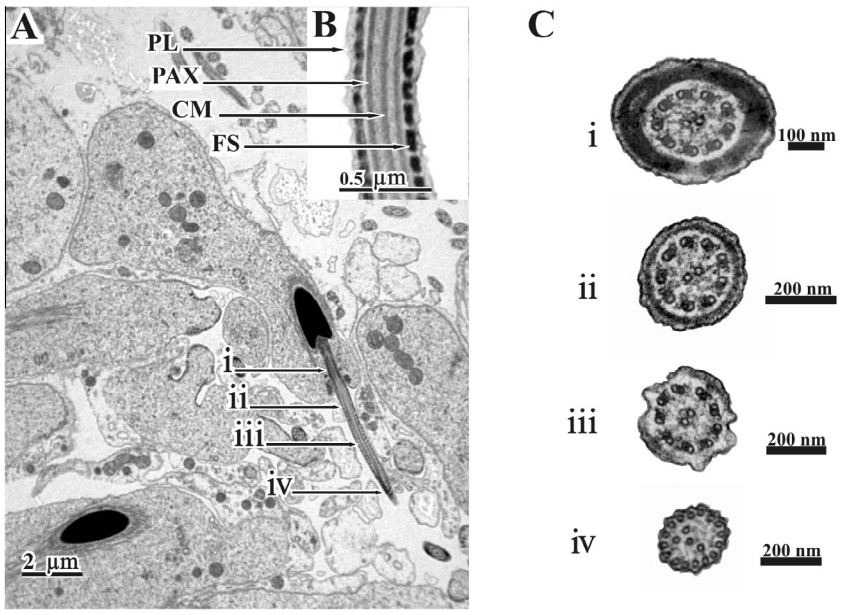

Figure 4 (A) An entire spermatozoon is shown with all its distinct features. In the same spermatozoa, tail has been marked as (i, ii, iii and iv) showing the different regions which are enlarged in (C). (B) Inset of the (A) an enlarge portion of longitudinal section of tail region is shown representing all its internal features such as plasma lemma (PL); fibrous sheath (FS); peripheral of axonemal complex (PAX) and central microtubules (CM). (C) Cross sections of tail region at different intervals with distinct features. In section (i) tail region with outer plasma lemma, thick fibrous sheath and the axoneme is seen. The microtubules are arranged in 9 + 2. In section (ii) tail region with outer thin plasma lemma, thin fibrous sheath and the axoneme are seen and same microtubules are arranged as shown in section (i). In section (iii) only axonemal complex sheath is surrounded by plasma lemma and in section (iv) axonemal complex sheath without plasma lemma are seen.

doublets of the midpiece axoneme were enclosed by a layer of dense staining granular material that was similar to that observed within the midpiece axoneme of *Sceloporus bicanthalis* (Rheubert et al., 2012); Caiman (Saita et al., 1987) and *A. boskinus* spermatids (Al-Dokhi, 2012). These dense staining materials around the microtubules of the midpiece were comparable to the striated columns found within mammalian spermatozoa (Lindemann, 1996; Rheubert et al., 2012) and most likely aid to strengthen the midpiece axoneme. The mitochondria of the midpiece, at least in the late elongating spermatids of *D zarudnyi*, have tubular cristae internally and concentric layers of cristae superficially. There are typically 7–9 concentric rows of mitochondria in a sagittal section of the *D zarudnyi* midpiece and 7–8 concentric mitochondria within each row in cross section. These numbers seem to be consistent with what has been observed in other species of reptilians (Rheubert et al., 2012; Saita et al., 1987; Al-Dokhi, 2012). The concentric layers of cristae were also observed within chelonians (Healy and Jamieson, 1992) and Sphenodon (Healy and Jamieson, 1994). It is possible that these concentric rings of cell membrane may provide energy for sperms to survive long and short periods of time within the female reproductive tract before fertilization. The midpiece ends at the annulus in *D. zarudnyi* spermatids as that described for many reptilian (Gribbins et al. 2013; Rheubert et al., 2012; Ferreira and Dolder, 2002). The end piece in *D. zarudnyi* is easily distinguished from the principal piece, as the axoneme is not surrounding by the fibrous sheath. Although the tail differentiation in *D. zarudnyi* were related to what has been described in other reptilians (Rheubert et al., 2012). The ultrastructural feature of the tail region of *D. zarudnyi* is to be unique, because it contains compound mitochondria with concentric layers of cortical cristae at the midpiece. *D. zarudnyi* spermatid is also having a distinguish endonuclear canal as reported in our recent publication (Al-Dokhi et al., 2013), which is present in some reptiles such as Sphenodon, chelonians, and archosaurs. These characteristic features have not been reported in major reptilians. The importance of such differences and similarities in the structure of the reptilian spermatids between closely and distantly related species is unknown as there are no sufficient studies from other reptilians. Until further, morphological date's produced from spermiogenesis within reptilians, answers to these questions will remain primitive and incomplete.

5. Conclusion

Observations on sperm tail differentiation of *D. zarudnyi* were of great significance, because that could be used to correlate the similarities within reptile phylogeny and may possibly be used as a supportive link in future studies to other limbless lizards.

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