Ultrastructure of the spermatozoon of the Chinese water snake, *Myrrophis (Enhydris) chinensis* (Reptilia: Homalopsidae)

S.-L. HAO¹,² & Y.-P. ZHANG*¹

¹College of Life and Environmental Sciences, Wenzhou University, Wenzhou, China, and ²The Sperm Laboratory, College of Life Sciences, Zhejiang University, Hangzhou, China

(Received 29 October 2017; accepted 23 July 2018)

Abstract
Identifying the polymorphism of the spermatozoon ultrastructure of squamates is vital for improving the accuracy of phylogenetic analyses. Multiple studies have been conducted to distinguish the similarities and differences among the sperms of squamates, but these studies have mainly focused on lizards. Thus, there is a need to update the database of the ultrastructure of snake spermatozoa. *Myrrophis chinensis* is a protected snake in China because of its economic and scientific value. In this study, we examined the ultrastructure of the *M. chinensis* epididymal spermatozoon. The ultrastructure of the *M. chinensis* spermatozoon was found to have the following characteristics: dense collar, multilaminar membranes, extracellular microtubules and extraordinary elongation of the midpiece, all of which are typical sperm characteristics of the synapomorphies of Serpentes. Although no unique trait was identified, the current findings could still provide valuable data for phylogenetic analysis.

Keywords: *Myrrophis chinensis*, spermatozoa, ultrastructure

Introduction

*Myrrophis (Enhydris) chinensis*, a member of *Myrrophis* (Olivier et al. 2008; Cai et al. 2015), is a Chinese water snake that is mainly found in East, South and Central China, including Hunan, Jiangsu, Anhui, Zhejiang, Fujian, Guangdong and Taiwan (Zhao et al. 1998). It is an ovoviviparous and poisonous snake. *Myrrophis chinensis* is a protected species in China because of its important economic and scientific value. It is currently listed in the Lists of Terrestrial Wildlife under State Protection. The animal is vulnerable to changes in the environment, and details of this vulnerability in relation to the specific kinds of environmental changes have been recorded in the “Red List of China’s Vertebrates”, which contains a comprehensive assessment of the endangered species of wild vertebrates in China (Jiang et al. 2016). Information obtained from the reproductive studies of *M. chinensis*, including the investigation of the spermatoza, would therefore be beneficial to the strategy of wildlife conservation aiming at saving this species from extinction. This kind of information may also provide some valuable data for evaluating the economic importance of this species.

Spermatozoa are highly differentiated reproductive cells and they possess complicated compartments, which include the acrosomal complex, nucleus, midpiece and flagellum (Callard & Callard 1999). The morphology of a spermatozoon is also linked to its swimming speed and fertilization ability. The analysis of sperm morphology can help to characterize the sperm and determine its fertilization potential (Tuset et al. 2008). Additionally, since the reproductive modes and strategies of the Serpentes are very diverse, their reproductive biology has been the focus of researchers for decades (Shine 2003). In this respect, variations in the morphology of Serpentes sperms are likely to be related to the reproductive characteristics of the animals (Vieira et al. 2004), such as the fertilization process, the position of sperm storage in the female reproductive tract (Server & Hamlett 2002; Cunha et al. 2008), and the mechanism of sperm release (Girling 2002). Thus, more research on the detail of sperm morphology would be of great value to uncover the biological mechanisms of fertilization. This information would also be useful for reproductive energetic research, and for improving our understanding of snake reproductive biology.

*Correspondence: Y.-P. Zhang, College of Life and Environmental Sciences, Wenzhou University, Wenzhou 325035, China, Email: zhangyp@wzu.edu.cn
© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
In the past three decades, ultrastructural descriptions of spermatozoa in amniotes have been applied to phylogenetic analysis (Jamieson 1995a; Jamieson et al. 1996; Oliver et al. 1996; Robson et al. 1997; Teixeira et al. 1999a,b,c), where significant polymorphism displayed by sperm ultrastructure has been found to improve the accuracy of phylogenetic analyses (Erséus 1999; Vieira et al. 2005, 2006). Polymorphism in the sperm ultrastructure has been detected, not only among different genera of the same family, but also among congeneric species, such as Tropidurus (Teixeira et al. 1999d), Tupinambis (Tavares-Bastos et al. 2002), Gekko (Hao et al. 2015) and Ramphotyphlops (Harding et al. 1995). Therefore, phylogenetic analysis based on sperm ultrastructural characteristics might provide an additional method to deal with the polymorphic traits in phylogenetic reconstructions.

Sperm ultrastructure has been reported for several species in the Serpentes. Austin (1965) published the first detailed description of the ultrastructure of sperm tails from Lampropeltis getulus, Coluber constrictor, Drymarchon corais, Crotalus adamanteus (Colubridae), Micrurus fulvius (Elapidae) and Constrictor sp. (Boidae). Subsequently, multiple studies have been conducted to distinguish the similarities and differences among the sperm in Serpentes, including Colubridae (Austin 1965; Hamilton & Fawcett 1968; Furieri 1970; Rheubert et al. 2010), Boidae (Tourmente et al. 2006; Tavares-Bastos et al. 2008), Viperidae (Furieri 1970; Cunha et al. 2008; Tourmente et al. 2008), Elapidae (Austin 1965), Typhlopidae (Harding et al. 1995; Tavares-Bastos et al. 2007), Leptotyphlopidae (Tavares-Bastos et al. 2007) and Anomalepididae (Tavares-Bastos et al. 2007). Even so, the sperm ultrastructure database still needs to be enriched and updated.

The aim of this research is to present a detailed description of the ultrastructure of the M. chinensis spermatozoon. The ultrastructural characteristics of the M. chinensis spermatozoa were compared with the ultrastructural characteristics of the sperm of other Serpentes to explore their similarities and differences. This will not only provide supporting evidence for the phylogenetic analysis of reptiles but also offer some basic information for further study on the molecular mechanism of reptile spermatogenesis and fertilization.

**Material and methods**

**Collection of sperm samples**

A total of five M. chinensis individuals were caught in May 2015 in Wenzhou (27°23′N, 119°37′E), Zhejiang Province, China. The animals were euthanized by intramuscular injection with a lethal dose of sodium thiopental (Hubei Xinkang Pharmaceutical Chemical Co., Ltd.) to minimize animal suffering. The epididymides were then extracted from the dead animals and placed in a petri dish containing phosphate-buffered saline solution (PBS, pH 7.2, Shanghai Zhanyun Chemical Co., Ltd.), and then minced to yield pieces less than 1 mm in length.

**Light microscopy (LM)**

Part of the minced epididymis was transferred into a microcentrifuge tube and gently homogenized in PBS using a glass rod. One drop of the homogenate was spread on a glass slide and allowed to dry in air. The slide was stained with 1% toluidine blue (Shanghai Lanji Technology Development Co., Ltd.) for 2 min and then washed with distilled water. The general morphology of the entire sperm was observed under a light microscope (Olympus BX51, Japan) and the images were captured with a Charge-coupled Device (CCD) camera (Olympus DP71, Japan). The lengths of the head and the entire sperm were recorded with a micro-ruler (Shanghai Taiyi Medical Apparatus Equipment Co., Ltd.) under a light microscope (Olympus BX51, Japan).

**Transmission electron microscopy (TEM)**

The remaining portion of the minced epididymis was fixed in 2.5% glutaraldehyde solution (Yonghua Chemical Technology Co., Ltd., Jiangsu) at 4°C overnight. Next, it was rinsed with 0.1 M PBS (pH 7.2) and then fixed in 1% osmium tetroxide (SPI Company) for 1 h. After that, it was washed twice with double-distilled water (5 min each), stained with 1% uranyl acetate (State-owned 404 factory in Lanzhou, China) for 2 h, and then dehydrated with a series of graded acetone (70, 80, 90 and 100%; Suzhou Hualida Fine Chemical Co., Ltd.), and subsequently embedded in epoxy resin (Ted Pella, Inc).

The embedded samples were sliced into ultrathin slices (90 nm thick) using an ultramicrotome (RMC PowerTomeXL). These slides were mounted on 200 mesh copper grids, stained with 1% lead citrate (Ted Pella, Inc) for 3 min, and allowed to dry at 45°C for 24 h in an oven (DHG 9245A, Ningbo Laifu Technology Co., Ltd.). The structural details of the specimens were observed and photographed with a transmission electron microscope (TEM; H7500, Hitachi) at various magnifications. The length and width of the acrosome complex and the diameter of the neck region were measured from the micrographs at different
magnifications following the techniques suggested by Zhang et al. (2006).

Results

Morphology of spermatozoon

The spermatozoon of *M. chinensis* is filiform, with a total length of about 111 μm as seen under a light microscope. It is clearly divided into head and tail (Figure 1A). The head is about 11.16 μm in length and 0.73 μm in diameter. It is narrow, elongated and slightly curved, and contains the acrosomal complex and nucleus (Figure 1B). The tail consists of the neck region, midpiece, principal piece and endpiece. The midpiece is extremely long and accounts for nearly half the length of the sperm. The principal piece is much thinner and is slightly shorter than the midpiece.

Ultrastructure of spermatozoa

Acrosomal complex. The acrosome complex is slightly curved and cone-shaped, with a length of about 3.2 mm, which covers the anterior portion of the nucleus (Figure 1B). The acrosome complex consists of two caps, an external acrosomal vesicle (av) and an internal subacrosomal cone (sc). The acrosomal vesicle consists of an outer cortex (co) surrounding a central medulla (me) (Figure 1C and D). The posterior extremity of the acrosomal vesicle is a thin and sleeve-shaped structure that extends almost to the nuclear shoulder (ns) (Figure 1B). The subacrosomal cone is separated from the acrosomal vesicle by an electron lucent area, the subacrosomal space (ss) (Figure 1B and E). Under the acrosomal medulla, the subacrosomal cone, which has a paracrystalline appearance, encloses the entire nuclear rostrum (Figure 1B and E–G). A posterolateral flange appears behind the base of the acrosome vesicle (Figure 1B, black arrow). At the apex of the subacrosomal cone, a slender and rod-shaped perforatorium (p) extends into the acrosomal medulla (Figure 1B and D). The shape of the perforatorium tip could not be observed clearly and the perforatorium baseplate is not visible. Anterior to the nuclear rostrum (nr), a narrow electron lucent area called the epinuclear lucent zone (ep) appears within the subacrosomal cone (Figure 1B and F). The acrosome complex is circular and lacks a unilateral ridge as shown by the cross-section (Figure 1C–G).

Nucleus. The nucleus is elongated and slightly curved, and it is composed of homogeneous and highly electron-dense chromatin. The nuclear lacuna is absent in the nucleus (Figure 1H). The anterior pole of the nucleus gradually becomes elongated and thin, forming a tapered nuclear rostrum that extends to the subacrosomal cone (Figure 1B and G). The round nuclear shoulder is present at the base of the nuclear rostrum (Figure 1B). The posterior end of the nucleus is marked by a slightly semicircular indentation, the nuclear fossa (nf), where the anterior fraction of the proximal centriole is located (Figure 1J and L). The entire nuclear body is surrounded by a circumferential multilaminar structure (ml) rather than a single-layer plasma membrane (Figure 1H). Extracellular microtubules (et) are also present outside the multilaminar structure (Figure 1J).

Neck region. The neck region has a diameter of about 0.72 μm and it comprises the proximal (pc) and distal centrioles (dc), dense collar (dco) and pericentriolar material (pcm) (Figure 1I, K, L, 2A and B). The neck region is also called the connecting region. It connects the posterior end of the nucleus and the anterior extremity of the midpiece. The dense structure within the center of the proximal centriole (dp) is obviously present (Figure 1I). The proximal centriole is surrounded by the pericentriolar material, a narrow zone of electron-dense material (Figure 1L). The pericentriolar material located in the nuclear fossa forms the capitulum (ca) (Figure 1L) and the laminar structure (ls) is linked to the pericentriolar dense area (Figure 2B).

The distal centriole lies posterior to the proximal centriole at approximately 90°. The distal centriole consists of two central singlets of microtubules and nine triplets of microtubules, and it forms the basal body at the start of the axonemes (Figure 1K). A dense collar surrounds the distal centriole (Figure 2A and B). Nine peripheral dense fibers are located at the inner surface of the dense collar (Figure 1K). No indented mitochondrion is found outside the dense collar (Figure 2A and B).

Midpiece. The midpiece consists of the mitochondrial sheath, axoneme (ax) and fibrous sheath (fs), as well as the neck region described above. Its posterior end is marked by the annulus (an) (Figure 2H). A plasma membrane is present throughout the midpiece, and it consists of a tortuous and un-compact multilamellar membrane structure (ml) (Figure 2B, E and F). The outpouch of the multilaminar membrane (op) is also visible (Figure 2A and G). There are extracellular microtubules (et) scattered outside the multilaminar membranes of the midpiece (Figure 2D). The axoneme is enclosed by a helically arranged fibrous sheath that begins at mitochondrial tier 2 (Figure 2A and B). It consists of the peripheral dense fibers (pf) and axoneme doublets (Figure 2B).
Figure 1. Microscopy analysis of *Myrrophis chinensis* spermatozoon. (A) Image of the whole sperm, taken with a light microscope. (B–L) Ultrastructure of the sperm, as observed by transmission electron microscopy: (B) Longitudinal section of the anterior region of the head showing acrosome vesicle, subacrosomal cone, subacrosomal space, perforatorium, epinuclear lucent zone, nuclear rostrum, nuclear shoulder and posterolateral flanges (black arrows). (C) Cross section through the apical portion of the acrosome vesicle showing the plasmalemma, acrosome cortex and acrosome medulla. (D) Cross section through the perforatorium showing the plasmalemma, perforatorium, acrosome cortex and acrosome medulla. (E) Cross section through the anterior region of the epinuclear lucent zone showing the plasmalemma, acrosome vesicle, subacrosomal cone and subacrosomal space. (F) Cross section through the anterior region of the subacrosomal cone showing plasmalemma, acrosome vesicle, subacrosomal cone, epinuclear lucent zone. (G) Cross section through the nuclear rostrum showing plasmalemma, acrosome vesicle, subacrosomal cone, nuclear rostrum. (H) Cross section through the nucleus showing nucleus and multilaminar membrane. (I) Longitudinal section of the neck region showing multilaminar membrane, neck cylinder, proximal centriole, distal centriole, dense structure within the center of the proximal centriole and peripheral dense fiber. (J) Transverse of the nucleus showing nucleus and nuclear fossa. (K) Transverse of the neck region showing multilaminar membrane, microtubules, dense collar and peripheral dense fiber. (L) Longitudinal section of the neck region showing nuclear fossa, caputim, proximal centriole, distal centriole, pericentriolar material, dense collar, peripheral dense fiber, mitochondria and fibrous sheath. av: acrosome vesicle; ca: caputim; co: cortex; dc: distal centriole; dco: dense collar; dp: dense structure within the center of the proximal centriole; ep: epinuclear lucent zone; et: extracellular microtubules; fs: fibrous sheath; ls: laminar structure; H: head; me: medulla; mi: mitochondria; ml: multilaminar membrane; n: nucleus; nf: nuclear fossa; nr: nuclear rostrum; ns: nuclear shoulder; p: perforatorium; pc: proximal centriole; pcm: pericentriolar material; pf: peripheral dense fiber; pm: plasmalemma; sc: subacrosomal cone; ss: subacrosomal space; T: tail.
and D). The axoneme is characteristically arranged in a 9 + 2 pattern (Figure 2D and G). The peripheral dense fibers at doublets 3 and 8 are thicker than the other fibers and are detached from their corresponding doublets (Figure 2D and G). Outside the fibrous sheath, the mitochondrial sheath contains the mitochondria and dense bodies (Figure 2B, D and F). In the sagittal longitudinal sections (SLS), the small round mitochondria are arranged neatly with the dense bodies encircling the fibrous sheath (Figure 2B and F), while in the parasagittal longitudinal sections (PLS) near the plasma membrane, the mitochondria are arranged in a zig-zag formation (Figure 2E and H). In cross section, the mitochondria appear oval and contain linear cristae (Figure 2D and G). The annulus, an electron-dense structure, appears at the end of the midpiece, with an irregular shape as seen in the longitudinal section (Figure 2F).

**Principal piece.** The principal piece starts at the region behind the annulus. It comprises the plasma membrane and axoneme and is surrounded by the fibrous sheath (Figure 2K). Compared to the midpiece, the principal piece has a much smaller diameter. The axoneme maintains the 9 + 2 microtubule arrangement, but the enlarged peripheral fibers 3 and 8 found in the axoneme are no longer present (Figure 2J and K). The fibrous sheath extends throughout the principal piece but gradually becomes thinner (Figure 2I). The multilaminar membranes, dense bodies and mitochondria are absent (Figure 2I–K). However, the extracellular microtubules (et) and the wide zone of granular cytoplasm (cy) are still present in the anterior portion of the principal piece (Figure 2J).

**Endpiece.** The endpiece comprises the plasma membrane and axoneme (Figure 2L and M). The fibrous sheath fades away at the junction between the principal piece and the endpiece (Figure 2N). The plasma membrane is closely associated with the axoneme. At the proximal section of the endpiece, the axoneme still shows the 9 + 2 structure, but gradually becomes disarranged toward the distal end (Figure 2L).

**Discussion**

**Comparison with other amniotes**

The ultrastructure of *M. chinensis* spermatozoon possesses the general characteristics found in the spermatozoa of all amniote classes (reptiles, birds and mammals), including a pointed-form acrosome, the presence of the subacrosomal cone, and the tapering of the tip of the cylindroid nucleus (Jamieson 1995a). A single wholly prenuclear perforatorium is present, while the endonuclear canal is absent in *M. chinensis* as in other species of Squamata (i.e. snake and lizard) (Jamieson 1995a; Oliver et al. 1996; Hao et al. 2015; Blenginia et al. 2017). However, in Sphenodontida, Chelonia and Crocodilia, one to three perforatoria usually penetrate the endonuclear canals (Hess et al. 1991; Healy & Jamieson 1994; Jamieson et al. 1997). In contrast, the perforatoria and endonuclear canals are absent in the sperm of Passeriformes (Jamieson et al. 2006), Piciformes (Henley et al. 1978), Apodiformes (Jamieson & Tripepi 2005), Charadriiformes (Saita et al. 1983) and Columbiformes (Jamieson et al. 2006). The perforatorium and endonuclear canal are typically present in the palaeognaths (Soley 1999), non-passerines and Galloanserae (Jamieson et al. 2006). The perforatorium also varies among the spermatozoa of mammals. For example, it is found in rats (Breed 2005) and bats (Beguelinia et al. 2011), but not in agoutis (Arroyo et al. 2015) or tree shrews (Jeong et al. 2006). The endonuclear canal characteristic of mammalian spermatozoa (Jamieson 1995a) is absent. The absence of the nuclear lacuna in Squamata spermatozoa is considered an ancestral characteristic of Squamata (Gribbins & Rheuert 2014), and this is also reflected in *M. chinensis* spermatozoa.

The proximal centriole is present in all amniote classes but is absent or degenerated in some therian mammals (Jamieson 1995a; Arroyo et al. 2015). The distal centriole extends through the midpiece in turtle, tuatara, crocodilians, and ratites, but it is short in squamates, and vestigial in mammals (Jamieson et al. 1997). In *M. chinensis*, the fibrous sheath extends into the anterior region of the midpiece, which is also seen in other squamates (Jamieson 1995a; Oliver et al. 1996; Hao et al. 2015; Blenginia et al. 2017). However, the fibrous sheath does not enter the midpiece in other amniotes (Jamieson 1995a; Jamieson & Tripepi 2005) and is even absent in some species of birds such as parrots and doves (Jamieson et al. 1995).

In *M. chinensis*, the peripheral fibers are present in the midpiece, but in amniotes such as turtles (Bian et al. 2013), crocodiles (Jamieson et al. 1997), tuatara (Jamieson & Healy 1992), ratites (Soley 1993), non-passerines (Jamieson et al. 1995) and monotremes (Carrick & Hughes 1982), the peripheral fibers are not restricted to just the midpiece, as some of them also extend into the principal piece. In *M. chinensis*, peripheral fibers 3 and 8 are enlarged and detached from their corresponding doublets, which are regarded as the synapomorphies of Squamata (Harding et al. 1995; Jamieson 1995b, 1999).
Figure 2. Posterior view of a mature spermatozoon from *Myrrophis chinensis*. (A) Longitudinal section of the neck region showing the dense collar, peripheral dense fiber, outpouch of multilaminar membranes, mitochondria and fibrous sheath. (B) Longitudinal section of the neck region and midpiece showing nuclear fossa, laminar structure, dense collar, proximal centriole, distal centriole, peripheral dense fiber, fibrous sheath, mitochondria and axoneme. (C) Cross section through the neck region showing multilaminar membrane, mitochondria and peripheral dense fiber. (D) Cross section through the midpiece showing the microtubules, multilaminar membrane, mitochondria, fibrous sheath, peripheral dense fiber and dense body. (E) Longitudinal section of the midpiece showing dense body, mitochondria and multilaminar membrane. (F) Longitudinal section of the midpiece showing axoneme, fibrous sheath, dense body, mitochondria and multilaminar membrane. (G) Cross section through outpouch of multilaminar membranes of the midpiece showing outpouch of multilaminar membranes, multilaminar membrane, dense body, peripheral dense fiber, mitochondria and fibrous sheath. (H) Longitudinal section of the midpiece and principal piece, showing multilaminar membrane, mitochondria, annulus, fibrous sheath and axoneme. (I) Longitudinal section of the principal piece showing axoneme and fibrous sheath. (J) Cross section through the anterior region of the principal piece showing microtubules, fibrous sheath and cytoplasm. (K) Cross section through the middle region of the principal piece showing fibrous sheath and plasmalemma. (L) Longitudinal section of the endpiece, showing axoneme. (M) Cross section through the endpiece showing axoneme. (N) Cross section through the junction between the principal piece and endpiece showing the axoneme. an: annulus; ax: axoneme; cy: cytoplasm; db: dense body; dc: distal centriole; dco: dense collar; et: extracellular microtubules; fs: fibrous sheath; ls: laminar structure; mi: mitochondria; ml: multilaminar membrane; mt: microtubules; nf: nuclear fossa; op: outpouch of multilaminar membranes; pc: proximal centriole; pf: peripheral dense fiber; pm: plasmalemma.
The mitochondria in *M. chinensis* spermatozoa have a sinuous appearance marked by linear cristae, which is a typical trait in squamates (Jamieson 1995b). However, in turtle, tuatara and crocodile spermatozoa, the mitochondria are spherical or subpherical in shape with concentric cristae (Hess et al. 1991; Healy & Jamieson 1994; Jamieson et al. 1997). In *M. chinensis*, the granular cytoplasm zone is found at the anterior part of the principal piece, which is similar to some lizard species (Giugliano et al. 2002; Teixeira et al. 2002; Colli et al. 2007). The dense collar, multilaminar membranes, extracellular microtubules and extraordinary elongation of the midpiece found in *M. chinensis* spermatozoa are distinguishing features between *M. chinensis* and Lacertilia spermatozoa, and they may belong to the synapomorphies of Serpentes (Harding et al. 1995; Oliver et al. 1996; Jamieson 1999; Tavares-Bastos et al. 2007).

The polymorphism of ophidian sperm ultrastructure

The polymorphic traits of sperm ultrastructure between families, genera and even congeneric species have been documented for the Serpentes. Table I summarizes the main features of the ultrastructure of spermatozoa from 20 snake species, including *M. chinensis*, which is described here for the first time. The acrosome vacuity subdivision is absent in the acrosomal vesicle of all the species listed in Table I, except for *Ramphotyphlops waitii* (Harding et al. 1995). The acrosomal ridge is commonly seen in some lizards such as Teiidae (Colli et al. 2007), Hoplocercidae and Opluridae (Vieira et al. 2007), but is absent in *M. chinensis*. It is also absent in other Colubridae species, but is present in *Liophyphlops beui* (Tavares-Bastos et al. 2007) and *Boa constrictor amarali* (Tavares-Bastos et al. 2008). The spermatozoon of *M. chinensis* contains a nucleus consisting of an electrodense structure without the lacunae, a typical trait found in the spermatozoon of many snakes, but which is not found in some species like *Crotalus durissus* (Cunha et al. 2008), *Agkistrodon contortrix* (Rheubert et al. 2017), *Leptotyphlops koppesi*, *Typhlops reticulatus* and *L. beui* (Tavares-Bastos et al. 2007). The multilaminar membrane and extracellular microtubules are present in almost all snakes, except in *Bothrops alternatus* and *Bothrops diporus*, and remain undetermined in *L. beui* (Tavares-Bastos et al. 2007). However, the location of the multilaminar membrane and extracellular microtubules in the mature sperm vary for different species (Table I). In cross section, the anterior region of the acrosome complex is circular in the case of *M. chinensis*, but is flattened in the case of *Typhlops reticulatus*, *Epicrates cenchria*, *Boa constrictor amarali* and *Corallus hortulanus* (Table I). The absence of a stopper-like perforatorial baseplate characteristic of the autapomorphy of Serpentes spermatozoa (Tourmente et al. 2008) has not been found in all Serpentes species. The epinuclear electron-lucent zone is present in all snakes listed in Table I, except in *R. waitii* and *B. constrictor occidentalis* (Tourmente et al. 2006; Tavares-Bastos et al. 2007). In *Aspidites melanocephalus*, this structure has not been ascertained (Oliver et al. 1996).

Interestingly, the dense collar is absent in *Agkistrodon piscivorus* (Gribbins et al. 2010). In *C. durissus* (Cunha et al. 2008), *Oxyuranus microlepidotus*, *Boiga irregularis* (Oliver et al. 1996) and *S. pygaea* (Rheubert et al. 2010), no data on the sinuous tubular mitochondria and its zigzagged arrangement was available to show the parasagittal longitudinal sections of the spermatozoa. Intermitochondrial dense bodies are present in all the snakes listed in Table I except for *Bothrops* and *Agkistrodon*. The starting location of the fibrous sheath is quite variable. In *M. chinensis*, the fibrous sheath appears to originate at mitochondrial tier 2, consistent with *Crotalus durissus* (Cunha et al. 2008). In contrast, the fibrous sheath in *Agkistrodon contortrix* starts at mitochondrial tier 4 (Rheubert et al. 2017), and at tier 1 in most snakes, such as *Colubroidea* (Rheubert et al. 2010), *Scolecodphidia* (Tavares-Bastos et al. 2007), *Viperidae* (Tourmente et al. 2008) and *Boidae* (Tavares-Bastos et al. 2008). Peripheral fibers 3 and 8 in *M. chinensis* extend beyond the midpiece, a feature also observed in many other snakes (Jamieson & Koehler 1994; Rheubert et al. 2010). The shape of the annulus in the sagittal longitudinal sections is diverse, including linear, triangular, irregular and oval shapes. Finally, among all the reported snakes, the granular cytoplasm zone at the anterior part of the principal piece in *M. chinensis* has only been observed also in *Typhlops reticulatus* (Tavares-Bastos et al. 2007).

Apparent, as indicated above, polymorphisms in Serpentes spermatozoa are determined by the following ultrastructural characteristics: location of multilaminar membrane, extracellular microtubules, and peripheral fibers 3 and 8; and absence or presence of acrosome vacuity subdivision, acrosomal ridge, nuclear lacuna, perforatorium baseplate, epinuclear electron-lucent zone, dense collar, dense body and granular cytoplasm zone. In addition, the shape of the acrosome complex, mitochondria and annulus also contributes to the polymorphisms in Serpentes spermatozoa.

The present study provides the first description of the ultrastructure of a Homalopsidae sperm. At present, sperm ultrastructure has only been described for about 20 snake species, but as many as 3691 snake species (26 families) are known worldwide (http://www.reptile-data base.org/). Therefore, further work on other Serpentes is necessary to reduce this information gap. Furthermore, since sperm ultrastructure has been introduced to increase the accuracy of phylogenetic analysis in
Table I. Comparison of sperm ultrastructure in species belonging to Serpentes.

| Species | Acrosome vacuity | Acrosomal ridge | Nuclear lacuna | Multilaminar membrane | Extracellular microtubules | Acrosome complex in TS | Perforatorium baseplate | Eptiuclear electron-lucent zone | Laminated structures |
|---------|------------------|-----------------|----------------|------------------------|--------------------------|------------------------|--------------------------|-------------------------------|------------------------|
| Anomalepididae | Liotrephlops beui | Absent | Present | Present | ? | ? | Circular | Present (stopper-like) | Present | Present |
| Leptotyphlopidae | Leptotyphlops koppesi | Absent | Absent | Present | Present (nucleus to annulus) | Present (all along the sperm) | Circular | Present (stopper-like) | Present | Present |
| Typhlopidae | Ramphotyphlops varius | Present | Absent | Absent | Present (nucleus to annulus) | Present | Circular | Absent | Absent | Absent |
| Typhlops reticulatus | Absent | Absent | Present | Present (nucleus to annulus) | Present (all along the sperm) | Flattened | Present (stopper-like) | Present | Present | Present |
| Boidae | Boa constrictor amarali | Absent | Present | Absent | Present (midpiece) | Present (midpiece) | Flattened | Present (stopper-like) | Present | Present |
| | Boa constrictor occidentalis | Absent | Absent | Absent | Present (midpiece) | Absent | Circular | Absent | Absent | Present |
| | Corallus hortulanus | Absent | Absent | Absent | Present (midpiece) | Present (midpiece) | Flattened | Absent | Present | Present |
| | Epicrates cenchria | Absent | Absent | Absent | Present (midpiece) | Present (midpiece) | Flattened | Present (stopper-like) | Present | Present |
| Pythonidae | Aspidites melanocephalus | Absent | Absent | Absent | Present (midpiece) | Present (all along the sperm) | Circular | Absent | ? | Present |
| Viperidae | Agkistrodon contortrix | Absent | Absent | Present | Present | Circular | ? | Present | Present |
| | Agkistrodon piscivorus | Absent | Absent | Absent | Present | Present | Circular | ? | Present | ? |
| Bothrops alternatus | Absent | Absent | Absent | Absent | Absent | Circular | Absent | Present | Present |
| Bothrops diporus | Absent | Absent | Absent | Present (nucleus to annulus) | Absent | Absent | Circular | Present (stopper-like) | Present | Present |
| Crotalus durissus | Absent | Absent | Present | Present (nucleus to annulus) | Present | Present | Circular | Present (stopper-like) | Present | Present |
| Elapidae | Oxyuranus microlepidotus | Absent | Absent | Absent | Present (midpiece) | Present (all along the sperm) | Circular | Absent | Present | ? |
| Colubridae | Boiga irregularis | Absent | Absent | Absent | Present (midpiece) | Present (all along the sperm) | Circular | Absent | Present | Present |
| Nerodia sipedon | Absent | Absent | Absent | Present (midpiece) | Present (all along the sperm) | Circular | ? | Present | Present | Present |

(Continued)
### Table I. (Continued).

| Species                     | Acrosome vacuity subdivision | Acrosomal ridge | Nuclear lacuna | Multilaminar membrane | Extracellular microtubules | Acrosome complex in TS | Perforatorium baseplate | Epinuclear electron-lucent zone | Laminated structures |
|-----------------------------|------------------------------|-----------------|----------------|------------------------|---------------------------|------------------------|--------------------------|-------------------------------|---------------------|
| *Seminatrix pygaea*         | Absent                       | Absent          | Absent         | Present (nucleus to annulus) | Present (midpiece and tail) | Circular               | Present                   | Present                      | Present             |
| *Stegonotus cucullatus*     | Absent                       | Absent          | Absent         | Present (midpiece)        | Present (all along the sperm) | Circular               | Present                   | Present                      |                |
| *Homalopsidae*              |                              |                 |                |                         |                           |                        |                          |                               |                    |
| *Myrrophis chinensis*       | Absent                       | Absent          | Absent         | Present (nucleus to annulus) | Present (nucleus to annulus) | Circular               | Present                   | Poorly developed            | Present             |

| Species                     | Dense collar | Peripheral fibers 3 and 8 | Mitochondria in SLS | Mitochondria in TS | Mitochondria in PLS | Annulus in SLS | Dense body | Granular cytoplasm zone | Elongated midpiece | Reference |
|-----------------------------|--------------|---------------------------|---------------------|-------------------|-------------------|----------------|------------|------------------------|-------------------|-----------|
| Anomalepididae              | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Triangular      | Present    | Absent                 | Present           | Tavares-Bastos et al. 2007 |
| *Liophthalmops beui*        |              |                           |                     |                   |                   |                |            |                        |                   |           |
| Leptotyphlopidae            | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Linear      | Present    | Absent                 | Present           | Tavares-Bastos et al. 2007 |
| *Leptozyphlops koppesi*     |              |                           |                     |                   |                   |                |            |                        |                   |           |
| Typhlopidae                 | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | ?           | Present    | Absent                 | Present           | Tavares-Bastos et al. 2007 |
| *Raphophyphlops macrurus*   |              |                           |                     |                   |                   |                |            |                        |                   |           |
| Typhlops reticulatus        | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Triangular  | Present    | Present                | Present           | Tavares-Bastos et al. 2007 |
| Boidae                      | Present      | Enlarged                  | Rounded             | Irregular         | Sinuous tubules, zigzagged | Irregular   | Scarce     | Absent                 | Present           | Tavares-Bastos et al. 2008 |
| *Boa constrictor amarali*   |              |                           |                     |                   |                   |                |            |                        |                   | Tourmente et al. 2006 |
| *Boa constrictor occidentalis* | Present     | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | ?          | Scarce     | Absent                 | Present           | Tavares-Bastos et al. 2008 |
| *Corallus hortulanus*       | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Irregular   | Scarce     | Absent                 | Present           | Tavares-Bastos et al. 2008 |
| *Epicrates cenchrda*        | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Irregular   | Scarce     | Absent                 | Present           | Tavares-Bastos et al. 2008 |
| Pythonidae                  | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Irregular   | Present    | Absent                 | Present           | Oliver et al. 1996   |
| *Aspidites melanocephalus*  |              |                           |                     |                   |                   |                |            |                        |                   |           |
| *Viperidae*                 | Absent       | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | Irregular   | Absent     | Absent                 | Present           | Rheubert et al. 2017 |
| *Agkistrodon contortrix*    | Absent       | Enlarged                  | Rounded             | ?                 | Sinuous tubules, zigzagged | Absent     | Absent     | Absent                 | Present           | Gribbins et al. 2010  |
| *Agkistrodon piscivorus*    | Absent       | Enlarged                  | Rounded             | ?                 | Sinuous tubules, zigzagged | Absent     | Absent     | Absent                 | Present           | Tourmente et al. 2008 |
| *Bothrops alternatus*       | Present      | Enlarged                  | Rounded             | Round             | Sinuous tubules, zigzagged | ?          | Absent     | Absent                 | Present           | Tourmente et al. 2008 |

(Continued)
Table I. (Continued).

| Species                  | Dense collar | Peripheral fibers 3 and 8 | Mitochondria in SLS | Mitochondria in TS | Mitochondria in PLS | Annulus in SLS | Dense body | Granular cytoplasm zone | Elongated midpiece | Reference                  |
|--------------------------|--------------|----------------------------|---------------------|--------------------|---------------------|----------------|------------|-------------------------|---------------------|--------------------------|
| *Bothrops diporus*       | Present      | Enlarged                   | Rounded             | Round              | Sinuous tubules, zigzagged | ?              | Absent     | Absent                  | Present             | Tourmente et al. 2008    |
| *Crotalus durissus*      | Present      | Enlarged                   | ?                   | Round              | ?                   | Linear         | Present    | Absent                  | Present             | Cunha et al. 2008        |
| *Elapidae*               | Present      | Enlarged                   | Rounded             | Round              | ?                   | Absent         | Present    | Absent                  | Present             | Oliver et al. 1996       |
| *Oxyuranus microlepidotus*| Present      | Enlarged                   | Rounded             | Round              | Sinuous tubules, zigzagged | Irregular      | Present    | Absent                  | Present             | Oliver et al. 1996       |
| *Boiga irregularis*      | Present      | Enlarged                   | Round               | Round              | Sinuous tubules, zigzagged | Irregular      | Present    | Absent                  | Present             | Jamieson & Koehler 1994   |
| *Nerodia pipidion*       | Present      | Enlarged                   | Round               | Round or oval      | ?                   | Triangular     | Present    | Absent                  | Present             | Rheubert et al. 2010     |
| *Stegmatopterus cucullatus* | Present       | Enlarged                   | Round               | Sinuous tubules, zigzagged | Irregular      | Present    | Absent                  | Present             | Oliver et al. 1996       |
| *Homalopsidae*           | Present      | Enlarged                   | Round               | Round or oval      | Sinuous tubules, zigzagged | Oval          | Present    | Present                  | Present             | This research             |
| *Myrrophis chinensis*    | Present      | Enlarged                   | Round               | Sinuous tubules, zigzagged | Oval          | Present    | Present                  | Present             | This research             |

PLS: parasagittal longitudinal section; SLS: sagittal longitudinal section; TS: transverse section.
Lacertilia (Teixeira et al. 1999b; Tavares-Bastos et al. 2002; Vieira et al. 2004, 2005), more research in this field would significantly improve the phylogenetic analysis of snakes as well as our understanding of their evolutionary history.

Acknowledgements

We thank Zhou-Xi Fang and Ling-Liang Fang from College of Life Science, Wenzhou Medical University for the assistance in electron microscopy work. We thank Alan K. Chang (Wenzhou University) for revising the language of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by grants from the Zhejiang Province Natural Science Foundation of China [grant number LY16C030001] and the National Natural Science Foundation of China [grant number 31170376].

References

Arroyo MAM, Silva FF, Santos PRS, Silva AR, Oliveira MF, Assis-Neto AC. 2015. Ultrastructure of spermatogenesis and spermatozoa in agoutis during sexual development. Reproduction, Fertility and Development 29:383–393.

Austin CR. 1965. Fine structure of the snake sperm tail. Journal of Ultrastructure Research 12:452–462. DOI:10.1016/S0022-5320(65)80112-5.

Beguelinia MR, Pugab CCI, Tabogaa SR, Morielle-Versute E. 2011. Ultrastructure of spermatogenesis in the white-lined broad-nosed bat, Platyrhinus lineatus (Chiroptera: Phyllostomidae). Micron 42:586–599. DOI:10.1016/j.micron.2011.02.004.

Bian XG, Gandahi JA, Liu Y, Yang P, Liu Y, Zhang L, Zhang Q, Chen QS. 2013. The ultrastructural characteristics of the spermatozoa stored in the cauda epididymidis in Chinese soft-shelled turtle Pelodiscus sinensis during the breeding season. Micron 44:202–209. DOI:10.1016/j.micron.2012.06.010.

Blenginia CS, Narettoa S, Cardozoa G, Giojalasb LC, Chiaraviglioa M. 2017. Comparative sperm ultrastructure of two tegu lizards (genus Salvator) and its relation to sperm competition. Zoological Anzeiger 267:63–68. DOI:10.1016/j.zooj.2017.02.004.

Breed WG. 2005. Evolution of the spermatozoon in murid rodents. Journal of Morphology 265:271–290. DOI:10.1002/(ISSN)1097-4687.

Cai B, Wang Y, Chen Y, Li J. 2015. A revised taxonomy for Chinese reptiles. Biodiversity Science 23:365–382. (In Chineses). DOI:10.17520/biods.2015037.

Carrick FN, Hughes RL. 1982. Aspects of the structure and development of monotreme spermatozoa and their relevance to the evolution of mammalian sperm morphology. Cell Tissue Research 222:127–141. DOI:10.1007/BF00218293.

Colli GR, Teixeira RD, Scheltinga DM, Mesquita DO, Wiederhecker HC, Bão SN. 2007. Comparative study of sperm ultrastructure of five species of teiid lizards (Teiidae, Squamata), and Cercosaura ocellata (Gymnophthalmidae, Squamata). Tissue & Cell 39:59–78. DOI:10.1016/j.tice.2006.12.001.

Cunha LD, Tavares-Bastos L, Bão SN. 2008. Ultrastructural description and cytochemical study of the spermatozoon of Crotalus durissus (Squamata, Serpentes). Micron 39:915–925. DOI:10.1016/j.micron.2006.12.008.

Erséus C. 1999. Sperm types and their use for a phylogenetic analysis of aquatic clitellates. Hydrobiologia 402:225–237. DOI:10.1023/A:1003752811830.

Furieri P. 1970. Sperm morphology of some reptiles: Squamata and Chelonias. Baccetti B, editor. Comparative Spermatology. New York: Academic Press. 115–131.

Girling JE. 2002. The reptilian oviduct: A review of structure and function and directions for future research. Journal of Experimental Zoology 293:141–170. DOI:10.1002/(ISSN)1097-010X.

Giugliano LG, Teixeira RD, Colli GR, Bão SN. 2002. Ultrastructure of spermatozoa of the lizard Ameiva ameiva, with considerations on polymorphism within the family Teiidae (Squamata). Journal of Morphology 253:264–271. DOI:10.1002/jmor.10002.

Gribbins KM, Rheubert JL. 2014. The architecture of the testis, spermatogenesis, and mature spermatozoa. Rheubert JL, Siegel DS, Trauth SE, editors. Reproductive biology and phylogeny of lizards and tuatara. Boca Raton: CRC Press. 340–424.

Gribbins KM, Rheubert JL, Anzalone ML, Siegel DS, Sever DM. 2010. Ultrastructure of spermogenesis in the cottonmouth, Agkistrodon piscivorus (Squamata: Viperidae: Crotalinae). Journal of Morphology 271:293–304.

Hamilton DW, Fawcett DW. 1968. Unusual features of the neck and middlepiece of snake spermatozoa. Journal of Ultrastructure Research 23:81–97. DOI:10.1016/S0022-5320(68)80033-4.

Hao SL, Pan LL, Fang ZX, Zhang YP. 2015. Comparative studies on sperm ultrastructure of three gecko species, Gekko japonicus, Gekko chinensis and Hemidactylus bosriji (Reptilia, Squamate, Gekkonidae). Asian Herpetological Research 6:189–198.

Harding HR, Aplin KP, Mazur M. 1995. Ultrastructure of spermatozoa of Australian blindsnakes, Ramphotyphlops spp. (Typhlopidae, Squamata): First observation on the mature spermatozoon of scolopendrid snakes. Jamieson BGM, Anzalone JL, editors. Advances in spermatozoal phylogeny and taxonomy. Paris: Muséum National d’Histoire Naturelle. 385–396.

Healy JM, Jamieson BGM. 1994. The ultrastructure of spermatozoa of Australian blindsnakes, Ramphotyphlops spp. (Typhlopidae, Squamata): First observation on the mature spermatozoon of scalelophidian snakes. Jameson BGM, Anzalone JL, editors. Advances in spermatozoal phylogeny and taxonomy. Paris: Muséum National d’Histoire Naturelle. 385–396.

Henley C, Feduccia A, Costello DP. 1978. Oscine spermatozoa: A light- and electron-microscopy study. Condor 80:41–48. DOI:10.2307/1367789.

Hess RA, Thurston RJ, Gist DH. 1991. Ultrastructure of the turtle spermatozoon. Anatomical Record 229:473–481. DOI:10.1002/ar.1092290406.
Jamieson BG. 1995a. Evolution of tetrapod spermatozoa with particular reference to amniotes. Jamieson BGM, Ausio J, Justine J, editors. Advances in Spermatozoal Phylogeny and Taxonomy. Paris: Muséum National d’Histoire Naturelle. 343–358.

Jamieson BGM. 1995b. The ultrastructure of spermatozoa of the Squamata (Reptilia) with phylogenetic considerations. Jamieson BGM, Ausio J, Justine J, editors. Advances in Spermatozoal Phylogeny and Taxonomy. Paris: Muséum National d’Histoire Naturelle. 359–383.

Jamieson BGM. 1999. Spermatozoal phylogeny of the vertebrata. Gagnon C, editor. The male gamete: from basic science to clinical applications. Vienna: Cache River Press. 303–331.

Jamieson BGM, Healy JM. 1992. The phylogenetic position of the Tuatara, Sphenodon (Sphenodontida, Anniota), as indicated by cladistic analysis of the ultrastructure of spermatozoa. Philosophical Transactions of the Royal Society B: Biological Sciences 335:207–219. DOI:10.1098/rstb.1992.0019.

Jamieson BGM, Hodgson A, Spottiswoode CN. 2006. Ultrastructure of the spermatozoon of Myrmecocichla formicivora (Vieillot, 1881) and Philtilusius socius (Latham, 1790) (Aves; Passeriformes), with a new interpretation of the passerian acrosome. Acta Zoologica 87:297–304. DOI:10.1111/j.1463-6395.2006.00242.x.

Jamieson BGM, Koehler L. 1994. The ultrastructure of the spermatozoon of the northern water snake, Nerodia sipedon (Colubridae, Serpentes), with phylogenetic considerations. Canadian Journal of Zoology 72:1648–1652. DOI:10.1139/z94-220.

Jamieson BGM, Koehler L, Todd B. 1995. Spermatozoal ultrastructure in three species of parrots (Aves, Psittaciformes) and its phylogenetic implications. Anatomical Record 241:461–468. DOI:10.1002/ar.1092410202.

Jamieson BGM, Oliver SC, Scheltinga DM. 1996. The ultrastructure of spermatozoon of Squamata I. Scincidae, Gekkonidae and Pygopodidae (Reptilia). Acta Zoologica (Stockholm) 77:85–100. DOI:10.1111/j.1463-6395.1996.tb01255.x.

Jamieson BGM, Scheltinga DM, Tuckor AD. 1997. The ultrastructure of spermatozoa of the Australian freshwater crocodile, Crocodylus johnstoni Krefft, 1873 (Crocodylidae, Reptilia). Journal of Submicroscopic Cytology and Pathology 29: 265–274.

Jamieson BGM, Tripepi S. 2005. Ultrastructure of the spermatozoon of Apus apus (Linnaeus 1758), the common swift (Aves; Apodiformes; Apodidae), with phylogenetic implications. Acta Zoologica 86:239–244. DOI:10.1111/j.1463-6395.2005.00204.x.

Jeong SJ, Park JC, Kim HJ, Bae CS, Yoon MH, Lim DS, Jeong MJ. 2006. Comparative fine structure of the epididymal spermatozoa from three korean shrews with considerations on their phylogenetic relationships. Biocell 30:279–286.

Jiang ZG, Jiang JP, Wang YZ, Zhang E, Zhang YY, Li LL, Xie F, Cai B, Cao L, Zheng GG, Dong L, Zhang ZW, Ding P, Luo ZH, Ding CQ, Ma ZJ, Tang SH, Cao WX, Li CW, Hu HJ, Ma Y, Wu Y, Wang YX, Zhou KY, Liu SY, Chen YY, Li JT, Feng ZJ, Wang Y, Wang B, Song XL, Cai L, Zang CX, Zeng Y, Meng ZB, Fang HJ, Ping XG. 2016. Red list of China’s vertebrates. Biodiversity Science 24:500–511. DOI:10.17252/biodivsci.20160706.

Oliver SC, Jamieson BGM, Scheltinga DM. 1996. The ultrastructure of spermatozoon of Squamata. II. Agamidae, Varanidae, Colubridae, Elapidae, and Boidae (Reptilia). Herpetologia 52:216–241.

Olivier SGP, Van W, Patrick D. 2008. Global diversity of snakes (Serpentes; Reptilia) in freshwater. Hydrobiologia 595:599–606. DOI:10.1007/s10750-007-9118-x.

Rheuert J, Messak J, Siegel DS, Gribbins KM, Trauth SE, Sever DM. 2017. Inter- and intraspecific variation in sperm morphology of Scorpaenus conobrinus and Scorpaenus undulates (Squamata: Phrynosomatidae). Biological Journal of the Linnean Society 121:355–364. DOI:10.1093/biolinneb/blw043.

Rheuert J, McMahan CD, Sever DM, Bundy MR, Siegel DS, Gribbins KM. 2010. Ultrastructure of the reproductive system of the black swamp snake (Seminatrix pygaea). VII. Spermatozoond morphology and evolutionary trends of sperm characters in snakes. Journal of Zoological Systematics and Evolutionary Research 48:360–375. DOI:10.1111/jz.2010.48.issue-4.

Robson SK, Rouse GW, Pettigrew JD. 1997. Sperm ultrastructure of Tarsius bancanus (Tarsiidae, Primates): Implications for primate phylogeny and the use of sperm in systematics. Acta Zoolologica 78:269–278. DOI:10.1111/azo.1997.78.issue-4.

Saita A, Longo OM, Tripepi S. 1983. Osservazioni comparative sulla spermogenesi. III. Aspetti ultrastrutturali della spermio- genesi di Jacana jacana (Charadriiformes). Accademia Nazionale Dei Lincei. (Rendiconti Della Classe Di Scienze Fisiche, Matematiche E Naturali) 74:417–430.

Server DM, Hamlett WC. 2002. Female sperm storage in reptiles. Journal of Experimental Zoology 292:187–199. DOI:10.1002/jez.1154.

Shine R. 2003. Reproductive strategies in snakes. Proceedings of the Royal Society B: Biological Sciences 270:995–1004.

Soley J. 1999. Reproduction. Deeming DC, editor. The ostrich: biology, production and health. Oxon: CAB International. 29–158.

Soley JT. 1993. Ultrastructure of ostrich (Struthio camelus) spermatozoon: I. Transmission Electron Microscopy. Ondersteboort Journal of Veterinary Research 60:119–130.

Tavares-Bastos L, Colli GR, Bao SN. 2008. The evolution of sperm ultrastructure among Boidae (Serpentes). Zoomorphology 127:189–202. DOI:10.1007/s00435-008-0062-8.

Tavares-Bastos L, Cunha LD, Colli GR, Bao SN. 2007. Ultrastructure of spermatozoa of scolecodophidian snakes (Lepidosauria, Squamata). Acta Zoológica (Stockholm) 88:189–197. DOI:10.1111/j.1463-6395.2007.00265.x.

Tavares-Bastos L, Teixeira RD, Colli GR, Bao SN. 2002. Polymorphism in the sperm ultrastructure among four species of lizards in the genus Tupinambis (Squamata: Teiidae). Acta Zoológica (Stockholm) 83:297–307. DOI:10.1007/j.1463-6395.2002.00119.x.

Teixeira RD, Colli GR, Bao SN. 1999a. The ultrastructure of spermatozoa of the lizard Polychrus acutirostris (Squamata, Polychrotidae). Journal of Submicroscopic Cytology and Pathology 31:387–395.

Teixeira RD, Colli GR, Bao SN. 1999b. The ultrastructure of the spermatozoa of the lizard Micablepharus maximiliani (Squamata, Gymnophthalmidae), with considerations on the use of sperm structures character in phylogenetic reconstruction. Acta Zoológica (Stockholm) 80:47–59. DOI:10.1046/j.1463-6395.1999.20010.x.

Teixeira RD, Colli GR, Bao SN. 1999c. The ultrastructure of the spermatozoa of the worm lizard Amphibolophus alba (Squamata, Gymnophthalmidae), and the phylogenetic relationships of Amphibolophus. Canadian Journal of Zoology 77:1254–1264. DOI:10.1139/z99-089.

Teixeira RD, Scheltinga DM, Trauth SE, Colli GR, Bao SN. 2002. A comparative ultrastructural study of spermatozoa of the teiid lizards Cnemidophorus gularisigularis, Cnemidophorus ocellifer, and Kontropyx altamazonica (Reptilia, Squamata, Teiidae). Tissue & Cell 34:135–142. DOI:10.1016/S0040-8166(02)00021-6.
Teixeira RD, Vieira GHC, Colli GR, Bão SN. 1999d. Ultrastructural study of spermatozoon of the neotropical lizards, Tropidurus semitaeniatus and Tropidurus torquatus (Squamata, Tropiduridae). Tissue & Cell 31:308–317. DOI:10.1054/tice.1999.0047.

Tourmente M, Cardozo G, Bertona M, Guidobaldi A, Giojalas L, Chiaraviglio M. 2006. The ultrastructure of the spermatozoa of Boa constrictor occidentalis, with considerations on its mating system and sperm competition theories. Acta Zoologica (Stockholm) 87:25–32. DOI: 10.1111/j.1463-6395.2006.00217.x.

Tourmente M, Giojalas L, Chiaraviglio M. 2008. Sperm ultrastructure of Bothrops alternatus and Bothrops diporus (Viperidae, Serpentes), and its possible relation to the reproductive features of the species. Zoomorphology 127:241–248. DOI:10.1007/s00435-008-0067-3.

Tuset VM, Trippel EA, De Monserrat J. 2008. Sperm morphology and its influence on swimming speed in Atlantic cod. Journal of Applied Ichthyology 24:398–405. DOI:10.1111/jai.2008.24.issue-4.

Vieira GHC, Colli GR, Bão SN. 2004. The ultrastructure of the spermatozoon of the lizard Iguana iguana (Reptilia, Squamata, Iguanidae) and the variability of sperm morphology among iguanian lizards. Journal of Anatomy 204:451–464. DOI:10.1111/j.0021-8782.2004.00300.x.

Vieira GHC, Colli GR, Bão SN. 2005. Phylogenetic relationships of corytophanid lizards (Iguania, Squamata, Reptilia) based on partitioned and total evidence analyses of sperm morphology, gross morphology, and DNA data. Zoologica Scripta 34:605–625. DOI:10.1111/j.1463-6409.2005.00208.x.

Vieira GHC, Cunha LD, Scheltinga DM, Glaw F, Colli GR, Bão SN. 2007. Sperm ultrastructure of hoplocercid and oplurid lizards (Sauropsida, Squamata, Iguania) and the phylogeny of Iguania. Journal of Zoological Systematics and Evolutionary Research 45:230–241. DOI:10.1111/jzs.2007.45.issue-3.

Zhang YP, Fang ZX, Ji X. 2006. A comparison of the ultrastructure of spermatozoa of two species of skinks Mabuya multifasciata and Sphenomorphus indicus. Acta Zoologica Sinica 52:591–602. In Chinese.

Zhao EM, Huang MH, Zong Y. 1998. Fauna Sinica: Reptilia, Vol. 3, Squamata: Serpentes. Beijing: Science Press (In Chinese).