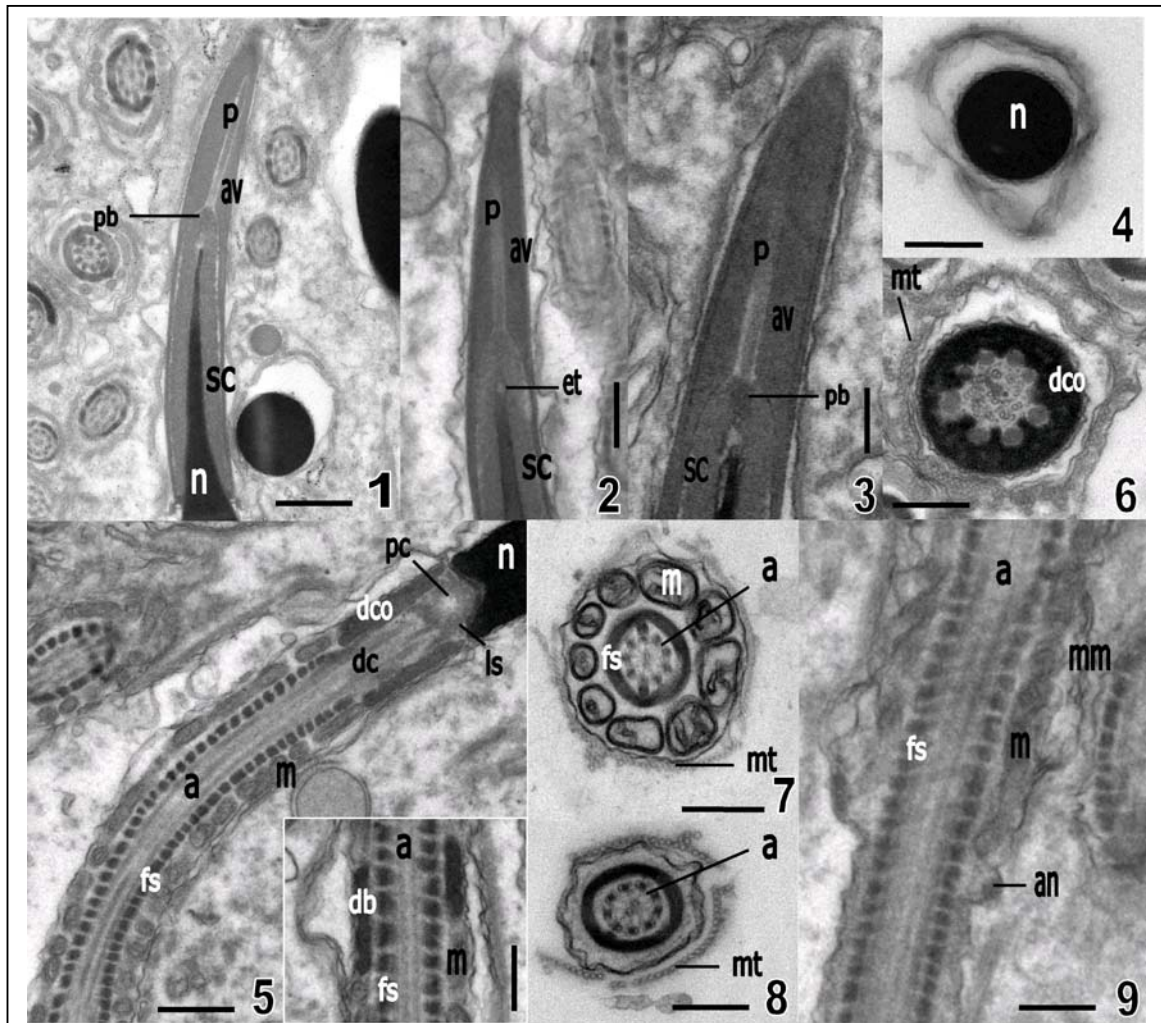


A comparative ultrastructural study of spermatozoa among three families (Boidae, Colubridae, and Typhlopidae) of snakes. Leonora Tavares-Bastos(1); Guarino Rinaldi Colli(2); Sônia Nair Bão(3). (1) Programa de Pós-Graduação em Biologia Animal, Universidade de Brasília. (2) Dep. de Zoologia, Universidade de Brasília, 70919-970, Brasília, DF, Brasil. (3) Dep. de Biologia Celular, Universidade de Brasília. Email: leonora@unb.br

Snakes occur on all continents except Antarctica, and have several families that are classified in Scolecophidia (Boidae and Colubridae.) and Alethinophidia (Typhlopidae) [1]. Detailed studies in Squamata families have revealed that the sperm ultrastructure provides a source of characters to phylogenetic analyses [2], and data of polymorphism were observed in the sperm ultrastructure indicating that it contains significant phylogenetic signal [3]. Herein we provide a detailed description of the ultrastructure of the spermatozoon of *Corallus hortulanus*, *Boa constrictor*, and *Epicrates cenchria*, and compare the general sperm morphology among the three snake families (Boidae, Colubridae, and Typhlopidae). Spermatozoa were obtained from reproductive individuals of *C. hortulanus*, *B. constrictor*, *E. cenchria* (Coleção Herpetológica da Universidade de Brasília, CHUNB 17494, 17495 and 17492, respectively), collected at Chapada dos Guimarães, Mato Grosso State, Brazil (14° 52'S, 55° 48'W). The specimens were killed by euthanasia by Tiopental®, and testes and epididymides were removed by dissection, and cut into small pieces. The tissues were fixed in a solution containing 2% glutaraldehyde-2% paraformaldehyde, with 3% sucrose and 5mM CaCl₂, in 0.1M sodium cacodylate buffer pH 7.2, at 4°C, for 24 h, and postfixed for 1 h in 1% osmium tetroxide, 0.8% potassium ferricyanide in 0.1M sodium cacodylate buffer pH 7.2. Tissues were dehydrated in a series of ascending acetone (30%-100%) and embedded in Spurr's epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observations were made in a Jeol 1011 transmission electron microscope. Images were acquired with a GATAN BioScan Camera, model 792. Spermatozoa of *C. hortulanus*, *B. constrictor*, *E. cenchria* are filiform, consisting of a head region (acrosome complex and nucleus), a midpiece, and a tail region (principal piece and endpiece). In the five species analysed (*C. hortulanus*, *B. constrictor*, and *E. cenchria* - our data; *N. sipedon* and *Ramphlotyphlops* spp. - data of literature [4, 5, respectively]), the head region lacks the lateral ridge. The acrosome vesicle is not subdivided in cortex and medulla in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Figs. 1,2,3), and in *Ramphlotyphlops* spp., whereas in *N. sipedon* it is subdivided in cortex and medulla. The subacrosomal cone appears as a paracrystalline structure in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Figs. 1,2,3), and in *Ramphlotyphlops* spp., while it is not paracrystalline in *N. sipedon*. The acrosome vacuity subdivision is present only in *Ramphlotyphlops* spp. The epinuclear lucent zone is observed in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Fig. 2), and in *N. sipedon*, while it is absent in *Ramphlotyphlops* spp. The perforatorium tip appears rounded in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Fig. 1), and in *Ramphlotyphlops* spp., whereas it is pointed in *N. sipedon*. Embedded in the subacrosomal material, the perforatorium base plate is observed in *B. constrictor*, *E. cenchria* (Figs. 1,3), and in *N. sipedon*, while it is absent in *C. hortulanus* (Fig. 2), and in *Ramphlotyphlops* spp. It is seen as a stopper-like structure in *N. sipedon*, whereas it appears as a knoblike structure in *B. constrictor*, *E. cenchria* (Figs. 1,3). The nucleus observed in the five species is elongate in longitudinal section (Figs. 1,2,3), and lacks lacunae (Fig. 4). In the midpiece region, the bilateral stratified laminar structure projecting on the proximal centriole was observed in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Fig. 5), and in *N. sipedon*, but it is absent in *Ramphlotyphlops* spp. The neck region in the five species contains the dense collar surrounding the distal centriole (Figs. 5,6); the fibers 3 e 8 appear grossly enlarged in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Fig. 7), and in *Ramphlotyphlops* spp., but in *N. sipedon* it is not grossly enlarged. In the five species analysed, the ends of the mitochondria, in longitudinal section, have a rounded shape (Fig. 5), and in cross-section, the mitochondria appear as an irregular structure with linear cristae (Fig. 7); the intermitochondrial dense bodies appear as solid structures (inset Fig. 5). A fibrous sheath beginning in the midpiece in the level of the mitochondria tier 1 in *C. hortulanus*, *B. constrictor*, *E. cenchria* (Fig. 5), whereas in *N. sipedon*, and in *Ramphlotyphlops* spp., it begins before of the mitochondria tier 1. In the five species analysed, the annulus is a small dense ring, with irregular shape in longitudinal section (Fig. 9); the multilaminar membrane (Fig. 9), and the extracellular microtubules are present (Figs. 6-8). Several differences in spermatozoa ultrastructure were observed between each of the snake families analysed here, indicating that these characters can provide useful information on the phylogenetic analyses. Studies of sperm ultrastructure are needed especially on snake families to cast light on the phylogenetic relationships.

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Spermatozoa of *Corallus hortulanus*, *Boa constrictor*, and *Epicrates cenchria*. **Figs. 1-3:** Longitudinal section of the acrosome complex showing the acrosome vesicle, subacrosomal cone, perforatorium, and nucleus. The perforatorium base plate is observed in *B. constrictor* (**Fig. 1**) and *E. cenchria* (**Fig. 3**), however its absence is observed in *C. hortulanus* (**Fig. 2**). **Fig. 2:** Longitudinal section showing the epinuclear lucent zone. **Fig. 4:** Transverse section of the nucleus. **Figs. 5-7, 9:** Midpiece. **Fig. 5:** Longitudinal section of the midpiece showing nucleus, proximal centriole, bilateral stratified laminar structure, dense collar, distal centriole, axoneme, fibrous sheath, and mitochondria. **Inset Fig. 5:** The intermitochondrial dense body. **Fig. 6:** Transverse section of the proximal centriole showing dense collar, and extracellular microtubules. **Fig. 7:** Transverse section of the midpiece showing axoneme, fibrous sheath, mitochondria, multilaminar membrane, and extracellular microtubules. **Fig. 8:** Principal piece: axoneme and extracellular microtubules. **Fig. 9:** The axoneme, the fibrous sheath, mitochondria, multilaminar membrane, and annulus. a: axoneme; av: acrosome vesicle; an: annulus; db: dense body; dc: distal centriole; dco: dense collar; et: epinuclear lucent zone; fs: fibrous sheath; ls: stratified laminar structure; m: mitochondria; mm: multilaminar membrane; mt: extracellular microtubules; n: nucleus; p: perforatorium; pb: perforatorium base plate; pc: proximal centriole; sc: subacrosomal cone. Scale bars: Figs. 1,3: 0.25 μm ; Figs. 2,4,5, inset Fig 5: 0.30 μm ; Figs. 6-9: 0.15 μm .

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