Zoologischer Anzeiger 267 (2017) 63–68

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Zoologischer Anzeiger

Research paper

Comparative sperm ultrastructure of two tegu lizards (genus *Salvator*) and its relation to sperm competition

C.S. Blengini[a,*], S. Naretto[a], G. Cardozo[a], L.C. Giojalas[b], M. Chiaraviglio[a]

[a] *Instituto de Diversidad y Ecología Animal (IDEA) CONICET and Laboratorio de Biología del Comportamiento, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, CP: X5000JJC, Argentina*

[b] *Instituto de Investigaciones Biológicas y Tecnológicas (IIByT) CONICET and Centro de Biología Celular y Molecular, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Velez Sarsfield 1611, CP: X5016GCA, Argentina*

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 18 February 2017
Accepted 19 February 2017
Available online 21 February 2017
Corresponding Editor: Alexander Kupfer.

Keywords:
Lizards
Mitochondria
Sexual selection
Spermatozoa
Ultrastructure

ABSTRACT

The knowledge of sperm ultrastructure of Squamata provides informative traits for phylogenetic analyses. Furthermore, several sperm ultrastructure traits are important for sperm motility and longevity. Here, we provided a detailed ultrastructural description of the spermatozoa of two closely related teiid lizards, *Salvator rufescens* and *S. merianae*. We carried out an interspecific comparison of sperm ultrastructure traits and discussed their possible relation to sperm competition risk. Sperm ultrastructure of the two species shared patterns with other lizards previously described. The lack of interspecific differences in most sperm subcellular components, suggested that sperm ultrastructure traits are conserved within the genus *Salvator* and thus pointed out an important phylogenetic influence. However, we detected interspecific differences in the number of mitochondria and dense bodies sets, suggesting differences in the amount of energy available for sperm motility and longevity. Further comparative studies are urgently needed to understand the sperm metabolic pathways in neotropical lizards in general.

1. Introduction

Sperm ultrastructure studies provide information about subcellular mechanisms that may influence the reproductive success, supplying useful traits to elucidate phylogenetic relationships among species (Teixeira et al., 1999, 2002; Giugliano et al., 2002; Tavares-Bastos et al., 2002; Colli et al., 2007; Tourmente et al., 2008). Furthermore, several sperm ultrastructure traits are important for sperm motility and longevity (Eddy et al., 2003; Turner, 2006). The outer dense fibers (ODFs) that surround the axoneme, in particular numbers 3 and 8, are enlarged in Squamata spermatozoa, providing flagellum rigidity during sperm movement (Gastmann et al., 1993; Turner, 2006). Moreover, the number of mitochondria in the midpiece have a key role to provide energy for sperm survival and movement (Tavares-Bastos et al., 2002; Turner, 2006; Tourmente et al., 2009). Finally, the fibrous sheath (FS) is involved in sperm movement, influencing flagellum flexibility and the flagellar beat pattern (Fawcett, 1975; Lindemann et al., 1992; Eddy et al., 2003). In addition, it is considered an important source of energy for the spermatozoa, because it has enzymes involved in glycolysis, allowing the production of ATP throughout the flagellum length (Narisawa et al., 2002; Miki et al., 2004; Turner, 2006). Tourmente et al. (2009) documented that species with higher levels of sperm competition, where selection on sperm performance may be particularly intense, the area of ODFs and FS are larger, suggesting an improvement in sperm quality in snakes.

Salvator merianae and *S. rufescens* (Daudin, 1802) (formerly *Tupinambis merianae* and *T. rufescens*) are excellent model systems to study the sperm ultrastructure in the context of sperm competition risk. They are closely related species (Cabaña et al., 2014), share many bioecological traits (Cardozo et al., 2012) and have a partially overlapping distribution (Cardozo et al., 2012; Lanfri et al., 2013), where reciprocal hybridization between these species and introgression by backcrossing occurs (Cabaña et al., 2014). In *Salvator* lizards, females can copulate with different males, even on the same day (Lopes and Abe, 1999). Moreover, follicular development is completed about 20 days after mating, suggesting sperm retention in female genital ducts (Garcia Valdez et al., 2011). Therefore, there is a high opportunity for sperm competition to occur in these species. Indeed, Blengini et al. (2014) confirmed that *S. rufescens* is exposed to higher levels of sperm competition risk than *S. merianae* and evidenced a large among- and within- male variation in sperm morphometric and dynamic traits, suggesting that

* Corresponding author.
E-mail addresses: ceciliablengini@gmail.com, cecilia_blengini@hotmail.com (C.S. Blengini).

http://dx.doi.org/10.1016/j.jcz.2017.02.004
0044-5231/

males vary in sperm competitive ability. Variation in the secondary sexual character, the relative testis mass and the length of sperm component was observed between allopatry and sympatry in each species, suggesting differences in the investment of reproductive traits (Naretto et al., 2016). Furthermore, a negative relationship between secondary sexual character with sperm principal piece length and seasonal flexibility of male reproductive strategies were observed in *S. rufescens* (Blengini et al., 2016). Then, we expect to find variation in sperm ultrastructure traits, being S. *rufescens* the species that could present greater number of mitochondria and larger area of outer dense fibers and fibrous sheath than S. *merianae*. Even though, the sperm ultrastructure of *S. merianae* has been previously described (Tavares-Bastos et al., 2002), there is evidence that sperm traits could vary among populations in different geographical areas (Lüpold et al., 2011). Then, the aim of our study is to provide a detailed ultrastructural description of the spermatozoa of *S. rufescens and S. merianae* to compare sperm ultrastructure traits between these species.

2. Materials and methods

2.1. Study species

Salvator merianae and *S. rufescens* are similar in body size and live in the southernmost area of genus distribution in South America (Lanfri et al., 2013). Both species are seasonal breeders, that reproduce from October to December (Fitzgerald et al., 1993; Naretto et al., 2014). They are included in the Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (Cites, 2008). We are authorized to perform animal capture for scientific purposes by the government environmental agencies. Specimens were killed for the legal skin trade, in accordance with AVMA Guidelines on Euthanasia (AVMA, 2007). Sampling was conducted at two sites of allopatry for each species (*S. merianae*: 31°28′W, 63°38′S to 31°45′W, 63°15′S; *S. rufescens*: 29°30′W, 64°15′S to 29°57′W, 63°55′S).

2.2. Sperm sampling procedure

Spermatozoa were obtained from the terminal portion of the epididymis (Depeiges and Dacheux, 1985). All the samples obtained were collected in a 1.5 mL plastic tube containing approximately 90 μL of phosphate buffered saline (PBS).

2.3. Spermatozoa ultrastructure

Sperm ultrastructure data was obtained from 3 males of each species of *Salvator*. Semen samples were washed twice with PBS and centrifuged for 7 min at 700G. The supernatant was eliminated and the resulting pellet was fixed for 3 h at room temperature in a 2% glutaraldehyde and 4% formaldehyde solution. Subsequently, the samples were postfixed in 1% osmium tetroxide in cacodylate buffer during 2 h at room temperature. The samples were washed with distilled water and dehydrated in a series of ascending acetone concentrations (50, 70, 90 and 100%), then transferred to a solution of 50% acetone and 50% Araldite epoxy resin followed by two final steps in 100% resin. The samples were then embedded in Araldite epoxy resin at 60 °C for 24–72 h. Ultrathin sections (approximately 60 nm) were mounted on 250 mesh nickel grids, and stained with a saturated solution of uranyl in ethanol, and in lead citrate. Finally, the samples were observed under a Zeiss LEO 906E transmission electron microscope. Ultrastructural morphometry was performed in the micrographs at different magnifications. The area (μm2) of the fibrous sheath and the outer dense fibers in position 3 and 8 was measured using Image J version 1.48 g (NIH, USA) in one spermatozoa per male. The area of the fibrous sheath was quantified

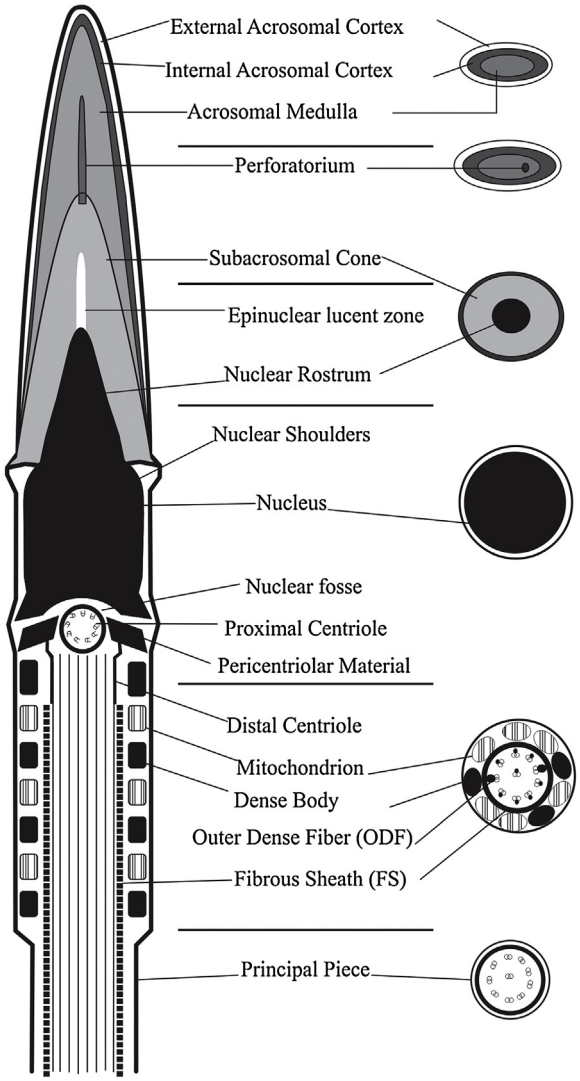

Fig. 1. Schematic representation of the spermatozoon of *Salvator merianae* and *S. rufescens* in longitudinal section with its corresponding transverse sections. The scale of some sections and structures has been changed for clarity.

making a measure of the area of two concentric circles, then the subtraction of the external circle less internal circle was made. Differences between species in the area of fibrous sheath and outer dense fibers were determined by non- parametric Kruskal-Wallis test. These statistical tests were conducted using InfoStat software (version 2012; Universidad de Cordoba, Argentina).

3. Results

Since there were no differences between species in the most subcellular components analyzed, a single schematic drawing including the major findings in both species was provided (Fig. 1).

3.1. Acrosome complex

In both species, the acrosome complex, which was located in the anterior most region of the head, was comprised of an external and elongate acrosome vesicle, an internal cap, the subacrosomal cone, and the perforatorium (Fig. 2A, B, H–J). In cross section, it was depressed and increasingly circular toward the base (Fig. 2C–G, K–O). The acrosome vesicle was divided into: a narrow cortex and a wide medulla at its anterior portion. The cortex consisted of two