

THE ULTRASTRUCTURE OF THE SPERMATOZOA AS A TOOL TO DETECT PHYLOGENETIC RELATIONSHIPS AMONG DIFFERENT SPECIES OF *Cynolebias* GENUS
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The ultrastructure of fish spermatozoa has been used as an alternative data to investigate the phylogeny of many taxa (Jamieson, 1991). They seem to be useful for groups where external morphology cannot be scored, because traditionally used characters are either too conserved or too variable.

Mature male specimens belonging to three species of annual fishes of *Cynolebias* genus: *C. charrúa*, *C. reicherti* and *C. viarius* (Cyprinodontiformes, Rivulidae) were collected in temporal ponds of Uruguay during the rainy season. Their gonads were processed for transmission and scanning electron microscopy and used to analyse the ultrastructure of spermatozoa.

Spermatozoa of all species studied showed one flagellum but its morphology had features that may provide useful systematic characters as it was previously reported for our research group. In the present study we report additional ultrastructural differences: nuclear chromatin condensation and organisation; angle of head flagellum insertion and mitochondria number and distribution in the mid piece.

In *C. charrúa*, *C. viarius* and *C. reicherti* the nucleus presented a nuclear space all around the highly condensed chromatin, that looked more visible in *C. reicherti*. In spite of chromatin electron density, several electron-lucent lacunae of different aspect and size are seen. In *C. charrúa* and *C. viarius* the chromatin appeared homogeneously electron-dense with these electron-lucent spaces (figs. 1a and 2a). In addition in *C. reicherti* it was observed several highly electron-dense areas of chromatin scattered on all nuclear volume. Besides this, the surface of condensed chromatin was undulated (fig.3a). The mid piece was very short in all studied species. In *C. charrúa* and *C. viarius*, the proximal centriole was contained inside a little nuclear fossa (fig. 2b). In both species, it lay at an angle of about 45° with respect to the longitudinal axis of the spermatozoa (fig. 1a and 2a). Only a little number of mitochondria ($\cong 5$ for *C. viarius* and 6 for *C. charrúa*) were distributed around the flagellar insertion. In *C. reicherti* nuclear fossa was not clearly observed and the longitudinal axis of flagellum looked perpendicular to proximal centriole (fig.3a). The number of mitochondria were higher ($\cong 7$, fig. 3b) and seemed to take a "horseshoe" disposition around the flagellum. Furthermore, the different external disposition of lateral fins observed at flagellar level, we found that this arrangement is lightly suggested at the beginning of flagellum formation at spermatid stage, when it was still inside in the cytoplasm channel (fig.1b).

The finding of differences in the ultrastructural morphology of the sperm, suggests the possibility that these results can be used to discern controversial phylogenetic relationship in related species of *Cynolebias*. The nuclear lacunae are probably formed during the condensation of chromatin. Nuclear inclusions contained a clear but not completely electron lucent substance and were usually bigger than lacunae. The angle of the proximal centriole to the distal centriole is species-specific among Cyprinidae species (Baccetti et al., 1984). The duration of sperm motility has been related to the size of the midpiece (Billard et al, 1995). The reduction in mitochondria number could be related to the reproductive behaviour of *Cynolebias*, and the success in sperm swimming could be complemented by the lateral fins.

Finally, the present results allow us to suggest that some aspects of the ultrastructural morphology in *Cynolebias* acquire evolutionary significance: reduction of the mitochondrial collar during the spermiogenesis and the absence of a juxta-axonemal fiber agrees with the proposition that a reduction in complexity is a major trend in the evolution of spermatozoa, and can contribute to achieve a major number of successful fertilisations (Lee and Jamieson, 1993)

References

- Lee and Jamieson *The ultrastructure of the spermatozoa of bufonid and hylid frogs (Anura, Amphibia): implications for phylogeny and fertilization biology*. Aool.Scr.,22, 309-323. (1993)
- Billard, R. et al, *Sperm physiology and quality*. In Bromage,N.R. and Roberts R.J. (Eds.): Broodstock Management and Egg and Larval Quality, pp.25-52. Blackwell Science, Oxford (1995).
- Baccetti et al., *Fish germinal cells. Comparative spermatology of seven cyprinid species*. In Gamete Research 10: 373-396. (1984)
- Jamieson, B.G.M. *Fish Evolution and Systematics: Evidence from Spermatozoa*. Cambridge University Press, Cambridge pp 1-319.(1991).

