Zeitschrift für Fischkunde	Band 8	Heft 1/2	15.10.2006	S. 83-88

A note on the spermatozoon ultrastructure of the foureyed fish *Anableps anableps* (Atherinomorpha, Cyprinodontiformes)

Zur Feinstruktur der Spermien des Vieraugenfisches *Anableps anableps* (Atherinomorpha, Cyprinodontiformes)

Hartmut Greven & Günter Schmahl
Institut für Zoomorphologie und Zellbiologie der Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, D- 40225 Düsseldorf; grevenh@uni-duesseldorf.de

Zusammenfassung: Spermien von *Anableps anableps* sind etwa 40 µm lang, bestehen aus einem abgerundetem, stumpfen Kopf von etwa 3,4 µm Länge, einem Mittelstück von 3,9 µm Länge, in dem das Axonem von einer Manschette mit Längsreihen lang gestreckter und teilweise verschmolzener Mitochondrien umgeben ist, und einem langen Schwanzfaden (etwa 32,6 µm lang) mit der typischer 9+2 Anordnung von Mikrotubuli. Der Kern besitzt eine tiefe Fossa, in der ein proximales und ein distales Centriol identifiziert werden können.

Schlüsselwörter: *Anableps*, spermatozoon, innere Besamung, „Introspermien“

Summary: Spermatozoa of *Anableps anableps* are approximately 40 µm long and consist of the blunt head (approx. 3.4 µm long), the midpiece of 3.9 µm length – here the axoneme is surrounded by a sleeve of longitudinal rows of elongated and partly fused mitochondria, and a long flagellum (approx. 32.9 µm) with the typical 9+2 arrangement of microtubules. The nucleus possesses a deep fossa, which contains the proximal and distal centriole.

Key word: *Anableps*, spermatozoon, internal insemination, introsperms

1. Introduction

Currently, the viviparous foureyed fish of the genus *Anableps* (Anablepidae, Atherinomorpha) comprise three species (Ghedotti 1998). Internal fertilization is achieved with the male anal fin that is modified in a fleshy tubular gonopodium. Relative deep insertion of the gonopodium into the female's genital pore during copulation (see Mattig & Greven 1994) minimizes loss of sperm. The consequence of the evolution of a tubular gonopodium was the loss of sperm package. *Anableps* species do not produce spermatozeugmata; only *A. dowi* produces partial sperm bundles (Grier et al. 1981). Further, there is a distinct laterality to the position of the gonopdium allowing the differentiation of lefts and rights. Closely related to *Anableps* is the genus *Jenynsia* which is also characterized by a fleshy gonopodium, laterality and production of free spermatozoa. (Garman 1895, Turner 1950, Parenti 1981, Mattig & Greven 1994, Ghedotti 1998).

As expected, the testis of *Anableps* as well as of *Jenysia* species (Grier 1981, Grier et al. 1981, Parenti & Grier 2004) is of the atherinimorph type, i.e. spermatogonia are restricted to the distal end of the testicular tubules (restricted type according to Grier 1981). Fine structural details of the male gametes of members of the Anablepidae have been reported from *Jenynsia lineata* only (Dadone & Narbaitz 1967). Spermatozoa of this species reveal characteristics typical of those in many inseminating teleosts (for a more recent review see Burns & Weitzman 2005).

In the present note we describe the ultrastructure of mature spermatozoa of *Anableps anableps* as a part of a wider study on the biology, in particular the reproductive biology of Ana-

blepidae (see among others MATTIG & GREVEN 1994, unpublished). As shown repeatedly morphological modifications of sperm may reflect the mode of reproduction and above that spermatozoal ultrastructure may help to discuss phylogenetic relationships (JAMIESON 1991, MATTEI 1991).

2. Material and Methods

Two males of *Anableps anableps* (total length 21 and 23 cm) were obtained from the Aquazoo – Löbbecke Museum, Düsseldorf. Fish were anaesthetized with MS222 and their gonads were excised. In addition, spermatozoa, obtained by gently pressing the abdomen before dissection, were collected in a glass vessel.

For transmission electron microscopy (TEM) pieces of the testes were fixed in 2.5% glutaraldehyde in 0.1 mol/l cacodylate buffer and free spermatozoa in a fixative according to KARNOVSKY (1965). Tissue and spermatozoa were postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature, dehydrated in a series of ethanol, and infiltrated and embedded (SPURR 1969). Ultrathin sections were stained with aqueous uranyl acetate and lead citrate. Sections were examined in a Zeiss 902-A transmission electron microscope.

For scanning electron microscopy (SEM) samples of the glutaraldehyde fixed spermatozoa were, dehydrated in ethanol, critical point dried, mounted on specimen holders, evaporated with gold and examined in a Zeiss Leo 1430 VP scanning electron microscope.

Sperm dimensions were measured directly from SEM micrographs.

3. Results

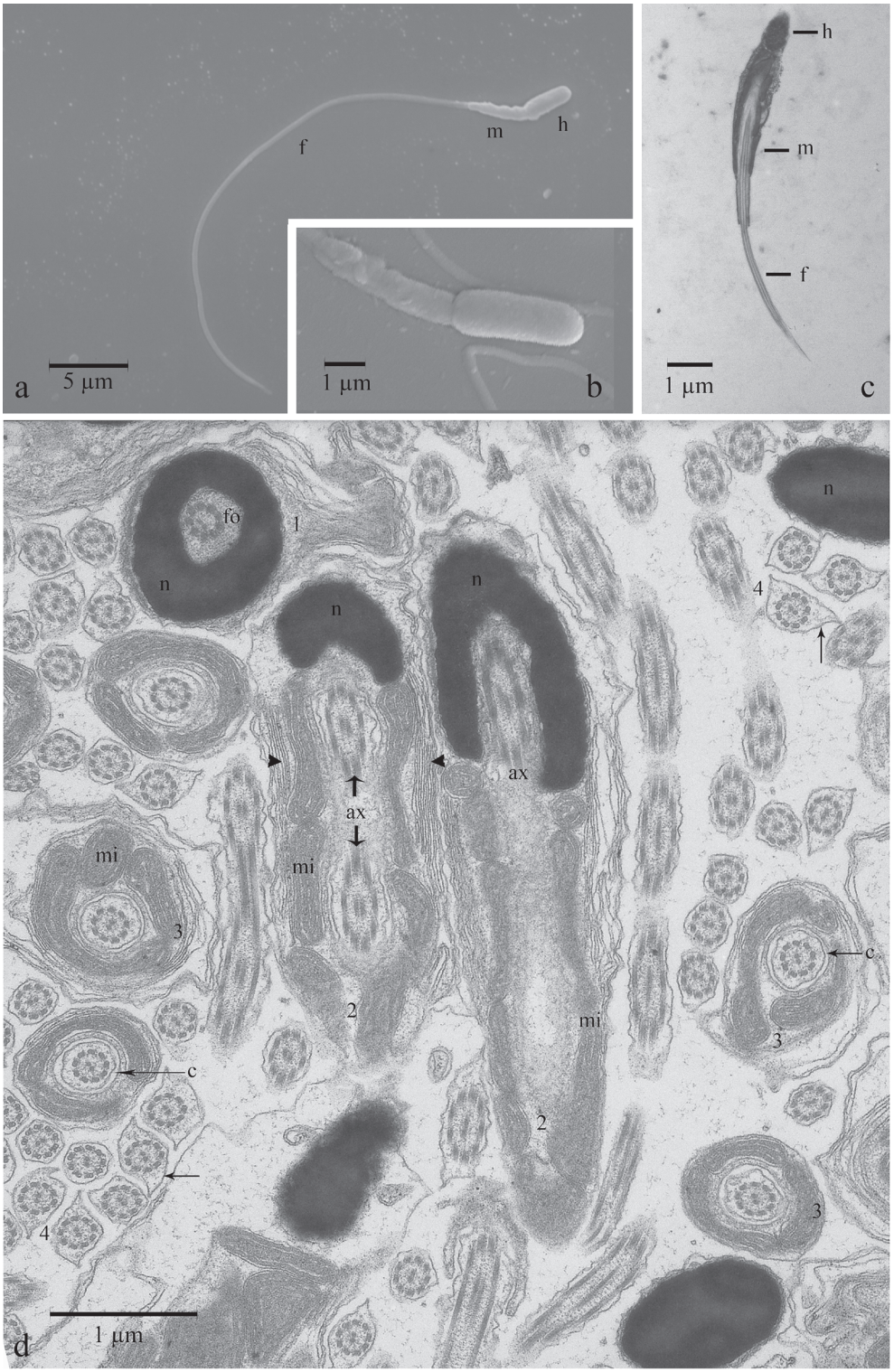
The mature spermatozoon of *Anableps anableps* is about 40 µm long and consists of a head not conspicuously elongated (approx. 3.4 µm long), a relatively long midpiece (approx. 3.9 µm long) and a single flagellum of approx. 32.6 µm length (figs. 1 a-c). The head is barely depressed as seen in transverse sections (fig. 1 d)

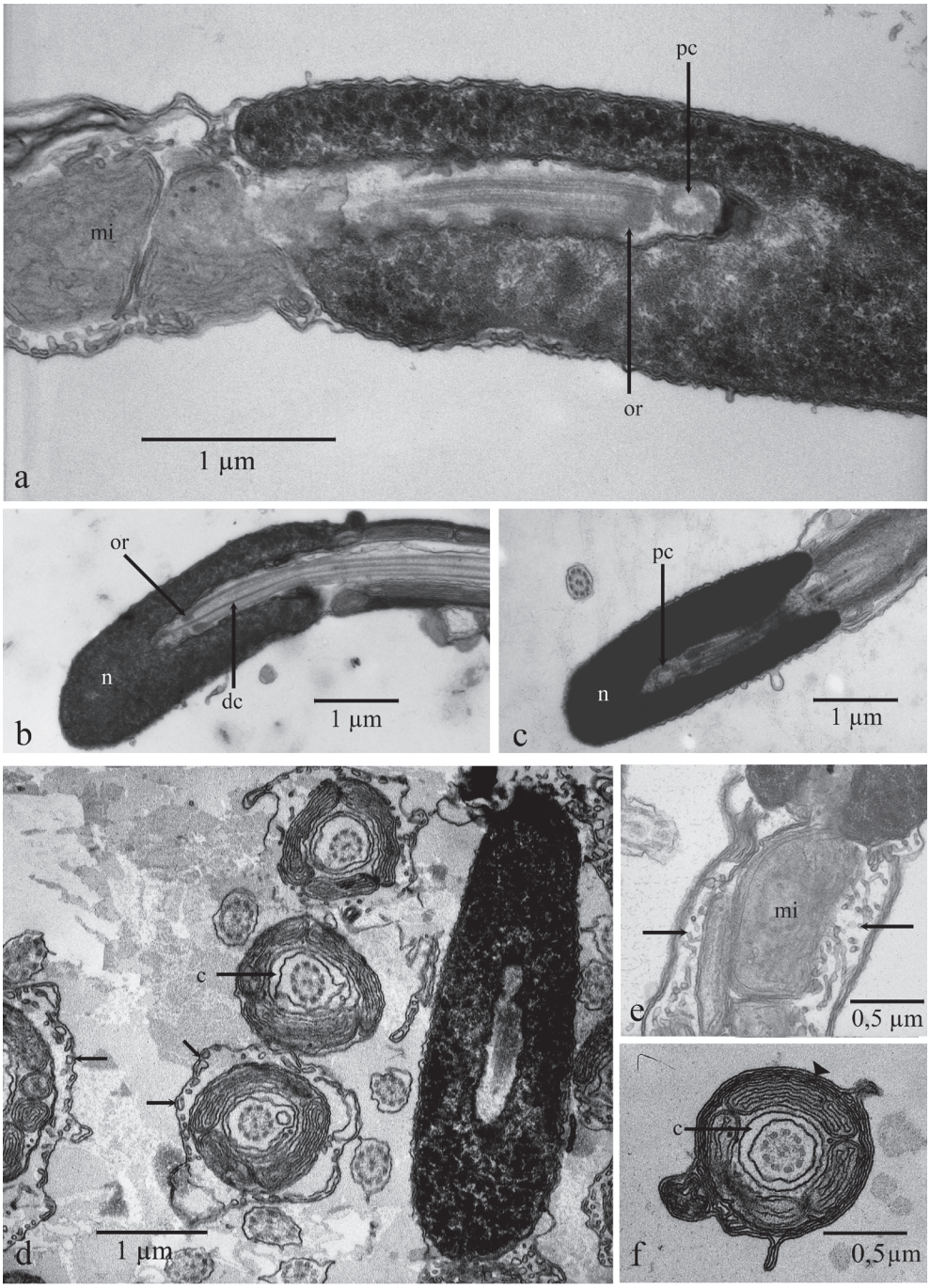
The head lacks an acrosome and has an electron dense nucleus with a deep axial nuclear fossa its length being about half or more the nuclear length (fig. 2 a). The chromatin consists of numerous large, electron dense, granular masses (figs. 2 a, b). The fossa contains the centriolar complex, i.e. the proximal and distal centriole; centrioles are arranged in a right angle to each other (figs. 2 a, b, c). The distal centriole serves as basal body of the axoneme. Its anterior end is embedded in an osmiophilic ring (figs. 2 a, b).

The midpiece contains longitudinal rows of modified mitochondria. In some transverse sections are three, in others two more or less separated mitochondria that obviously are partly fused forming a network (figs. 1 d, 2 d, c). In transverse sections of the midpiece the mitochondrial cristae are largely vertical and paral-

Figs. 1 a-d: The spermatozoon of *Anableps anableps*; SEM micrographs (**a,b**) and low power TEM micrograph (**c**) of isolated spermatozoa showing the blunt head (h), the relatively long midpiece (m), and the single flagellum (f); **d** mature spermatozoa in a cyst sectioned at various levels.; 1 transverse section of the nucleus with the fossa (fo); 2 undefined sections showing the nucleus (n), the axoneme (a), the mitochondrial sleeve (mi) and peripheral membranes (arrowheads), 3 transverse sections of the midpiece with mitochondria (mi) and the cytoplasmic canal (c); 4, transverse section of the free flagellum; some show small side fins (arrows).

Abb. 1 a-d: Das Spermium von *Anableps anableps*; REM-Fotos (**a,b**) und TEM-Foto (**c**) isolierter Spermien mit stumpfem Kopf (h), relativ langem Mittelstück (m) und einem einzigen Flagellum (f); **d** reife Spermien in einer Cyste in unterschiedlichen Regionen angeschnitten; 1 Querschnitt durch den Kern mit Fossa (fo); 2 ungerichtete Anschnitte mit Kern (n), Axonem (ax), der mitochondrialen Manschette (mi) und peripheren Membranstapeln (Pfeilköpfe); 3 Querschnitte des Mittelstücks mit Mitochondrien (mi) und dem cytoplasmatischen Kanal (c); 4 Querschnitte des freien Flagellums; einige Flagellen sind seitlich leicht ausgezogen (Pfeile).


a
5 µm
f
m
h
b
1 µm
c
1 µm
h
m
f
d
1 µm
fo
1
n
n
n
n
4
ax
ax
mi
mi
mi
3
2
2
3
3
4
c
c



Figs. 2 a-f: TEM micrograph of the spermatozoon of *Anableps anableps*; **a** nucleus (n) with fossa and proximal centriol (pc) and osmiophilic ring (os); mitochondria (mi); peripheral microtubules (arrows); **b, c** longitudinal section of the nucleus (n) with fossa and proximal (pc) and distal (dc) centriol; osmiophilic ring (or); **d** transverse sections of the midpiece with peripheral microtubules (arrows); note three individual mitochondria in the .uppermost horizontal profile of the midpiece; cytoplasmic canal (c); nucleus (n);

lel. At the outer periphery there are many membranes forming cisternae that appear to be continuous with the mitochondria (figs. 1 d, 2 f). Mitochondria form a sleeve (= mitochondrial collar) that surrounds the proximal region of the flagellum. The space between the mitochondrial sleeve and the axoneme is the cytoplasmic canal, which runs longitudinally from the anterior to the posterior end of the midpiece (figs. 1 d, 2 d, f), where it opens to the exterior. Beginning near the posterior end of the nucleus and extending along the length of the midpiece, a row of accessory perhaps stabilizing microtubules is present at the periphery of the flagellum (figs. 2 d, e). Glycogen seems to be absent in the midpiece.

The flagellum has an axoneme composed of the 9+2 microtubular doublet construction (fig. 1 d). The central pair of microtubules is absent from the origin of the flagellum to its entrance in the cytoplasmic canal. The flagellum ends in a small tip; the free portion of the flagellum has very small side fins (fig. 1 d).

4. Discussion

As revealed by our SEM and TEM-micrographs, the spermatozoon of *Anableps anableps* represents an anacrosomal introsperm (for terminology see Jamieson 1991). The spermatozoon has a moderately elongated, but not pointed nucleus and a midpiece slightly longer than the head with numerous modified mitochondria. Such characters are frequently observed in spermatozoa of inseminating species (Jamieson 1991, Mattei 1991).

The single TEM-picture of a mature spermatozoon available from the related *Jenynsia lineata* (see Dadone & Narbaitz 1967) does not allow estimating dimensions such as the length of spermatozoa and to asses exactly the shape of the head. However, as in *A. anableps*, the fossa appears deep containing two centrioles. The authors describe modified mitochondria as "a system of parallel membranes", which "appears to be continuous in longitudinal sections while in transverse sections it shows a lateral interruption", p. 215. They resemble those found in *A. anableps*. Here, but to our opinion also in *J. lineata*, in transverse sections occasionally individual mitochondria can be distinguished, which however, appear to fuse in part forming a network. A submitochondrial highly ordered network found in *J. lineata* spermatozoa between the mitochondrial sleeve and the cytoplasmic canal was not found in *A. anableps*.

The most conspicuous differences of *Anableps* spermatozoa, e.g. to the highly modified spermatozoa of poeciliids, are the broad and blunted head in combination with a relatively long midpiece, presence of the proximal centriole and absence of a complex submitochondrial network (see Jamieson 1991), which is interestingly present in *J. lineata* (see above). The selective advantage of an elongated and pointed sperm head and the elongate midpiece (e.g., for sperm package, side-to-side alignment, energy supply for spermatozoa) has been discussed repeatedly and speculations regarding this topic have been summarized more recently for ostariophysan fishes by Burns et al. (2005).

---

e anterior end of the midpiece with peripheral microtubules (arrows); mitochondrium (mi), nucleus (n); **f** transverse section of the midpiece showing the cytoplasmic canal (c) and the peripheral membrane system continuous with the mitochondrial sleeve (arrowhead).

Abb. 2 a-f: TEM-Aufnahmen der Spermien von *Anableps anableps*. **a** Kern (n) mit mit Fossa und proximalem Centriol (pc) und osmiophilem Ring (or); Mitochondrien (mi); periphere Mikrotubuli (Pfeile); **b,c** Längsschnitt durch den Kern (n) mit Fossa sowie proximalem (pc) und distalem (dc) Centriol; osmiophiler Ring (or); **d** Querschnitte des Mittelstücks mit peripheren Mikrotubuli (Pfeile); im oberen Querschnitt liegen drei voneinander getrennte Mitochondrien; cytoplasmatischer Kanal (c), Kern (n); **e** Vorderende des Mittelstücks mit peripheren Mikrotubuli (Pfeile); Mitochondrium (mi), Kern (n); **f** Querschnitt des Mittelstücks mit cytoplasmatischem Kanal (c) und einem peripheren Membransystem, das eng mit den Mitochondrien assoziiert ist (Pfeilkopf).

We did not find noticeable amounts of glycogen in the midpiece of *A. anableps* spermatozoa. Thus, the energy source for sperm movement (and storage that, however, is unknown in *Anableps* species to our knowledge) may be mainly exogenous, e.g. from sugars in the ovarian cavity as discussed for the viviparous *Poecilia reticulata* (Cyprinodontiformes), and the perciform, *Cymatogaster aggregata* (Perciformes) (see GARDINER 1978).

5. Acknowledgements

We are indebted to Mrs. Marion NISSEN and Mr. Marcel BRENNER for technical help.

6. Literature

BURNS, J.R. 1991. Testis and gonopodium development in *Anableps dowi* (Pisces: Anablepidae) correlated with pituitary gonadotropic development. J. Morphol. 210, 45-53.

BURNS, J.R., & S.H. WEITZMAN. 2005. Insemination in ostariophysan fishes, pp. 105-132. In: Viviparous fishes (URIBE, M.C., & H.J. GRIER, eds). New Life Publications, Homestead, Florida.

DADONE, L., & R. NARBAITZ. 1967. Submicroscopic structure of spermatozoa of a cyprinodontiform teleoost, *Jenynsia lineata*. Z. Zellforsch. 80, 214-219.

GARDINER, D.M. 1978. Utilization of extracellular glucose by spermatozoa of two viviparous fishes. Comp. Biochem. Physiol. 59A, 165-168.

GARMAN, S. 1895. Sexual rights and lefts. Amer. Nat. 29, 1012-1014.

GHEDOTTI, M.J. 1998. Phylogeny and classification of the Anablepidae (Teleostei: Cyprinodontiformes), pp. 561-582. In: Phylogeny and classification of Neotropical fishes (MALABARBA, L.R., R.E. REIS, R.P.VARI, Z.M.S. LUCENA, & C.A.S. LUCENA, eds). Museu de Ciencias e Tecnologia, PUCRS, Porto Alegre, Brazil.

GRIER, H.J. 1981. Cellular orgnization of the testis and spermatogenesis in fishes. Amer. Zool. 21, 345-357

GRIER , H.J., J.R. BURNS, & J.A. FLORES. 1981. Testis structure in three species of teleosts with tubular gonopodia. Copeia 1981: 797-801.

JAMIESON, B.G.M. 1991. Fish, evolution and systematics: evidence from spermatozoa. Cambridge University Press, Cambridge.

KARNOVSKY, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 17, 137A-138A.

MATTEI, X. 1991. Spermatozoon ultrastructure and its systematic implications in fishes. Can. J. Zool. 69, 3038-3055.

MATTIG, S., & H. GREVEN. 1994. Beobachtungen zur Balz und Paarung des Vieraugenfisches *Anableps anableps* (LINNÉ, 1758) (Anablepidae, Cyprinidontiformes). Z. Fischkunde 2, 167-192.

PARENTI, L.R. 1981. A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull. Amer. Mus. Nat. Hist. 168, 335-557.

PARENTI, L.R., & H.J. GRIER. 2004. Evolution and phylogeny of gonad morphology in bony fish. Integr. Comp. Biol. 44, 333-348.

SPURR, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31-43.

TURNER, C.L. 1950. The skeletal structure of the gonopodium and gonopodial suspensorium of *Anableps anableps*. J. Morphol. 86, 329-366.

Received: 01.04.2006
Accepted: 02.05.2006