

# Extraordinarily long sperm in the socially monogamous cichlid fish *Pelvicachromis taeniatus*

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**Abstract** The main function of the spermatozoon is the transfer of the male haploid genome during fertilisation. In animals in general and in fishes in particular, there is huge variation in sperm size. In fishes, sperm size ranges from 13  $\mu\text{m}$  in *Mugil cephalus* to nearly 100  $\mu\text{m}$  in the channel catfish *Ictalurus punctatus*. We examined intra-specific variation in sperm morphometry in the socially monogamous cichlid *Pelvicachromis taeniatus* using scanning electron microscopy. The mean total sperm length of nearly 70  $\mu\text{m}$  was extraordinarily large for cichlids. Furthermore, within-male variation was remarkably high. To our knowledge, *P. taeniatus* produces the longest cichlid sperm ever documented. Several hypotheses concerning the adaptive significance of these results are presented.

**Keywords** *Pelvicachromis taeniatus* · Cichlid fish · Sperm length · Sperm competition

## Introduction

The main function of the spermatozoon is the transfer of the male haploid genome during fertilisation. In the vast majority of fish species, fertilisation is external and sperm morphology should be adapted to ensure efficient fertilisation of all eggs in a given environment. A spermatozoon consists of a head, which contains the male haplotype, a mid-piece with mitochondria and a flagellum, which is driven by the energy the mitochondria supply. In animals in

general and fishes in particular, there is huge variation in sperm size (Mattei 1991; Stockley et al. 1997; Snook 2005). In fishes, sperm size ranges from 13  $\mu\text{m}$  in *Mugil cephalus* to nearly 100  $\mu\text{m}$  in channel catfish *Ictalurus punctatus* (Stockley et al. 1997) and in cichlids, sperm size ranges from 15  $\mu\text{m}$  to 32  $\mu\text{m}$  (Balshine et al. 2001; Fishelson 2003).

Most studies concerning sperm variation focus on comparisons across species. The present study deals with intra-specific variation of sperm morphometry in the socially monogamous cichlid, *Pelvicachromis taeniatus* (Boulenger).

## Materials and methods

### Fish

*P. taeniatus* is a small, stream-dwelling cichlid of Western Africa, which breeds in caves. Males used in this study were unrelated F1-descendants of wild-caught fish of the Moliwe population near Limbe, West Cameroon. Fish were raised in full-sib groups at  $25\pm 1^\circ\text{C}$  under 12/12 L/D lighting regime. Fifteen brightly coloured, courting males in the reproductive age were killed by decapitation, testes were carefully excised and sperm investigated.

### Sperm morphometry

Spermatozoa were prepared for scanning electron microscopy by fixing them for at least 6 h at  $4^\circ\text{C}$  in 2.5% glutaraldehyde in 200  $\text{mmol}^{-1}$  cacodylate buffer containing 4% sucrose. Glutaraldehyde fixation was washed out and samples were dehydrogenated along an ascending series of ethanol (40–98%), dried, evaporated with gold and inves-

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tigated with a scanning electron microscope (Leitz AMR 1000).

Pictures of sperm were scanned and digitalised using DISS (Digital Image Scanning System, Point Electronic, Halle, Germany) and DIPS (Digital Image Processing, Point Electronic, Halle, Germany), respectively. Sperm tails were magnified up to  $\times 3,000$ , sperm heads and mid-pieces up to  $\times 10,000$ . The mid-piece was measured from the anterior end of the head to the beginning of the flagellum. Sperm measurements were conducted using the Diskus software (C.H. Hilgers, Königswinter, Germany).

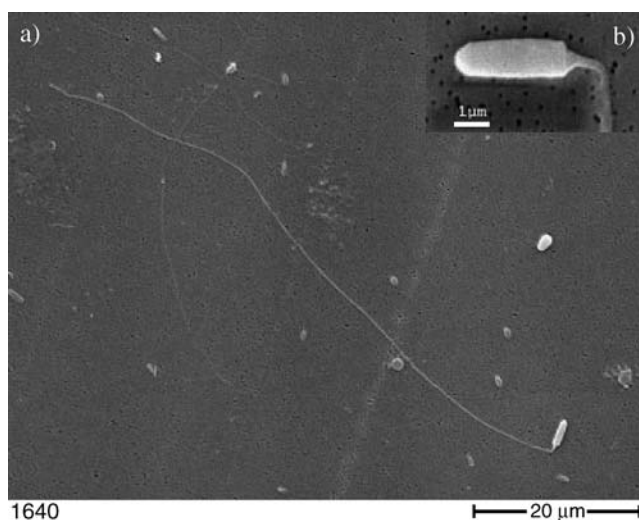
#### Statistical analysis

Parametric statistics were applied because sperm dimensions were normally distributed and their variances homogeneous. To estimate within and among male variation, repeatabilities were calculated as coefficients of intra-class correlation (Sokal and Rohlf 1995). The standard errors for repeatability values were calculated after Becker (1992).

#### Results

The total sperm length per male averaged  $69.65 \mu\text{m} \pm 11.35$  ( $N=15$ ; Fig. 1). The length of the head, mid-piece and flagellum per male averaged  $3.46 \mu\text{m} \pm 0.31$ ,  $0.44 \mu\text{m} \pm 0.08$  and  $65.75 \mu\text{m} \pm 11.28$ , respectively. There was a significant positive association between mean flagellum length and mean head length ( $r=0.61$ ,  $N=15$ ,  $P=0.012$ ).

There was significant between-male variance in sperm head length (ANOVA,  $F_{14, 209}=2.26$ ,  $P=0.007$ ), mid-piece length ( $F_{14, 209}=5.98$ ,  $P<0.0001$ ) and flagellum length ( $F_{14, 209}=2.42$ ,  $P=0.004$ ). Consistent differences between males were



**Fig. 1** Scanning electron micrographs of a spermatozoon of *P. taeniatus*. **a** Habitus, magnification  $\times 1,640$ ; **b** head and mid-piece, magnification  $\times 4,800$

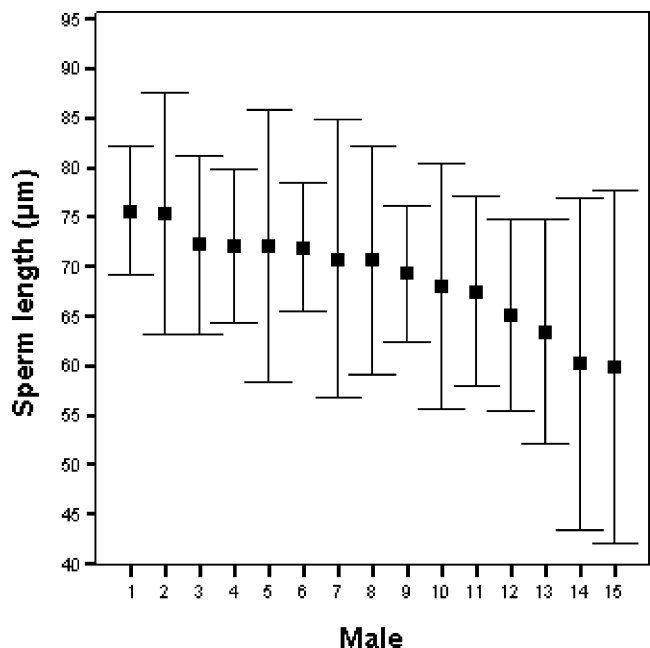
also found for the total sperm length ( $F_{14, 209}=2.48$ ,  $P<0.003$ ; Fig. 2). The repeatabilities for the single sperm components were  $0.0825 \pm 0.0529$  (head),  $0.2567 \pm 0.0900$  (mid-piece),  $0.0928 \pm 0.0557$  (flagellum) and  $0.0962 \pm 0.0574$  (total length).

#### Discussion

To our knowledge, *P. taeniatus* produced the longest cichlid sperm ever documented. The spermatozoa were twice as long as in *Telmatochromis vittatus*, the cichlid with the largest sperm known until now of 35 reported species. There was a positive isometric relationship between mean sperm head length and mean sperm flagellum length, which indicates similar selection pressures or a positive genetic correlation of the two components, respectively. A similar correlation between sperm head length and sperm flagellum length was found for example in bank voles *Clethrionomys glareolus* (Morrow and Gage 2001), whereas a reversed association was observed in Atlantic salmon *Salmo salar* (Gage et al. 2002).

Repeatability of each sperm component was relatively low. Of the total sperm variation, 75–90% could be explained by variation within males. This result was surprising because similar investigations on other taxa showed only a narrow range of variation within males (Gage et al. 1998; Morrow and Gage 2001; Birkhead et al. 2005).

What could be the adaptive significance of this extraordinarily large sperm size? Recent research stresses the



**Fig. 2** Mean total sperm length per male in  $\mu\text{m}$  ( $\pm$ SD) for 15 males arranged as to decreasing sperm length. Per male 6–18 sperm were measured

impact of sperm competition, i.e. the competition between sperm of different males in fertilising an egg (Parker 1970), on the evolution of sperm phenotype (Bernasconi et al. 2004; Snook 2005). Sperm competition theory provides several hypotheses concerning sperm size, which need to be tested in *P. taeniatus*. With increasing risk of sperm competition, an increase in sperm size is expected under certain conditions (Parker 1993) when males producing longer sperm are more competitive. In a comparative study in African cichlids, Balshine et al. (2001) documented that males of polygamous species feature longer sperm than males of monogamous species, which was interpreted as an adaptation to sperm competition. *P. taeniatus* typically spawns in pairs and the observed small relative testes size (Thünken et al., unpublished data) suggests a rather monogamous mating system. However, sneaking (Taborsky 1998) cannot be ruled out completely and therefore, further investigations are necessary to clarify the relation between sperm competition and sperm size in *P. taeniatus*.

Generally, it is assumed that sperm competition occurs between sperm of different males. However, as Parker and Begon (1993) pointed out, sperm competition can also arise within sperm of a single male, which can lead to the evolution of large sperm in the absence of inter-male sperm competition. Because the observed large within-male variation is possibly a further subtle hint for haploid control of sperm size (Morrow and Gage 2001), it would be interesting to explore the potential role of haploid selection (Joseph and Kirkpatrick 2004) on sperm size evolution in *P. taeniatus*.

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