Micron 69 (2015) 56–61

Contents lists available at ScienceDirect

Micron

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

Comparative ultrastructure of spermatozoa of the redclaw *Cherax quadricarinatus* and the yabby *Cherax destructor* (Decapoda, Parastacidae)

Antonín Kouba*, Hamid Niksirat, Martin Bláha

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, CZ-389 25 Vodňany, Czech Republic

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 30 October 2014
Accepted 6 November 2014
Available online 14 November 2014

Keywords:
Acrosome
Crayfish
Decapoda
Spermatozoon
Transmission electron microscopy

ABSTRACT

Ultrastructure of spermatozoa of redclaw *Cherax quadricarinatus* and yabby *Cherax destructor* were described and compared. The acrosome complex and nucleus are located at the anterior and posterior region of the spermatozoon, respectively. The acrosome is a complex vesicle divided into two parts: the main body of the acrosome appears as a dense cup-shaped structure in longitudinal sagittal view, with the subacrosome zone occupying the central area of the vesicle. The acrosome is larger in *C. quadricarinatus* (width 2.37 ± 0.27 μm, length 1.31 ± 0.23 μm) than in *C. destructor* (width 1.80 ± 0.27 μm, length 1.01 ± 0.15 μm). There was no significant difference in L:W ratios of the studied species. The subacrosome zone in both species consists of two areas of different electron density. The nucleus is substantially decondensed and irregular in shape, with elaborate extended processes. The examined species exhibited a well-conserved structure of crayfish spermatozoon, similar to those of *Cherax cainii* and *Cherax albidus*. Small acrosome size, the absence of radial arms, and an extracellular capsule seem to be the morphological features that mostly distinguish *Cherax* from the Astacidae and Cambaridae.

1. Introduction

The spermatozoon ultrastructure is an important aspect of basic knowledge of animal reproductive biology (Bian et al., 2013; Santos et al., 2013; Shi et al., 2014), and has traditionally been employed in taxonomic as well as phylogenetic studies (Jamieson, 1991; Justine, 1991; Mattei, 1991; Tudge, 1997, 2009).

The freshwater crayfishes, a group comprising over 640 identified species in 3 families, play key roles in physical and biological modification of ecosystems (Crandall and Buhay, 2008). Detailed studies on spermatozoon ultrastructure of crayfish are scarce and include those of *Astacus astacus*, *Astacus leptodactylus*, *Austropotamobius torrentium*, and *Pacifastacus leniusculus* in the Astacidae (Dudenhausen and Talbot, 1979, 1982; López-Camps et al., 1981; Niksirat et al., 2013a,b) and *Cambaroides japonicus*, *Cambarus* sp., *Procambarus clarkii*, *Procambarus leonensis*, and *Orconectes limosus* in Cambaridae (Moses, 1961a,b; Yasuzumi et al., 1961; Anderson and Ellis, 1967; Felgenhauer and Abele, 1991; Niksirat et al., 2013a).

The second largest crayfish family, Parastacidae, comprises over 170 recognized species (Crandall and Buhay, 2008). Spermatozoon ultrastructure has been reported only for two parastacid species (Beach and Talbot, 1987; Jamieson, 1991), *Cherax cainii*[1] and *Cherax albidus*, both belonging to the phylogenetic group of this genus inhabiting southwestern Australia (Munasinghe et al., 2004). An et al. (2011) provides description of the male reproductive system and spermatogenesis with some notes on sperm ultrastructure in *Cherax quadricarinatus*. The aim of present study is to specifically describe and compare spermatozoa of *C. quadricarinatus* and *Cherax destructor*, the best known and most economically important representatives of *Cherax* inhabiting northern and eastern Australia, respectively.

2. Materials and methods

Adult male redclaw (*C. quadricarinatus*) and yabby (*C. destructor*) crayfish were purchased from a pet retailer. Freshly ejaculated

* Corresponding author. Tel.: +420 389 034 745.
E-mail address: akouba@frov.jcu.cz (A. Kouba).

http://dx.doi.org/10.1016/j.micron.2014.11.002
0968-4328/

[1] We assume that the smooth marron, a common and widespread species largely involved in aquaculture formerly called *C. tenuimanus* was examined. See Austin and Ryan (2002) for details.

spermatophores from 3 males per species were obtained via electrical stimulation (AC250K2D, Diametral, Czech Republic; Jerry, 2001), immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 4 days at 4 °C, washed in buffer, post-fixed in 4% osmium tetroxide for 2 h, washed in buffer, dehydrated through an acetone graded series (30, 50, 70, 90, 95, and 100% for 15 min each), and embedded in resin (EMbed 812). A series of ultra-thin sections were cut using an UCT ultramicrotome (Leica Microsystems GmbH, Austria), mounted on copper grids, double-stained with uranyl acetate and lead citrate, and examined with a 1010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) operating at 80 kV. The length (L) and width (W) of the acrosome and the L:W ratio (Jamieson, 1991; Klaus et al., 2009) were determined from the recorded micrographs using ImageJ software (U. S. National Institutes of Health, Bethesda, MD, USA). After confirming normality and homoskedasticity with Kolmogorov–Smirnov and Cochran's C tests, respectively, differences among conspecific males were assessed by one-way ANOVA. As no differences were detected (n = 22, 42, 27 and 32, 42, 30 spermatozoa from each of 3 specimens of *C. quadricarinatus* and *C. destructor*, respectively), data for each species were pooled and compared by Student's t-test. For all statistical tests, $P < 0.05$ was considered significant. Data are expressed as the mean ± s.d.

3. Results

3.1. Comparison of acrosome dimensions

There was no significant (P = 0.20) difference in acrosome L:W ratio of *C. quadricarinatus* (0.57 ± 0.08, range 0.39–0.75, n = 104) from that of *C. destructor* (0.55 ± 0.08, range 0.40–0.73, n = 91). The width of the acrosome was significantly ($P < 0.01$) greater in *C. quadricarinatus* (2.37 ± 0.27 μm, range 1.58–3.27) than in *C. destructor* (1.80 ± 0.27 μm, range 1.13–2.55). A similar significant ($P < 10^{-6}$) pattern was observed in the acrosome length in *C. quadricarinatus* (1.31 ± 0.23 μm, range 0.87–1.94) compared to *C. destructor* (1.01 ± 0.15 μm, range 0.73–1.39).

3.2. Morphological features

In both species, an acrosome complex and the nucleus are located in the anterior and posterior region of the spermatozoon, respectively. The acrosome complex consists of the acrosome main body and subacrosome zone. The membrane lamellar complex is mostly located between the acrosome complex and the nucleus (Figs. 1A and 2A). Although the appearance of the spermatozoon is similar in the species, their shapes vary and are asymmetric.

The main body of the acrosome appears as a dense cup-shaped structure in longitudinal sagittal section (Figs. 1A–B and 2A). In *C. quadricarinatus*, it consists of two homogenous layers of different electron densities. The denser layer occupies the inner area of the acrosome base and extends anteriorly (Fig. 1B–C). Such a clear pattern is not obvious in *C. destructor* (Fig. 2B). In a minority of observed *C. quadricarinatus* spermatozoa, regions of lower electron density were observed at the base of the main acrosome body within the denser layer. These regions contain moderately and highly electron dense materials resembling the material in the subacrosome zone (Fig. 1C).

Electron-lucent canals at the acrosome base of *C. destructor* are narrower, less electron-lucent, and more abundant compared to regions of lower electron density in *C. quadricarinatus* (cf. Figs. 1C and 2B). Electron-lucent canals in *C. destructor* were often seen to possess a single small electron-dense component at the periphery (Fig. 2B). In rare instances in both species, these electron-lucent areas were observed at the apical region of the acrosome (Fig. 1D and Fig. 2C).

The subacrosome zone is divided in two regions distinct in structure and electron density. Most of the subacrosome zone was usually occupied by flocculent material of moderate electron density. An area of reduced mass is present adjacent to the inner area of the acrosome main body, formed by a fibrillar and granular network containing components of differing electron densities (Figs. 1A–D and 2A–C).

In both species, a membrane lamellar complex composed of stacks of highly folded, convoluted membranes often surround the moderately electron-dense material (Figs. 1E and 2D). Its position is more often lateral to the acrosome, but may appear distributed asymmetrically in longitudinal sagittal section (Figs. 1A–B and 2A). The membrane lamellar complex may be partly or almost completely missing on one or both sides of acrosome and observed adjacent to the nucleus (Figs. 1A and 2D–E).

Membranous lamella, a recognizable asymmetric structure appearing as electron lucent and dense concentric lines, is present in both species (Figs. 1F and 2F). There is usually one membranous lamella, but two smaller membranous lamellae may be present, usually located within the membrane lamellar complex close to the acrosome. Functional mitochondria characterized by cristae were not observed. A single centriole was recorded in one *C. destructor* specimen (Fig. 2E).

The subacrosome zone abuts on the nucleus, but is clearly separated by a multi-layered membrane (Fig. 3A–B). In the *C. quadricarinatus* spermatozoon, the subacrosome zone extends toward the nucleus to a greater extent than seen in *C. destructor*. The nucleus is enclosed by a continuous irregularly shaped, substantially decondensed nuclear envelope (Fig. 1B) occupying a broad area (Figs. 1A and 2A). Elaborate processes, sometimes with convoluted infoldings, extend from the nuclei of the studied species (Figs. 1A and 3C–D). Deposits of highly electron-dense material were observed, although rarely, on the surface of the nucleus in *C. destructor* (Fig. 2A). The extracellular matrix surrounding spermatozoa is similar in *C. quadricarinatus* and *C. destructor*, containing small granules and filaments (Fig. 1A–B), with additional lamellae in *C. destructor* (Figs. 2A and 3C).

4. Discussion

Acrosomes of described *C. quadricarinatus* and *C. destructor* spermatozoa are the smallest reported in any crayfish (mean width 2.37 and 1.80 μm, respectively). Beach and Talbot (1987) reported the width of the *C. albidus* and *C. cainii* acrosome to be approximately 2 μm, and Jamieson (1991) confirms this for *C. cainii*. Thus, a small acrosome seems to be typical for this genus. However, confirmation of this for the Parastacidae in general requires investigation of a greater range of species and genera, as measurements in Cambaridae species indicate substantial variability (mean width 2.45 and 4.77 μm in *P. clarkii* and *O. limosus*, respectively; Niksirat et al., 2013a). On the contrary, much larger acrosomes have been reported in Astacidae *A. torrentium* (mean width 8.01 μm) and *A. astacus* (mean width 11.66 μm) (Niksirat et al., 2013a,b). The shape of the acrosome in *C. quadricarinatus* and *C. destructor* (mean L:W ratio 0.55 and 0.57, respectively) is within a range usually reported for crayfish (0.5–0.6) suggesting a depressed acrosome (Jamieson, 1991; Niksirat et al., 2013a,b). The most depressed acrosome has been reported in *O. limosus* (mean L:W ratio 0.36) (Niksirat et al., 2013a).

Although representing different phylogenetic groups of *Cherax* (Munasinghe et al., 2004), spermatozoa of both *C. quadricarinatus* and *C. destructor* show similarity in general ultrastructure to *C. cainii*, and *C. albidus* (Beach and Talbot, 1987; Jamieson, 1991).