

Sexual reproductive biology of *Brachionus quadridentatus* Hermanns (Rotifera: Monogononta)

Estudio de la biología sexual reproductiva del rotífero *Brachionus quadridentatus* Hermanns (Rotifera: Monogononta)

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ABSTRACT

This study examined important aspects of the sexual reproductive biology of the monogonont rotifer *Brachionus quadridentatus* Hermanns. Observations on the following was made: 1) Morphological description of the male, 2) An analysis of mating behavior, 3) An analysis of female and male life-span at 25°C, and 4) Morphometric characterization of the three types of eggs known in this species and determination of hatching percentages of sexual eggs at 20 and 25°C. SEM photographs of the male are included, the female and parthenogenetic and sexual eggs. Some complementary photographs with the light microscope are also included. The mating behavior of *B. quadridentatus* is similar to those of other brachionids. Attempted copulations lasted on average 12.4 s, and completed copulations lasted on average 71.4 s. *B. quadridentatus* is the *Brachionus* species with the longest duration of copulation recorded so far. Photographs showing episodes of mating attempts and copulations are included. Mating attempts and copulations are similar to those of other members of *Brachionus*. A comparison of the mating of *B. quadridentatus* with that of other brachionids is also included.

Key words: Rotifer mating behavior, rotifer, evolution, taxonomy.

RESUMEN

Este estudio examinó aspectos importantes de la biología sexual y reproductiva del rotífero monogononte *Brachionus quadridentatus* Hermanns. Se han hecho las siguientes observaciones: 1) Descripción morfológica del macho, 2) Un análisis del comportamiento sexual 3) Un análisis de la longevidad de hembras y machos a 25°C, y 4) Una caracterización morfométrica de los tres tipos de huevos que se conocen en esta especie, y se han determinado los porcentajes de eclosión de los huevos sexuales a 20 y 25°C. Se ha documentado con fotografías (empleando microscopía electrónica de barrido), al macho, la hembra, y los huevos partenogenéticos y sexuales. Algunas microfotografías complementarias realizadas con el microscopio de luz, también se incluyen. El comportamiento sexual de *B. quadridentatus* es similar al de otros brachionidos. Los intentos de cópula duraron en promedio 12.4 segundos, y las copulaciones completas duraron en promedio 71.4 segundos. *B. quadridentatus* es la especie del género *Brachionus* con la mayor duración de cópula que se haya registrado hasta miembros del género *Brachionus*. Se incluye una comparación del comportamiento sexual de

B. quadridentatus con la de otras especies de ahora. Se ha incluido una secuencia de microfotografías con los diferentes episodios de la cópula. Los intentos de cópula son similares a los de otros brachionidos.

Palabras clave: Comportamiento sexual de rotíferos, evolución, taxonomía.

INTRODUCTION

Rotifers are cyclic parthenogenetic animals that are important components of the zooplanktonic community of a typical lake. Rotifer populations are commonly represented by females with males appearing sporadically (Pennak, 1989). That is perhaps the reason that mating behavior of rotifers is poorly known in spite of being studied for over thirty years (Velázquez-Rojas *et al.* 2002). Actual understanding of mating behavior in rotifers started with Gilbert (1963) who demonstrated that contact chemoreception is used by male rotifers to identify conspecific females in *Brachionus angularis* Gosse, and *B. calyciflorus* Pallas. In 1983, Snell & Hawkinson described the mating behavior of *B. plicatilis* Müller, and described its different steps. Mating behavior in *B. plicatilis* is divided into five phases which correspond to: encounter, circling, coronal localization, sperm transfer, and dissociation (Nogradi *et al.* 1993). Snell *et al.* (1995) have demonstrated that the mating behavior of male *B. plicatilis* is based on the recognition of a mate recognition pheromone (MRP) a glycoprotein that is both necessary and sufficient to elicit male mating behavior, representing a highly efficient mechanism to recognize conspecifics.

Given the great diversity of rotifers, it is reasonable to expect important differences in the mating behavior of rotifers (Velázquez-Rojas *et al.* 2002). Aloia & Moretti (1973) concluded that the mating behavior of *Asplanchna brightwelli* Gosse is different in four aspects to that of the two *Brachionus* species described by Gilbert (1963). Velázquez-Rojas *et al.* (2002) argued that the coronal localization step is irrelevant to several species of rotifers, including some members of the family Brachionidae.

Mating behavior is known in six different families of monogononts (Asplanchnidae, Brachionidae, Epiphaniidae, Euchlanidae, Lecanidae, and Trichocercidae), eight genera and about twenty species (Rico-Martínez & Snell, 1997). Interspecific crosses have been performed among members of three families (Asplanchnidae, Brachionidae, and Lecanidae), but only in one family (Brachionidae), have cross-mating tests been made between members of different genera. In fact, the genus *Brachionus* is the most studied regarding sexual reproductive behavior. Several species of the genus have been studied; *B. angularis* (Gilbert, 1963), *B. bidentatus* Anderson (Rico-Martínez, 1999), *B. calyciflorus* (Gilbert, 1963), *B. patulus* Müller (Rao & Sarma, 1985), *B. plicatilis* (Snell & Hawkinson, 1983; Rico-Martínez & Snell, 1995a & b), *B. quadridentatus* (Ruttner-Kolisko,

1969), *B. rotundiformis* Tschugunoff (Snell & Hawkinson, 1983; Rico-Martínez & Snell, 1995a & b), *B. rubens* Ehrenberg (Halbach & Halbach-Keup, 1972; Pilarska, 1972), and *B. urceolaris* Müller (Ruttner-Kolisko, 1969).

The only data about the mating behavior of *Brachionus quadridentatus* in the literature is from the work of Gómez & Serra (1995) where they used a euryhaline strain of *B. quadridentatus* to compare mating with several clones of *B. plicatilis*. In this work they reported a 34.6 percentage of attempted mating for *B. quadridentatus* homogamic mating. Also, Ruttner-Kolisko (1969) reported the presence of hybrid females between *B. quadridentatus* and *B. urceolaris*. However no description of the mating or of the sexual reproductive characteristics of *B. quadridentatus* were included in these works.

Several aspects of the sexual reproductive biology of *Brachionus quadridentatus* are described making a detailed description of the male, and the mating behavior. A brief discussion of the mating behavior in the genus *Brachionus* is also included.

MATERIALS AND METHODS

Rotifer Culture. *Brachionus quadridentatus* was collected in a pond at the Water Treatment Plant of the Universidad Autónoma de Aguascalientes. The approximate geographical coordinates of this sampling site are 21° 53' 10" N and 102° 28' 54" W (geopositioner GPS 4000 XL Satellite Navigator, Magellan Inc., 1997). *B. quadridentatus* was cultured in EPA medium (192 mg NaHCO₃, 120 mg CaSO₄·2H₂O, 120 mg MgSO₄·7H₂O, and 8 mg KCl in 2 L; USEPA, 1985) prepared with deionized water (16-18 megaohms) from a Water Pro PS deionizer (Labconco Co.), and fed the green algae *Selenastrum capricornutum* Printz (strain UTEX No. 1648) grown in Bold's Basal Medium (Nichols, 1973).

Mating behavior test. Cross-mating assays were done according to Snell and Hawkinson (1983) with some modifications. Briefly, this assay involves placing two neonate females and two neonate virgin male (both < 18 hours old), into 50 µl of EPA medium. Then, the number of male mating attempts and completed copulations in five minutes was recorded in each of five replicates using different males and females. A mating attempt was recorded if a male circled around a female maintaining contact with his corona. A copulation was recorded when a male attached his penis to a female. Many complete sequences of the mating behavior of this species were video-

taped ($n = 16$) and recorded: a) duration of copulation, b) sites of mating behavior initiation and c) sites of copulation. Additionally, we obtained photographs of several steps of the mating behavior using a Hitachi Color Video Printer (Hitachi Co.).

Swimming speed estimation. Swimming speed was estimated by videotaping ten males at 75X magnification for several minutes. Then, the video was replayed with a clear acetate sheet taped to the monitor and the swimming path of a rotifer was traced for ten seconds. A cartometer was then used to measure the length of the path.

Life span data of the female and male. The life-span of the females and males (virgin and non-virgin) was recorded at 25°C. Parthenogenetic eggs were placed in 500 μ l of EPA medium in individual wells of a 24-well culture polystyrene plate (Costar Co.) kept in a bioclimatic chamber (Revco Co.) with fluorescent light (600-1100 lux) and a light/darkness cycle of 16:8 hours, and were reviewed each 2 hours before hatching, then newly born females were kept in an individual well with 1 ml of EPA medium with 1×10^6 cells/ml of *Selenastrum capricornutum*, placed in the same conditions of the eggs (25°C, 600-1100 luxes, 16:8 of L:D cycle) and observed each 12 hours. Similarly, unfertilized sexual eggs were placed in 300 μ l of EPA medium in individual wells and kept under the same conditions of the females, male eggs were reviewed each 2 hours before hatching, then newly born males were observed each 12 hours under the same conditions.

The non-virgin male treatment consisted of a set of two newly born females and a male per each individual well. The hatching percentages of 10 sets of 10 sexual eggs were recorded at two different temperatures (20 and 25 $\pm 2^\circ$ C), under the same conditions of light and L:D cycle. These temperatures were selected because our particular strain grew well under these conditions.

Morphology of males, females and eggs. Morphometric characterization of males and the three types of eggs known in this species: a) male unfertilized sexual egg, b) female parthenogenetic egg, and c) female fertilized sexual egg was performed by direct observations at 40X in a light microscope with a micrometer (Olympus Co., USA). SEM photographs of the female, male and the sexual and parthenogenetic eggs were taken with a JEOL LV 5900 Scanning Electron Microscope at the Universidad Autónoma de Aguascalientes. Samples were prepared for SEM as follows: the animals are placed in a small 1-cm X 1-cm vial with a 54 μ m mesh size. Samples were dehydrated by 10 ml gradual changes in alcohol (70, 80, 90, 96%), and then samples were left 24 to 48 hr in absolute alcohol. Later, the vial with the sample was placed in the critical point chamber (Tousimis Co.), to remove any humidity leftover, using liquid CO₂. Once all humidity has been removed from the sample, the later is mounted in small metal cylinders stubs where the sample has been previously adhered with tape. Finally the sample is covered with gold and observed at the electron microscope.

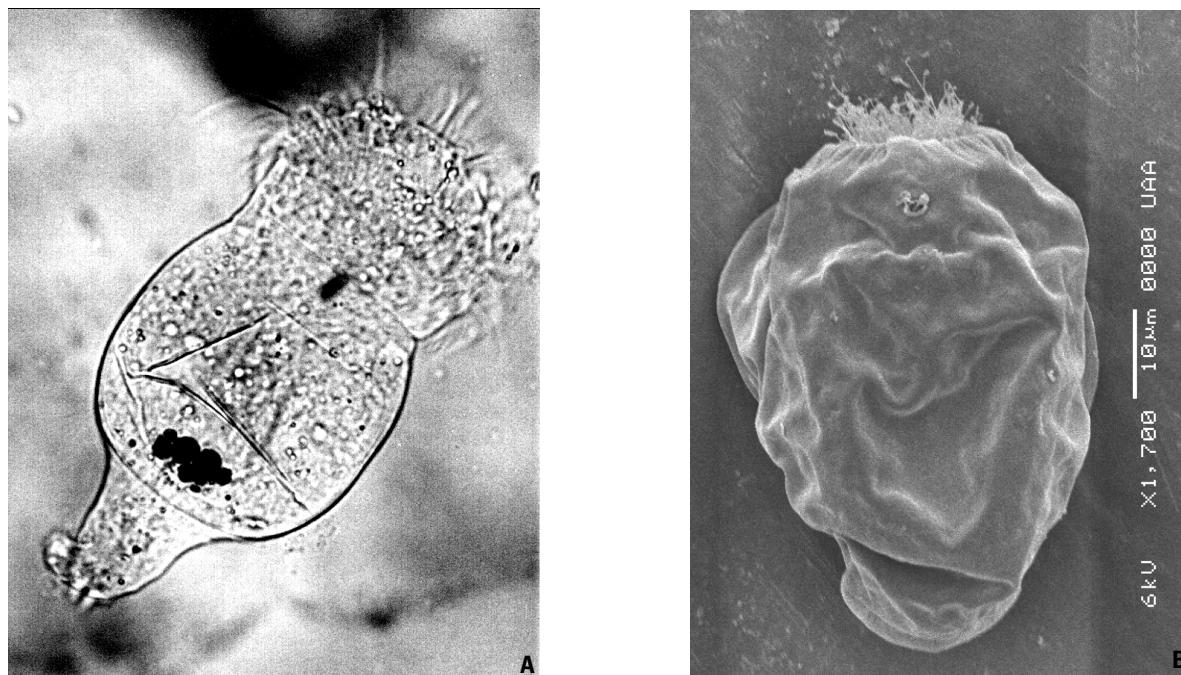
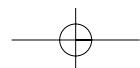


Figure 1. Male *Brachionus quadridentatus*. A) Photograph taken with the light microscope showing the corona, the foot with two toes, the stigma, and the sperm cells. B) SEM Photograph of the male showing the corona. The white bar at the bottom of the light microscope photographs equals 20 μ m.



RESULTS

Description of male *Brachionus quadridentatus*

The body consists of three regions: a) a retractile head where the ciliar corona is located (Figure 1B), b) the cylindrical trunk with a digestive system lacking a mastax and a primitive reproductive system with a single testis with two prostate glands and a short penis, c) the foot with two separate and mobile toes (Figure 1A).

Mating behavior

More than 30 complete episodes were observed and videotaped (total observation time was about twenty hours during one month) of mating behavior in *Brachionus quadridentatus*. Males initiated matings at the corona and foot opening. Attempted copulations lasted 12.4 s on average and copulations lasted 71.4 s on average, this difference is significant ($p = 0.038$; $df = 7$). Average 36.9 encounters every five minutes was observed (Table 1). Most copulation occurred at the corona, but almost all attempted copulations started at the foot opening. Curiously from the five replicates of the mating behavior test in this work, all attempted matings (18) occurred at the foot opening and none of them resulted in a completed copulation. The percentage of encounters becoming attempted matings was 7.86 and attempted mating becoming completed copulations was 0% (Table 1).

As in all brachionid species, the *B. quadridentatus* female assumes a passive role during mating while the male circles around the female, the coronal localization step is clear in this species and copulation occurs at the corona and foot opening.

Swimming speed

Males swam faster than females ($p = 0.003$, $n = 10$), but the swimming speed of *B. quadridentatus* males (Table 1) falls in the

range reported for other brachionid males (Rico-Martínez & Snell, 1997; Velázquez-Rojas *et al.*, 2002).

Life history data of the female and male

Female rotifers live longer than both virgin and non-virgin male rotifers at 25°C ($p < 0.001$; see Table 1). There was no significant difference between the life-span of virgin and non-virgin males ($p = 0.4738$, $df = 19$).

Morphology of females, males, and eggs

Males of *B. quadridentatus* are produced from sexual unfertilized eggs, as male brachionids are produced in general. Figure 2A shows a female carrying a fertilized sexual egg (or cyst). The cyst is longer and wider than the parthenogenetic eggs ($p < 0.01$, $n = 20$ in both cases). Figure 2B shows a female carrying three parthenogenetic eggs which are longer and wider than the male-producing eggs (Table 2). Figure 2C shows a female carrying a sexual unfertilized egg. This is the smallest of all eggs ranging from 40-60 mm long and 20-30 μm wide. The figure 2D shows a SEM photograph of a female. Females are longer and wider than males (Table 2; $p < 0.001$, $n = 20$, in both cases). Figure 3A shows the sexual unfertilized egg. Figures 3B and D shows the parthenogenetic egg with both light and scanning electron microscopy respectively. Figures 3C and 3D shows the fertilized sexual egg in light and scanning electron microscopy respectively.

DISCUSSION

The mating behavior of *B. quadridentatus* showed most of the characteristics of that of brachionid rotifers: a) Females are two to three times bigger than males, b) Males are faster swim-

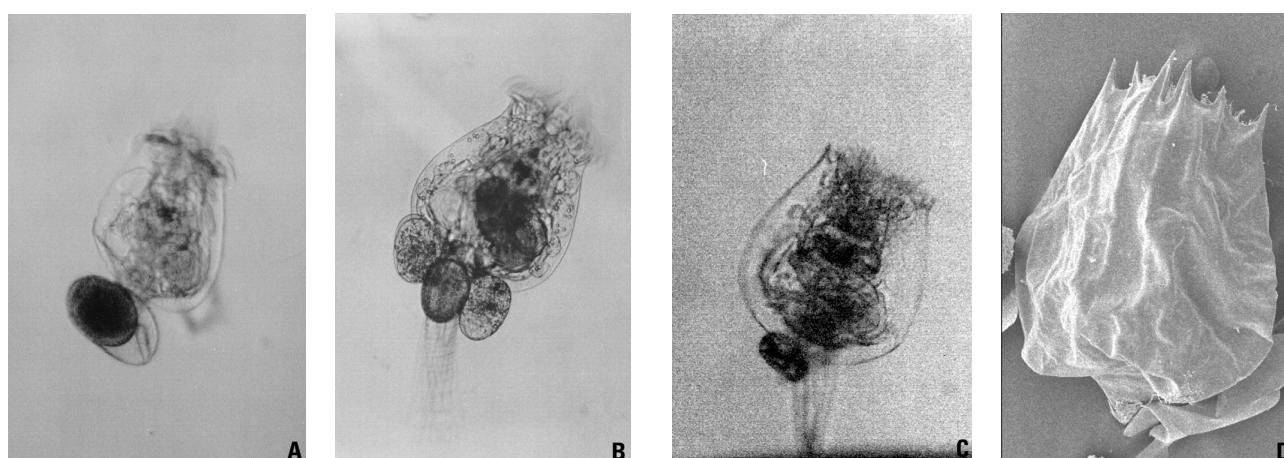


Figure 2. Female *Brachionus quadridentatus*. A) Female carrying a sexual fertilized egg. B) Female carrying three parthenogenetic eggs. C) Female carrying one unfertilized sexual egg. D) SEM photograph of a female in dorsal view showing details of the anterior spines. The white bar at the bottom of the light microscope photographs equals 20 μm .

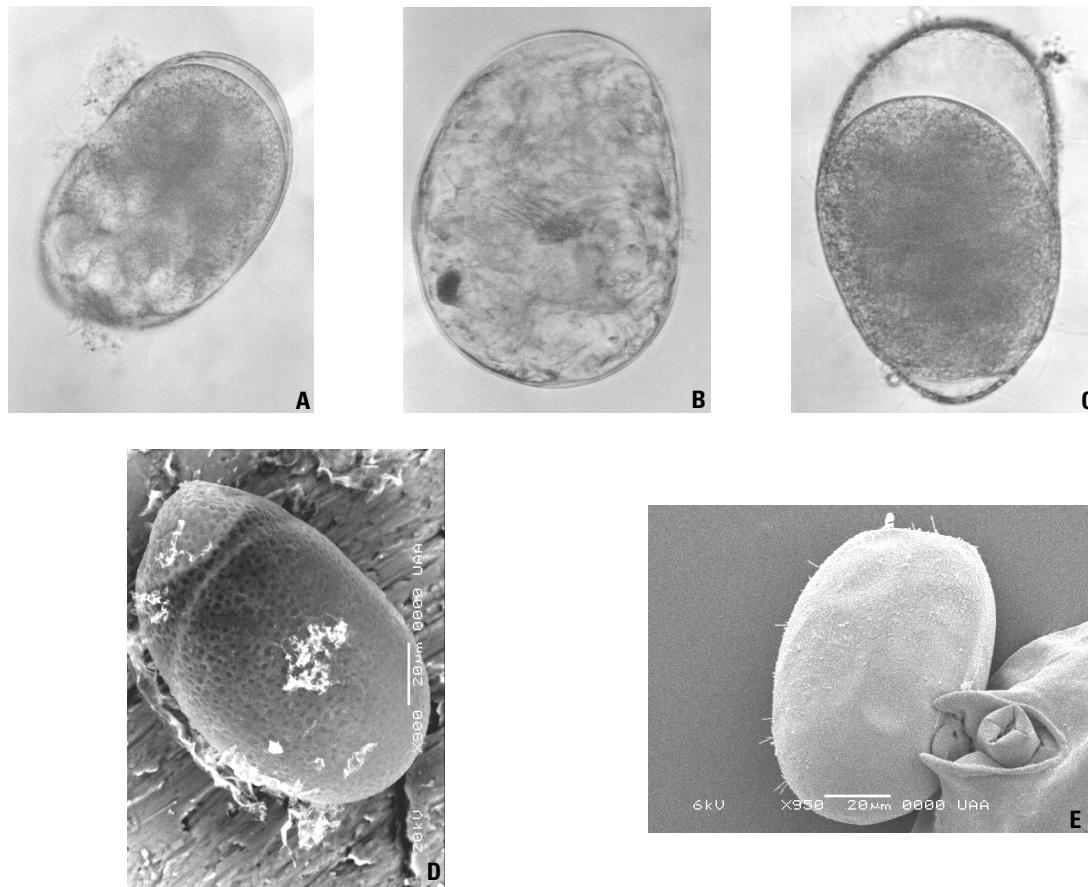
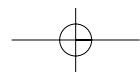


Figure 3. The different types of eggs of *Brachionus quadridentatus*. A) Unfertilized sexual egg with light microscopy. B) Parthenogenetic egg with light microscopy. C) Fertilized sexual egg with light microscopy. D) SEM photograph of the fertilized sexual egg. E) SEM photograph of the parthenogenetic egg. The white bar at the bottom of the light microscope photographs equals 20 μ m.

Table 1. Characteristics of the mating behavior, life span of females and males, and hatching percentages of sexual eggs of *Brachionus quadridentatus*. Numerical data correspond to the mean \pm one SD.

Duration of copulation in seconds (s)	71.38 \pm 61.26 (n=8)
Duration of attempted matings (s)	12.38 \pm 11.61 (n=16)
Number of encounters in 5-min periods	36.88 \pm 7.72 (n=8)
Female swimming speed (mm/s)	7.58 \pm 0.96 (n=10)
Male swimming speed (mm/s)	9.69 \pm 1.30 (n=10)
Percentage of encounters becoming mating attempts	7.86 \pm 10.23 (n=5)
Percentage of mating attempts becoming copulations	0 (n=5)
Sites of initiation of mating attempts on females	Mostly at foot opening (about 95%) and corona
Sites of copulation on females	Corona and foot opening
Life span of females at 25°C (h)	23.55 – 87.93 (n=22)
Life span of non-virgin males at 25°C (h)	15.75 – 35.25 (n=20)
Life span of virgin males at 25°C (h)	17.68 – 29.70 (n=21)
Hatching Percentage of sexual eggs at 25°C (after 192 h)	8.00 \pm 16.2 (n=10)
% Hatching Percentage sexual eggs 20°C (after 154 h)	32.00 \pm 10.33 (n=10)

mers than females, c) Copulation lasts from 25 to 340 seconds, d) The preferred sites of attempted matings and copulations are the corona and foot opening. However, *B. quadridentatus* showed the lowest percentage of encounters becoming mating attempts (7.86%) recorded so far for a brachionid rotifer attempting to copulate with conspecific females. Gómez & Serra (1995) reported a 34.6% value. However, they used a different technique to record mating behavior (one male and 25 females in 50 µl of Instant Ocean Medium), and they used a euryhaline strain of *B. quadridentatus*. *Keratella americana* Carlin with 14% (Rico-Martínez & Snell, 1997) and *Platyias quadricornis* Ehrenberg with 19.3% (Velázquez-Rojas et al. 2002) are the freshwater brachionid species with the lowest percentage after *B. quadridentatus*. In contrast percentages as high as 66% have been reported for *B. plicatilis* (Snell et al., 1995).

The sequence of mating behavior for *B. quadridentatus* is the typical complete sequence of mating behavior in brachionid rotifers. However, the authors caution about the meaning of "typical" since we know the mating behavior in as few as ten species of brachionids. Among rotifers, this family represents the best studied regarding mating behavior.

Regarding duration of copulation, *B. quadridentatus* represents the *Brachionus* species with the longer period (71.38 seconds). It is known that *Platyias quadricornis* is an "atypical" brachionid (regarding mating behavior), with copulation taking place mostly at the juncture of ventral and dorsal plates (as in *Lecane*), and duration of copulation is over 300 seconds (in all other brachionids this duration is less than 100 seconds). Then again, only *Lecane* spends more time copulating than *Platyias* (Rico-Martínez & Snell, 1997). Velázquez-Rojas et al. (2002)

argued that the duration of copulation is related to the hardness of the lorica. This observation comes from the fact that both *Lecane* and *Platyias* females have a very thick lorica. Perhaps, the lorica of *B. quadridentatus* females is thicker than that of other *Brachionus* species. However, we can not rule out other factors like the number of sperm cells or efficiency of other copulatory structures that can influence the duration of copulation.

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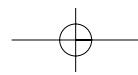
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Table 2. Morphometric characterization of male, female, and two of the three types of eggs; parthenogenetic and sexual egg of *Brachionus quadridentatus*. Abbreviations correspond to the following: ML = Maximum length, MW = Maximum width. All measurements are in µm.

Characteristic	Mean	SD	n
ML parthenogenetic egg	129.50	12.05	20
MW parthenogenetic egg	81.75	9.90	20
ML sexual egg	146.59	9.24	11
MW sexual egg	91.59	4.65	11
ML female	162.95	27.34	20
MW female	73.55	9.44	20
ML male	53.82	9.31	40
MW male	33.60	6.10	40
ML foot male	3.22	0.70	27



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