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RESEARCH ARTICLE

Study of morphotypes and life history of six clones of *Lecane bulla* (Gosse, 1851) from Quintana Roo, Mexico

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Abstract – The genus *Lecane* is highly diverse, there are 209 species, most of which inhabits tropical aquatic systems. In Quintana Roo 42 species have been reported, one of these is *Lecane bulla* described at the widest distribution throughout the Yucatan peninsula however; their morphotypes and demographic features are unknown. Therefore, the objective of this work was identify the presence of morphotypes *L. bulla* and their life history traits. We evaluated life history and morphometric data of females and asexual and sexual eggs from the populations were established from clonal strains, which remained in laboratory conditions for 6 months. They were kept in a bioclimatic chamber with photoperiod of 12 hours of light and 12 hours darkness, at a 25 ± 2 °C, and were feeding with the green algae *Nannochloropsis oculata* at 1×10^6 cell/ml. Thirty-four clonal strains from six locations were analyzed. Statistical analysis determined significant differences between morphometric measurements ($p < 0.001$) in the six localities as well as showed statistically significant differences in all demographic parameters. In conclusion, this study indicates the possible coexistence in the same geographical area of two different morphotypes of *L. bulla*, one is a small-sized distributed in the northwest of Quintana Roo and another large-sized in the southwest.

Keywords: Clonal culture / invertebrates / species complex / karst aquatic systems

1 Introduction

The diversity of the rotifers that inhabit aquatic ecosystems is underestimated (Ortells *et al.*, 2000) because phenotypic plasticity might hinder species abundance and challenges to their taxonomy. Phenotypic plasticity refers to an organism's ability to change its morphological structures when changes occur in biotic factors such as food type, density, predation among others and/or abiotic such as temperature, salinity, pH, *etc*. and when conditions are repositioned these organisms return to their normal structure (Stelzer, 2017).

In consequence, to estimate the diversity of rotifers using morphological information is fundamental, that species undergo morphological variations, intraspecific variation overlaps with interspecific variation (Fontaneto *et al.*, 2005). Morphological characteristics are traditionally observed under the microscope and from discontinuities in the variation of these, it is possible to distinguish between species (Leliaert *et al.*, 2014). Classical morphometry measures identifies morphological changes that occur in organisms based on their linear dimensions. Moreover, it is necessary to identify demographic parameters for each morphotype or strain to understand its population ecology. By developing life tables is possible understand the dynamics of the species (Xi *et al.*, 2013). Because, demographic studies can provide valuable information on the adequacy of environmental conditions (Ma *et al.*, 2010); such as mortality, fertility, survival, average lifespan, generation time, and/or population growth rate (Saucedo-Ríos *et al.*, 2017).

The present study is focus on the genus *Lecane* one of the most diverse genera of the Monogononta (Segers, 2008). It is expected that this genus has genetic evidence of 13 cryptic species (García-Moralez and Domínguez-Domínguez, 2020). The species *L. bulla* is one of the 209 species described for the genus, which is considered one of the richest genera in rotifer species (Segers, 2008). Some authors like Koste (1978) classified *L. bulla* in the genus *Monostyla*, one of three proposed genera derived from the degree of finger fusion (Koste, 1978). However this character was considered

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Table 1. Environmental data of sampled localities: number of strains; temperature (°C), conductivity (μs/cm), and pH.

Sampling site	Key	Number of strains	Geographical coordinates	Temperature	Conductivity	pH
El corchalito	COR	7	20° 55' 47" N; 87° 15' 47" W	27.9 ± 0.1	8.5 ± 1.1	6.1 ± 0.6
Parque del Cenote	PC	9	21° 10' 31.4' N; 86° 50' 55" W	27.7 ± 0.3	0.8 ± 0.2	7.1 ± 0.3
El secreto	SCR	4	20° 46' 55.9" N; 86° 57' 09" W	30.5 ± 0.7	1.1 ± 0.1	6.1 ± 0.6
Muyil	MYL	4	19° 58' 40.4" N; 87° 44' 0.1"W	30.2 ± 0.2	2.3 ± 0.2	7.9 ± 0.6
Punta Laguna	PL	4	20° 38' 50.2" N; 87 38' 04.2" W	31.1 ± 0.1	3.3 ± 0.1	8.2 ± 0.1
Chemuyil	CHM	6	21° 22' 22.2" N; 87° 19' 50.3" W	27.1 ± 0.3	8.2 ± 0.1	7.4 ± 0.3

Values are mean ± one SD. N = 3.

insufficient to establish different genera and so, it was decided to combine these into a single genus *Lecane* (Sharma, 1978).

As for its distribution *L. bulla* is widely distributed predominantly in tropical or subtropical environments (Segers, 2008). It is characterized by living mainly in the shallow coastal parts of aquatic ecosystems where they feed on bacteria and detritus (Serranía-Soto, 2003). According to Saucedo-Ríos *et al*. (2017) they are thermospecific (25 °C), with an average life cycle of seven days and a positive intrinsic growth rate.

As for males, the reports are few due mainly to the rarity of their appearance, which is a consequence of their breeding mechanisms (Segers, 1995). Within the genus, 11 males have been reported including *L. bulla* (Alvarado-Flores *et al*., 2017). Males generally have a lagged body, ringed with completely separate fingers (Segers and Rico-Martínez, 2000). It has been proposed that due to these morphological characteristics males can be used as a diagnostic character to delimit species (Sudzuki, 1999).

Therefore, *L. bulla* is an excellent ecological indicator; common species frequently recorded in the plankton samples collected in the coastal areas of aquatic systems worldwide (Segers and Rico-Martínez, 2000). It is also known that *L. bulla* is a cosmopolitan species adapted to live in wide environmental conditions, many specialized in a given environment that have high interspecific variability (Saucedo-Ríos *et al*., 2017).

For example, the study conducted by Walsh *et al*. (2009) identified three different haplotypes of *L. bulla* from the Chihuahua desert with a genetic divergence between 12 and 15%. García-Morales and Elías-Gutiérrez (2013) found that *L. bulla* presented eight different haplotypes distributed in Campeche (1), Mexico City (1), Veracruz (3) and Quintana Roo (3). Finally Moreno *et al*. (2017) estimated the diversity of rotifer species testing the application of the DNA barcode on resistance eggs in the Mediterranean region. They found 35 operational taxonomic units (OTU), revealing four complex cryptic species, where *L. bulla* presented two different haplotypes, which affirms the fact that it is a complex of cryptic species. Therefore, the objective of this work is to describe the variability in morphology, and the demographics of the life history of the species of the *L. bulla* that inhabit the aquatic ecosystems of Quintana Roo.

2 Materials and methods

L. bulla specimens were collected in Quintana Roo, Mexico from August to November 2017. The name of the localities abbreviated, geographical location and their physical and chemical conditions at the time of sampling are presented in Table 1. The populations were established from a single female, which remained in laboratory conditions for 6 months before conducting the experiments. The strains were kept in a photoperiod of 12 h of light and 12 h darkness, at a stable temperature of 25 ± 2 °C, were fed with the green algae *Nannochloropsis oculata* at 1×10^6 cell/ml, cultivated according to Nichols (1973), adding micro growth medium following the suggestions of AquaFarm®. The EPA medium was prepared by dissolving 96 mg $NaHCO_4$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, and 4 mg KCL, with a pH of 7.3, which was prepared using type I water (ultrapure). The concentration of algal cells was measured with a Neubauer Improved Marienfeld chamber (0.0025 mm^2). Life history studies began with 100 asexual eggs. Eggs were observed every 5 h until we collected 12 individuals. The hatching percentages were recorded at 24 h. The neonates were then transferred to the individual wells in a 24-well polystyrene plate (Corning®), with *N. oculata* at 1×10^6 cell/ml as food and incubated at a temperature of 25 ± 2 °C with a photoperiod of 12:12 light: darkness. The total volume in each well was 950 μl. In total, 72 individuals were observed (12 individuals per location, means 12 replicates per site). After the start of the experiment, in each interval of 12 h we count and eliminate the number of parthenogenetic eggs, the production of male eggs, and the presence of males. For intrinsic growth rate (r) studies we use the same experimental design as life table studies except that, we started with 5 neonates per localities (sites) with a total of 18 replicates (90 neonates per localities, in total 540 neonates analyzed from six localities), during this phase, the production of resistance eggs is counted.

Based on the data collected, we derive the following variables: life expectancy in days (L); age-specific survival (lx), fertility (mx), reproductive value (vx), net reproductive rate (R0), generation time (T), and intrinsic growth rate (r). The following formulas were used the following formula according to Krebs (1985) and Begon *et al*. (1996):

$$\text{Survivorship } (lx): \frac{nx}{12}$$

$$\text{fecundity } (mx): \frac{fx}{nx}$$

$$\text{Reproduction value } (vx): (lx * mx)$$

$$\text{Net reproduction rate } (R_0): \sum (mx * lx)$$

Table 2. Measurements of females and males.

	Female μm (Mean ± one SD)			Male μm (Mean ± one SD)		
	Lorica Length	Lorica Width	Foot Length	Lorica Length	Lorica Width	Foot Length
COR	131.4 ± 4.2	92.4 ± 4.7	69.5 ± 3.6	109.6 ± 2.7	60.0 ± 2.7	22.3 ± 1.5
PC	125.7 ± 5.5	87.8 ± 5.0	68.1 ± 8.4	106.3 ± 2.1	57.2 ± 2.6	16.5 ± 1.9
SCR	124.7 ± 4.5	86.4 ± 3.8	58.4 ± 2.8	108.1 ± 2.2	57.4 ± 3.3	18.2 ± 1.3
MYL	108.1 ± 5.6	73.5 ± 7.6	51.3 ± 4.8	82.1 ± 2.6	44.1 ± 1.2	18.8 ± 0.5
PL	107.1 ± 3.9	79.4 ± 5.8	50.3 ± 3.8	81.6 ± 2.4	46.4 ± 2.7	18.1 ± 0.5
CHM	102.9 ± 4.5	71.8 ± 5.8	48.4 ± 2.8	78.4 ± 2.6	43.4 ± 2.6	18.2 ± 1.3

60 females and 20 males.

$$\text{Generation time } (T): \frac{\ln(R_0)}{r}$$

where: x' age structure; nx − number of females alive on a given day; fx − number of offspring/eggs laid on a given day.

$$\text{Intrisic growth rate } (r): \frac{\ln[N(t2) - N(t1)]}{(t2 - t1)}$$

where: Natural Logarithm; No number of individuals; t1: initial time; t2: end time.

To analyze the possible effects related to body size with egg size and life table demographics, we measure the body and egg sizes of the six *L. bulla* populations, measured the length and width of the lorica, and two diameters of parthenogenetic eggs, male eggs and resistance eggs under a composite microscope Axiostar Plus, ZEISS® to a magnification of 20× using the micrometric rule calibrated in the SE64ReL 4.8 software. The volume of the egg (Ve) was calculated from linear measurements using the formula Ve-4/3 × (a2b + ab2)/16, where a and b are the two diameters, assuming that the eggs were general ellipsoids (Ma *et al.*, 2010).

Morphometry measurements were linear. 60 females and 20 males of each strain were randomly selected, in terms of parthenogenetic eggs, resistance and males, only 20 of each were selected by locality. On each photograph, using the micrometric ruler of the AxioVision SE64ReL 4.8 Inc 2003 software, the following sections of each organism were measured: A) length, B) width and C) foot length. The morphometric measurements were analyzed using a variance analysis (ANOVA). We tested the differences in the morphometric measurements within each location, for which a Tukey HSD post hoc test was applied to compare each value ($p < 0.05$) using R software version 2.6. While for demographic parameters and hatching percentage to determine between which populations there are differences the Duncan Test was used using Statistica 7.0 software. The relationship between body size against demographic parameters and egg volume was analyzed using Pearson's correlation test using Excel's commercial package, Microsoft License.

3 Results

The ANOVA result for body size was statistically significant since the value of $p < 0.001$ indicated differences in the size of the *L. bulla* specimens between the aquatic ecosystems of Quintana Roo. These results indicate that the females from the COR, PC and SCR sites have larger sizes with size range of 120–130 μm in the length of the body, 80–90 μm in the width and feet 65–70 μm in length with respect to the females of the MYL, CHM and PL sites. Females had a body size of 100–110 μm long, with a width of 60–70 μm and a foot length of 45–50 μm (Tab. 2). The same morphological data occurred in the measurements for the males of the COR, PC and SCR sites. These were larger than the males of MYL, CHM and PL (Tab. 2).

Sixty individuals per laboratory-established strain were analyzed from a total of 34 clonal strains from six locations. Statistical analysis determined significant differences between locality morphometric measurements (ANOVA: asexual females $p < 0.00$, asexual eggs $p < 0.007$, unfertilized sexual eggs $p > 0.001$ and fertilized sexual eggs $p < 0.001$). Morphometric data of parthenogenetic females and asexual and sexual eggs of *L. bulla* are presented in Table 2. We test the differences within each locality by comparing the measurements to each other, using a post hoc analysis of Tukey HSD with a $p < 0.05$. The analysis determined that parthenogenetic females, asexual and sexual eggs from COR, PC, and SCR localities are significantly larger than MYL, PL, and CHM localities ($p < 0.00$) (Tab. 3).

Our results about demographic analysis, showed that in six localities there are statistically significant differences in all demographic parameters except for generational time (T) (Tab. 3). Specifically, COR, PC, and SCR locations had a lower life expectancy (L; in days) compared to PL, MYL, and CHM. All localities had an intrinsic growth rate (r) positive, with CHM showing the highest r and lowest COR. The shortest T was SCR (3.7 days) and the longest of MYL (5.4 days); but there were no significant differences. In the case of the net reproductive rate (R0) there were differences among localities, namely if COR, PC and SCR were obtained with MYL, PL and CHM, the previous had a lower reproductive rate compared to the latter. Males were present in all localities; the total number of males that a female can produce throughout its life is presented in Table 3. A female COR can produce up to ten males throughout its life, while a female CHM only produces three.

Age-specific survival (lx) and reproductive value (vx) of the six localities are presented in Figure 1. The behavior of both parameters showed differences between COR, PC, and SCR locations concerning MYL, PL, and CHM locations. The former had the shortest survival, with a reproductive value curve that began from the hatching of females, with

Table 3. Morphological characterization of individuals of *Lecane bulla*.

Sites	Length (AF)	Width (AF)	Length (AE)	Width (AE)	Length (USE)	Width (USE)	Length (FSE)	Width (FSE)
COR	131.4 ± 4.2	92.8 ± 4.7	78.2 ± 2.9	67.8 ± 4.3	55.3 ± 3.1	53.5 ± 2.8	80.1 ± 1.5	76.9 ± 3.1
PC	125.7 ± 5.5	88.4 ± 5.0	76.6 ± 2.7	66.0 ± 3.0	55.5 ± 2.5	54.2 ± 2.8	78.3 ± 2.2	75.4 ± 2.5
SCR	124.7 ± 4.5	86.4 ± 3.8	75.6 ± 2.0	62.2 ± 2.3	54.1 ± 2.0	52.2 ± 2.0	74.7 ± 1.9	72.2 ± 2.0
MYL	108.1 ± 5.6	73.5 ± 7.6	51.7 ± 3.0	43.8 ± 2.0	45.7 ± 2.6	45.2 ± 2.4	55.5 ± 2.0	49.3 ± 2.1
PL	107.2 ± 3.9	79.4 ± 5.8	52.0 ± 2.6	55.6 ± 3.0	41.2 ± 3.0	48.8 ± 3.6	56.4 ± 1.8	54.2 ± 2.3
CHM	102.9 ± 4.5	71.8 ± 5.8	48.2 ± 2.3	42.1 ± 3.6	39.0 ± 2.2	38.3 ± 4.2	51.8 ± 2.4	49.3 ± 3.3

Measurements are given in micrometers with ± one SD. AF = Asexual females; AE = Asexual eggs; USE = Unfertilized sexual eggs; and FSE = Fertilized sexual eggs.

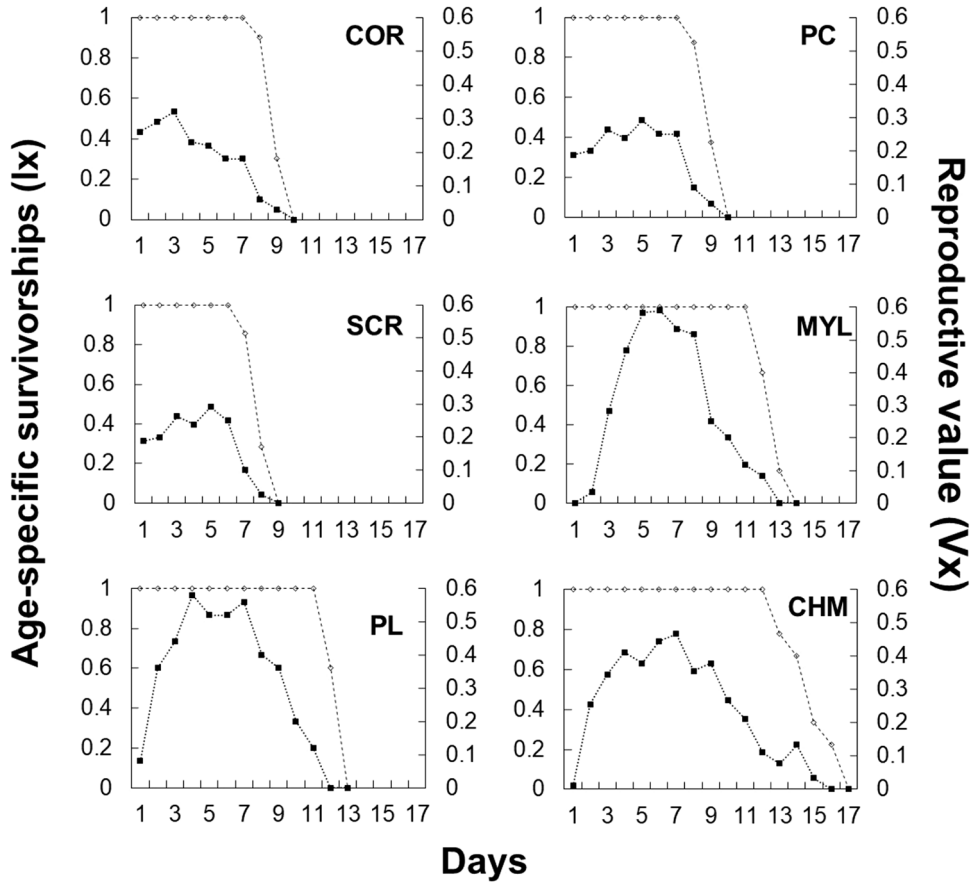

Fig. 1. Survivorship (diamond) and reproductive value (square) of *Lecane bulla* from six sites in Quintana Roo. COR: Corchalito, PC: Parque del cenote, SCR: El secreto; PL: Punta Laguna, MYL: Muyil, CHM: Chemuyil. X axis days.With a total of 18 replicates.

a production of up to 2.1 ± 0.1 of parthenogenetic eggs per day, these females continued to contribute reproductive value to the population over time including the day of their death. The latter showed the longest survival, their productive value began between the second and third days of birth, which presents reproductive values of up to 4.3 ± 0.3 parthenogenetic eggs per day, but their production stops 2 days before their death (Tab. 4).

The hatching percentages of parthenogenetic eggs, male eggs, and the average production of resistance eggs are shown in Table 5. The parthenogenetic eggs of *L. bulla* were incubated quickly and generally exhibited a success of hatching, except for MYL and PL were less than 50% manage to hatch. For male eggs, their varying hatching rate between COR, PC, and SCR relative to MYL, PL, and CHM, the former had higher hatching percentages compared to seconds were only less than 55% of males came to hatch. In terms of the production of resistance eggs, the locality with the highest number of cysts was COR (12.6 ± 1.6), while CHM had the lowest number with 3.7 ± 0.1 resting egg in total.

Table 4. Demographic parameters reported for *Lecane bulla*: lifespan (L; days), instantaneous growth rate of the population (r), generation time (T; days), net reproductive rate (R0) and number of male offspring total (male).

Site	L (days)	r	T (days)	R0	male
COR	8.2 ± 0.6	0.68 ± 0.02	4.0 ± 0.05*	15.1 ± 0.1	10
PC	8.3 ± 1.0	0.69 ± 0.02	3.9 ± 0.01*	15.6 ± 0.3	9
SCR	7.9 ± 0.5	0.67 ± 0.03	3.7 ± 0.04*	14.6 ± 0.2	8
MYL	11.5 ± 0.9	0.76 ± 0.06	5.4 ± 0.04*	40.4 ± 0.1	4
PL	11.8 ± 0.9	0.78 ± 0.01	4.9 ± 0.04*	48.2 ± 0.1	4
CHM	15.2 ± 0.8	0.82 ± 0.02	4.9 ± 0.08*	56.5 ± 0.2	3

The mean ± followed by the standard deviation for each value is reported. The demographic parameters are statistically different to $p < 0.05$, with the exception of those with a super index *, which did not show significant differences.

Table 5. Percentage of asexual eggs and hatched unfertilized eggs after 24 h.

Site	Asexual eggs (hatching)	Unfertilized eggs (hatching)	Fertilized sexual eggs (production)
COR	70%	77%	12.6 ± 1.6
PC	70%	80%	10.2 ± 0.5
SCR	74%	73%	9.3 ± 0.2
MYL	25%	50%	4.7 ± 1.0
PL	50%	54%	4.0 ± 1.5
CHM	80%	55%	3.7 ± 0.1

Average production of fertilized eggs. Eggs were randomly collected from the cultures. The number of wells in all cases was a three and each well contained 100 eggs. In the case of the resistance egg average, it corresponds to the total number counted during the intrinsic growth rate (r) experiments.

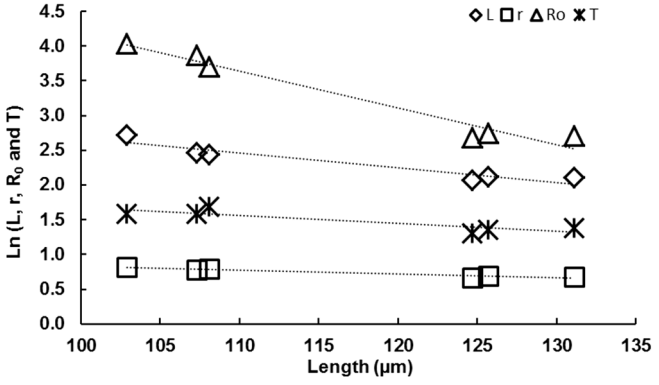

Fig. 2. Relationship between the demographic parameters (LN) and the body size (length, µm) of the six localities of *Lecane bulla* of Quintana Roo. N = 20.

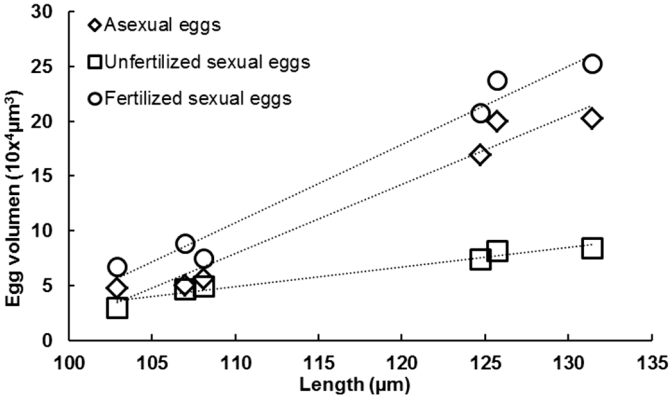

Fig. 3. Relationship between the egg volumen ($10 \times 4\ \mu m^3$) and the body size (length, µm) of the six localities of *Lecane bulla* of Quintana Roo. N = 20.

3.1 Size correlation with demographic parameters

Life expectancy (L), generation time (T), net reproductive rate (R0), and intrinsic population rate (r) correlated significantly with females' body size; r^2 was 0.90, 0.78, 0.95 and 0.94 (Fig. 2). That is, it can be observed that females who had the smallest body size have higher demographic parameter values and otherwise with the larger body-sized females had the lowest values. Also, the volume of parthenogenetic eggs, male eggs, and resistance eggs were correlated, with the size of the parthenogenetic females, the values of r^2 were 0.97, 0.96 and 0.96, respectively (Fig. 3). These correlations mean that the volume of the asexual and sexual egg is positively related to the size of the female, that is, small- females will produce small eggs, and large females will produce large eggs.

4 Discussion

According to Segers and Rico-Martínez (2000), *Lecane bulla* is one of the most common and frequently recorded

species in plankton samples, however little is known about its morphology, as the available information corresponds to specific research related mostly to taxonomic listings (Koste, 2000; Alvarado-Flores *et al*., 2017). In this study the significant differences between the sizes of the six locations analyzed, indicate the possible coexistence in the same geographical area of two different sizes.

Morphological differentiation does not occur in all rotifer complexes, so it is not always possible to distinguish different morphotypes. For this reason, it is common for several morphotypes to be grouped together in the same species (Kordbacheh *et al*., 2018). In the *L. bulla* complex isolated from different aquatic ecosystems in Quintana Roo, this is not the case, since two morphotypes were identified using the analysis of the body size of females and males, as well as the identification of finger, nail and claw structures. The combined use of morphometric measurements with morphological features has been useful to differentiate morphotypes in other complexes of rotifer species such as *Brachionus calyciflorus* (Mills *et al*., 2017), *B. plicatilis* (Michaloudi *et al*., 2018), *Testudinella clypeata* (Leasi *et al*., 2013) or *Polyarthra dolichoptera* (Obertegger *et al*., 2014).

L. bulla from COR, PC, and SCR locations distributed in the northwest area of Quintana Roo belong to the largest morphotype. These measurements are consistent with those recorded for *L. bulla* (Koste, 2000), while strains from MYL, PL, and CHM distributed in the southwest are the smaller morphotypes, which is similar in size to that reported for Sri Lankan *L. bulla* (Chengalath and Fernando, 1973).

Demographic parameters were shown to have a direct relationship to female size. It was determined that the larger the female's body size, the demographic values tend to be lower, which is consistent with other members of the *Lecane* genus. The large morphotypes of *Lecane* species such as: *L. papuana*, *L. quadridentata,* and *L. luna* rang from 120 to 200 μm in the length of the lorica, and have a shorter life expectancy ranging from 5 to 8 days. While small morphotypes of *Lecane* species such as *L. pyriformis*, *L. tenuiseta* and *L. cornuta* with measurements from 50 to 110 μm in the length of the lorica tend to have a higher expectation ranging from 15 to 26 days (Hummon and Bevelhymer, 1979; Perez-Legaspi and Rico-Martínez, 1998; Serranía-Soto *et al*., 2011, Saucedo-Rios *et al*., 2017). The instantaneous rate of population growth (r) is a parameter that represents a population's ability to grow and thrive in an environment (Campillo *et al*., 2011). In our study, the r values were all positive, indicating population growth but differ with the known ranges of previous studies of other species of the genus *Lecane*, since those of this work are higher than those reported, including *L. bulla* from Aguascalientes, Mexico (Saucedo-Ríos *et al*., 2017).

The percentages of egg hatching are also different among morphotypes. The hatching of parthenogenetic eggs has already been reported in different cryptic species (Gabaldón *et al*., 2015) but in males, this percentage is poorly known (Xu-Wang *et al*., 2016). The hatching of resistance eggs was not determined in this study, derived from the difficulties of the species to achieve it (Segers, 1995), but the average production is presented which, as far as the authors know, had not been documented. It has been suggested that the success of hatching is directly related to egg size, *i.e*. the development of small organisms is faster than that of large individuals (Gillooly *et al*., 2002). However, this idea does not match the results obtained in this work.

Hatching appears to be related to other factors such as the similarity between haplotypes (Gabaldon *et al*., 2015), morphological characteristics including body size (Ma *et al*., 2010), the integrity of eggs in the shell, size of the embryo and the color of eggs (García-Roger *et al*., 2006) and mainly to life expectancy and survival (Sarma *et al*., 2017). Females with shorter life cycles had the best percentages of hatching and production. This possibly relates to high rates of asexual reproduction, which leads to rapid population growth, and as a result, females can produce large numbers of parthenogenetic eggs that hatch to remain in the ecosystem. A strategy to compensate for these short periods of life has been observed in rotifers such as *L. tenuiseta* and *L. pyriformis* (Hummon and Bevelhymer, 1979).

Age-specific survival curves (lx) and reproductive value (vx), exhibit distinct peaks, large females had present from the first day of hatching, indicating that most animals quickly gained reproductive maturity, it is observed that *Lecane* species that have a short life cycle, and thus invest their energy in producing eggs in the first 24 hours. While small species do after 48 h.

In conclusion, the estimated diversity of rotifers from Quintana Roo is possibly much higher than previously proposed. The results of this small survey of six populations of the rotifer *Lecane bulla* from the northern and northwestern region of the Quintana Roo state suggest that. There are morphological differences that allowed for the first time to determine two morphotypes: a large-type morph (G) and small-type morph (P) which is an important finding considering that in many complexes morphological differentiation does not often occur in studies of rotifer diversity worldwide. It is necessary to study the morphological and demographic characteristics, together with genetic differences to establish the total species pool before they are lost due to ecosystem fragmentation or climate change. Also, rotifers like *Lecane bulla* are excellent ecological indicators that are easy to grow and use in reproductive, physiological, and toxicological tests.

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