Aquaculture Reports 26 (2022) 101309

Contents lists available at ScienceDirect

Aquaculture Reports

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

Genetic identification and comparative study on life history parameters of two strains belonging to *Brachionus plicatilis* species complex (Rotifera: Monogononta)

Luana Granada [a,b,*], Marco F.L. Lemos [a], Peter Bossier [b], Sara C. Novais [a,*]

[a] *MARE – Marine and Environmental Sciences Centre & ARNET – Aquatic Research Infrastructure Network Associated Laboratory, ESTM, Polytechnic of Leiria, 2520-641 Peniche, Portugal*

[b] *Laboratory of Aquaculture & Artemia Reference Center, Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Ghent 9000, Belgium*

ARTICLE INFO

Keywords:
16S rRNA gene
Aquaculture
Cryptic species
Ecotoxicology
Fecundity and lifespan
Intrinsic rate of population growth

ABSTRACT

The rotifer *Brachionus plicatilis* is an important species for aquaculture, due to its use as food for bivalves, and fish and crustaceans larvae in hatcheries. However, being a species complex, it has become increasingly important to catalogue and describe the species and biotypes that constitute this complex. Therefore, the purpose of this study was to genetically identify two morphologically identical strains and evaluate their suitability to be used in aquaculture under the studied conditions. A correct identification and knowledge of life history characteristics of the biotypes and isolated strains is vital to avoid mass mortalities in aquaculture and to better interpret the responses of the organisms when these are used as a bioindicator and model for ecotoxicology. Strains MRS10 and IBA3 were identified as *B. koreanus* and, even though they have been maintained under the same laboratory conditions for several years, significant differences in several life history parameters were observed. A life table assay showed IBA3 rotifers to be larger at first reproduction, and to have longer post-reproductive period and mean lifespan. On the other hand, MRS10 rotifers produced less non-viable eggs and had higher population growth rate. Both strains showed to be a potential model for ecotoxicological and molecular studies, mainly due to the ease of maintenance, short generation time, and reproduction via parthenogenesis. However, MRS10 might present better characteristics than IBA3 to be reared in aquaculture as live food.

1. Introduction

Monogonont rotifers are common planktonic invertebrates (Gómez et al., 1997) and, among brachionid rotifers, *Brachionus plicatilis* (Müller, 1786) is one of the most studied taxon (Anitha and George, 2006). *B. plicatilis* is an euryhaline and cosmopolitan species, typically found in salt lakes and coastal brackish waters (Gómez et al., 2002). It presents high commercial importance and value. Its small size, shape, and slow swimming speed make it suitable to be used as live food for several marine species larvae (Korstad et al., 1989; Lawrence et al., 2012; Lubzens, 1987).

Aquaculture provided 46 % of global fish production in 2018, almost the double compared to 2000. About 54.3 million tonnes of finfish were farmed, with an estimated value of USD 139.7 billion (FAO, 2020). *Brachionus* has been indispensable to sustain the farming of several important marine finfish species since the 1960's (Hagiwara and Yoshinaga, 2017). Since this rotifer can feed on a variety of food sources, it can be used as a "living capsule" in hatcheries, where it grows on formulated diets, prepared specifically to fulfil the nutrients required by the larvae (Lubzens, 1987; Lubzens et al., 2001). This way, the culture of rotifers has been crucial for the development of an aquaculture more independent of wild resources, enabling a stable production of aquatic seedling (Hagiwara and Yoshinaga, 2017). Moreover, monogonont rotifers reproduce via cyclical parthenogenesis, being parthenogenetic reproduction with asexual females predominant (Gómez et al., 1997). This results in a rapid growth population, another important aspect for their use in aquaculture (Moha-León et al., 2015; Rico-Martínez et al., 2016). It has been known for several decades that phylum Rotifera has a high level of cryptic diversity. Although cryptic species have different ecological preferences and genetic differences, the lack of morphological

* Correspondence to: Edifício CETEMARES, Avenida do Porto de Pesca, 2520-630 Peniche, Portugal.
E-mail addresses: luana.almeida@ipleiria.pt (L. Granada), sara.novais@ipleiria.pt (S.C. Novais).

https://doi.org/10.1016/j.aqrep.2022.101309
Received 4 May 2022; Received in revised form 15 August 2022; Accepted 18 August 2022
Available online 25 August 2022

variance makes it difficult to identify them unless using biochemical and molecular methods (Bączkiewicz et al., 2017; Dooms et al., 2007). Thus, DNA sequencing has been utterly important to catalogue the species and biotypes previously referred only as *B. plicatilis* (Gómez et al., 2002; Mills et al., 2017). Several morphotypes – large (L-type), medium (SM), small (SS) (designations commonly used in aquaculture) – and biotypes have been discovered and described, showing this complex systematics still has to be clarified (Anitha and George, 2006; Guerrero-Jiménez et al., 2019; Hagiwara et al., 1995).

Life table studies provide extremely important insights that will contribute to the expansion of economically viable commercial aquaculture industries (Kostopoulou and Vadstein, 2007), since different climatic zones of origin may result in different adaptation capabilities of rotifers (Xiang et al., 2016). Also, a correct identification of the cultured biotype can be greatly advantageous, since it was already shown that some biotypes, due to their growth efficiency, can be more suitable to be used by hatcheries (Kostopoulou and Vadstein, 2007). All these data can also be very helpful to explain the organisms' responses to abiotic and biotic factors, since *B. plicatilis* is widely used as a model organism in basic research, as a bioindicator and model for ecotoxicology (Kostopoulou et al., 2012).

Therefore, the main purpose of this work was to compare life history characteristics of two strains of rotifers from *B. plicatilis* species complex, through life table assays, and their suitability to be used in aquaculture and as models for further studies. Since these strains were not distinguishable by morphological characteristics and were both isolated more than a decade ago and maintained in laboratory at the same conditions since then, a genetic identification was also performed.

2. Material and methods

2.1. Rotifer cultures

Living samples of two rotifer strains (MRS10 and IBA3) of the *B. plicatilis* complex were obtained from the Laboratory of Aquaculture and Artemia Reference Center (ARC, Ghent University, Belgium). MRS10 strain was isolated from the Italian hatchery Maricoltura di Rosignano Solvay, Livorno, Italy. Information on geographical origin of IBA3 strain is unknown.

Due to several years under constant and near-optimal conditions at 25 psu (practical salinity units) and 25 °C, both cultures were only constituted by parthenogenetic females – sexual females produce males and diapausing eggs, which have not been observed for several years in these cultures on the weekly inspections under a binocular (personal observation of technicians responsible for stock maintenance). Stock tubes and respective rotifers were always handled separately to ensure isolation between strains. Rotifer clone cultures of MRS10 and IBA3 were established in 2017 by randomly isolating five individual females, with at least one amictic egg, from each strain, in falcon tubes with 30 mL of 25 psu artificial seawater (ASW, dissolving Instant Ocean Sea Salt with deionized water at the intended salinity, followed by autoclaving) (Dooms et al., 2007). The success ratio was 80 %, resulting in four clone cultures of each strain. Clone cultures were fed daily with *Tetraselmis* sp. at a final concentration of 10^5 cells mL^{-1}. Before feeding the rotifers, the microalgae was concentrated by centrifugation. This microalgae was semi-continuously cultured in autoclaved 32 psu natural seawater with F/2 medium, with a 16:8 light:dark photoperiod of 40.5 μmol m^{-2} s^{-1} lights. After concentration, density was estimated using a hemocytometer (Chamber Neubauer-Improved, Depth 0.100 mm, 0.0025 mm^2, Germany), and then stored at 4 ºC (Xiang et al., 2016).

The tubes with rotifer stock and clone cultures were placed in front of cool white tube lights with a constant light intensity of 34 μmol m^{-2} s^{-1} and at a room temperature of 25 ± 1 °C. Every week, stock cultures were restarted and clone cultures cleaned.

2.2. Strains identification

Due to the long period of maintenance of both strains since their isolation and to confirm that rotifers' stocks were constituted only by one species and had not been contaminated by other species, strains identification was done using all clone cultures established previously, four per strain.

2.2.1. Sample collection and DNA extraction

To minimize the amount of *Tetraselmis* sp. in the samples for DNA identification, rotifers were rinsed with artificial seawater several times and then placed in clean artificial seawater for 24 h to allow them to digest and excrete any remaining microalgae. In the same day of collection, density of rotifers in each clone culture was determined. Approximately 1000 individuals of each clone culture (4 clones from each strain) were collected from a sieve (30 μm pore size), rinsed with Milli-Q water, immediately frozen in liquid nitrogen, and stored at −80 ºC. DNA extractions were performed using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific™, USA), accordingly to manufacturer's instructions.

2.2.2. PCR and sequencing analysis

A 388-bp segment of the 16S rRNA gene was amplified, using *Brachionus*-specific primers: the forward primer Brach16S_F (5′-AGATGGATCCAAAGTATCTTCTGCCCACT-3′) and the reverse primer Brach16S_R (5′-ATAGGATCCGATAATCCAACATCGAGGTAGTAG-3′) (Papakostas et al., 2006). PCR reactions were performed in 20 μL final volume containing template DNA, 10 μL of JumpStart™ REDTaq® ReadyMix™ Reaction Mix (Sigma Aldrich, Belgium), and 20 pmol of each primer. Amplification was performed under the following cycling conditions: 2 min of initial denaturation at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C; one cycle of 4 min at 72 °C (Papakostas et al., 2006).

Obtained amplicons were purified with the High Pure PCR Product Purification Kit (Roche Diagnostics, Belgium), and the resulting purified PCR fragments were sent to STABvida (Caparica, Portugal) for sequence analysis. Sequencing was performed for 8 amplicons in both directions for a more accurate sequence confirmation. SeqTrace sequence analysis software was used to analyse the data, where forward and reverse sequences were aligned and checked for quality. Ambiguous base pairs were manually checked on the chromatograms (Dooms et al., 2007). For the identification of rotifer biotypes, the obtained 16S sequences were submitted to a BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2.3. Phylogenetic assessment

Phylogenetic analysis was performed using *Brachionus* 16S rRNA sequences deposited in GenBank, representatives from each phylogroup, along with the best BLAST hits for each clone of this work and respective sequences. Sequences were obtained from GenBank for *Brachionus* sp. Manjavacas (accession number AY647201), *Brachionus* sp. Austria (AY647202), *Brachionus* sp. Nevada (AY647203), *Brachionus* sp. Cayman (AY647204), *Brachionus* sp. Australia (AM180760), *B. ibericus* (AM180761), *B. rotundiformis* (AM180762), *Brachionus* sp. Tiscar (AM292537), *Brachionus* sp. Almenara (AM292538), *B. koreanus* (KC603851), *Brachionus* sp. SP-2007a (EU046256), and *B. plicatilis* strain ARC (AJ748693). A total of 20 nucleotide sequences were aligned with ClustalW algorithm and, using the Kimura 2-parameter model, a Maximum Likelihood (ML) tree was constructed in MEGA-X software (Kumar et al., 2018). Nodal support was assessed through 1000 bootstrap replicates.

2.3. Life table assay

After the successful establishment of clone cultures, one culture from each strain (MRS10.5 and IBA3.4) was scaled-up to 1 L to ensure enough

individuals to proceed with the life table assays. These 1 L cultures were fed and maintained at the same conditions as the stock cultures.

Life table experiment was conducted for both strains at the same time, in 48-well microplates. Each well was inoculated with one amictic neonate (< 2 h old), into 0.5 mL ASW at 25 psu, with 6×10^5 cells mL^{-1} of *Tetraselmis* sp., allowing *ad libitum* feeding regime (Gribble and Mark Welch, 2017). One microplate per strain was used, each with 48 individuals (1 organism per well), and incubated at 25 ± 1 °C under constant light. Rotifers were checked every 3 h until the first eggs were laid and hatched. Then, every 12 h, rotifers were checked, and the number of eggs and neonates produced by the original individuals was recorded. Neonates were subsequently removed. Every 24 h, the original live rotifers were transferred into new 0.5 mL ASW at 25 psu, with 6×10^5 cells mL^{-1} of *Tetraselmis* sp. (Xiang et al., 2016). At a separated microplate, 24 neonates of each strain were inoculated and were observed under a stereomicroscope to record their size at the beginning of the reproductive stage (camera AxioCam with 1.4 MP of resolution, connected to the ZEN software, Zeiss Microscopy), and then discarded. Survival of the original individuals was checked every time the microplates were observed. The experiment ended when all original organisms had died.

2.4. Comparative population growth in continuous cultures

A culture growth experiment was performed to study the reproductive performance of both strains (MRS10.5 and IBA3.4). Contrary to life table assay, here reproductive behaviour was assessed under conditions of increasing density and food limitation, two important factors when organisms are in culture. For this, erlenmeyers with 500 mL ASW at 25 psu, with 10^5 cells mL^{-1} of *Tetraselmis* sp., were inoculated with 12,500 rotifer individuals (final density of 25 rotifers mL^{-1}), and incubated at 25 ± 1 °C, under constant light. The experiment was performed in triplicates for both strains. Every 12 h, 1 mL of the cultures was collected, and the density was estimated for all replicates. Cultures were fed every 24 h. These procedures were carried for 7 days or until cultures reached the stationary phase.

2.5. Statistical analysis

Several life table parameters were calculated and compared between strains, including age-specific survivorship (l_x), age-specific fecundity (m_x), age at first reproduction (α), and age at last reproduction (ω). Based on these data, other parameters, such as duration of juvenile period (JP), reproductive period (RP), post-reproductive period (PP), mean lifespan (ML), and hatching rate (HR) were determined. Moreover, calculation of the gross reproductive rate (GRR), net reproductive rate (R_0), generation time (Ƭ), and intrinsic rate of population growth (r), was performed as follows (Pan et al., 2016; Snell et al., 2014; Walz, 1983; Xiang et al., 2016):

$$\text{Gross reproductive } rate: \quad GRR = \sum m_x; \tag{1}$$

$$\text{Net reproductive } rate: \quad R_0 = \sum_0^\infty l_x m_x; \tag{2}$$

$$\text{Generation } time: \quad T = \sum l_x m_x x \Big/ R_0; \tag{3}$$

$$\text{Intrinsic rate of population growth}: \quad r \approx \ln R_0 / T. \tag{4}$$

Data from the comparative population growth in continuous cultures were used to calculate the rate of population increase (r) (Nandini et al., 2007; Rebolledo et al., 2020):

$$\text{Rate of population } increase: \quad r = (\ln N_t - \ln N_0)/t, \tag{5}$$

where N_0 is the initial population density, and N_t is the density of population after time t (days).

For the determination of differences between strains for the above parameters, Levene's test for equality of variances and independent samples t-tests were performed using IBM SPSS Statistics version 27 for Windows. For all tests, the significance level was set at $p \leq 0.05$.

3. Results

3.1. Strains identification and phylogeny

According to the BLAST results of sequence identity and expected values (Table 1), all clones corresponded to *B. koreanus*, showing that strains MRS10 and IBA3 correspond to the same biotype.

The phylogenetic tree clustering based on Maximum Likelihood method resulted in three major groups (Fig. 1). The clone cultures sequenced in the 16S rRNA gene region were clustered with the pre-existing groups and their identification obtained by BLAST was corroborated. The order of species and biotypes was, in general, consistent with the three morphotypes (SS, SM, and L, in this order) described for *B. plicatilis* species complex (Gómez et al., 2002; Gribble and Welch, 2012). Being *B. koreanus* associated to SM morphology, as well as *B. ibericus* and biotypes Cayman, Tiscar, and Almenara, it can be inferred that MRS10 and IBA3 correspond to this morphotype.

3.2. Demographic parameters

Results showed a survival of nearly 100 % for both strains until day 5 (Fig. 2a and Table S1). From day 5.5, strain MRS10 had a significant higher mortality compared to IBA3 (l_x, $p < 0.05$). This can also be seen by the difference in mean lifespan between strains of 6.34 ± 1.96 and 7.98 ± 2.69 days, for MRS10 and IBA3 respectively (ML, Table 2; $t = -3.41$, $p = 0.001$).

Concerning the age-specific fecundity (m_x, Table S1), it was possible to observe the same overall initial pattern for both strains (Fig. 2b). For both, reproduction started after 24 h, number of eggs peaked at day 2 and, consequently, number of neonates peaked at day 3 (m_x, Table S1). However, at day 3.5, MRS10 females produced more offspring than IBA3 (m_x, Table S1; $t = 2.25$, $p = 0.048$), with MRS10 having 3.47 neonates $female^{-1}$, while for IBA3 2.88 neonates $female^{-1}$ were observed for this same age. On the other hand, although without statistically significant differences, IBA3 had its last reproduction at day 10.5 while for MRS10 was at day 6 (Table S1). Nevertheless, IBA3 also presented a longer post-reproductive period (3.70 days) than MRS10 (2.31 days) (PP, Table 2; $t = -3.22$, $p = 0.002$).

There were no differences between strains regarding the juvenile period, corresponding to the number of hours since a rotifer was born until it laid the first egg (JP, Table 2), and age at first reproduction (age of rotifers when their first neonate was born; α, Table 2). However, differences of size at first reproduction were observed (lorica length (μm), Table 2; $t = -5.28$, $p < 0.0001$), with IBA3 females being larger than MRS10.

The gross reproductive rate (GRR) represents the total number of offspring that would be produced by an average female in absence of mortality (theoretical fecundity), and the net reproductive rate (R_0) represents de average number of age class zero offspring produced by an average neonate during its entire lifetime (realized fecundity) (Pianka, 2011). The difference between these parameters was higher for IBA3 (1.66) than for MRS10 (0.98) (Table 2), suggesting a higher number of non-viable eggs in IBA3 comparing with MRS10.

Although MRS10 and IBA3 had similar net reproductive rates (R_0, 19.46 and 20.50 offspring $female^{-1}$), the fact that MRS10 had slightly shorter generation time (Ƭ, 3.14 days) than IBA3 (3.40 days) resulted in a higher intrinsic rate of population growth (r, 0.94 vs 0.89 day^{-1}), although these differences were not statistically significant.

Table 1
First two BLAST results for the 16S rRNA sequences of all the tested clone cultures of the *Brachionus plicatilis* species complex.

Clone	Description	Max score	Total score	E value	Ident	Accession number	Biotype
MRS10.1	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	688	688	0.0	99.73 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	625	625	7e-175	99.42 %	EU046256.1	Cayman
MRS10.2	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	688	688	0.0	99.73 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	625	625	6e-175	99.42 %	EU046256.1	Cayman
MRS10.4	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	688	688	0.0	99.47%	KC603851.1	Koreanus
	Brachionus sp. Cayman 16 S ribosomal RNA gene, partial sequence; mitochondrial	628	628	5e-176	100.00 %	AY647204.1	Cayman
MRS10.5	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	686	686	0.0	98.96 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	621	621	9e-174	99.42 %	EU046256.1	Cayman
IBA3.1	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	682	682	0.0	99.73 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	619	619	3e-173	99.41 %	EU046256.1	Cayman
IBA3.3	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	688	688	0.0	99.73 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	625	625	7e-175	99.42	EU046256.1	Cayman
IBA3.4	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	689	689	0.0	98.96 %	KC603851.1	Koreanus
	Brachionus sp. SP-2007a clone 15 16 S ribosomal RNA gene, partial sequence; mitochondrial	625	625	7e-175	99.42 %	EU046256.1	Cayman
IBA3.5	Brachionus sp. 'koreanus chromosome I mitochondrion, complete sequence	682	682	0.0	99.21 %	KC603851.1	Koreanus
	Brachionus sp. Cayman 16 S ribosomal RNA gene, partial sequence; mitochondrial	628	628	5e-176	99.71 %	AY647204.1	Cayman

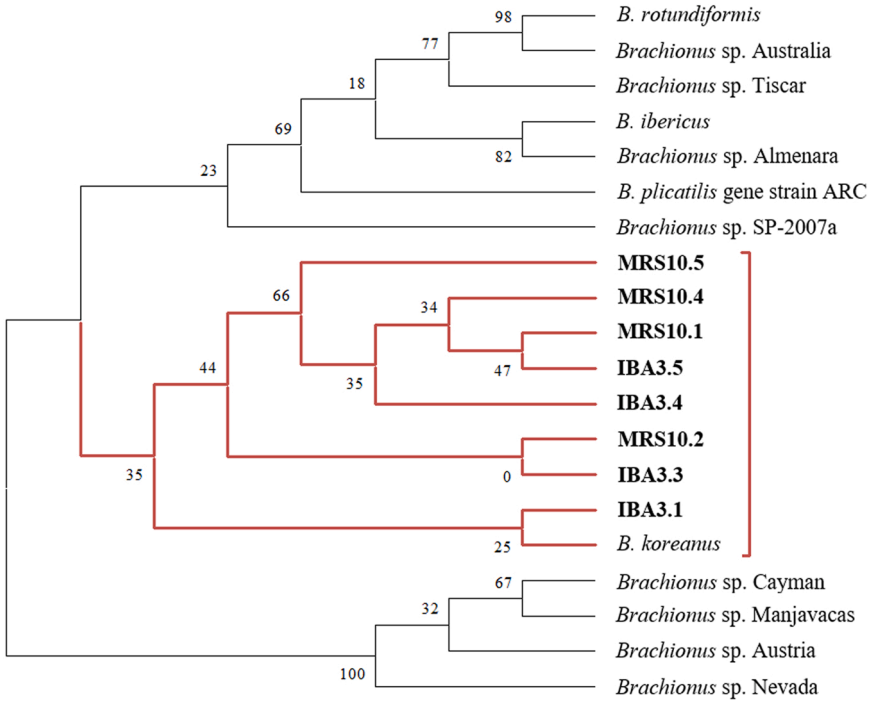

Fig. 1. Branch tree representing the phylogenetic relationship of clones of MRS10 and IBA3 strains (in bold) belonging to the *Brachionus plicatilis* species complex, based on the Maximum Likelihood method. Clones belong to the same clade as *B. koreanus* (branch lines in red). Next to the nodes is indicated the bootstrap support (1000 pseudoreplicates).

3.3. Growth rate in continuous cultures

Regarding the population growth under culturing conditions (Fig. 3), strain MRS10 appeared to have a better reproductive performance when fed a limited amount of microalgae per day. The density of rotifers increased steadily with time in the early phase of culture. After four days of culture, IBA3 density began to stabilize, reaching a maximum density of 232.2 ± 8.39 ind. mL^{-1}, while MRS10 density continued to increase until it reached a peak of 348.9 ± 19.53 ind. mL^{-1}, 33% more than observed for IBA3 ($t = 9.506$, $p = 0.001$). At the end of the experiment, after seven days of culture, the rate of population increase was higher for MRS10 (0.38 ± 0.008, $t = 10.44$, $p < 0.0001$) comparing to IBA3 (0.32 ± 0.005). At this point, MRS10 cultures appeared not to have reached their saturation level yet.

4. Discussion

Since the early 2000's, it became evident that *B. plicatilis* was in fact a

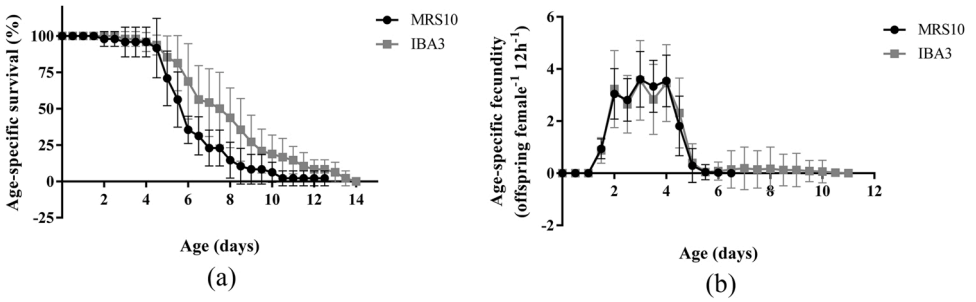

Fig. 2. Age-specific a) survival and b) fecundity (mean ± SD) of two asexual *Brachionus* sp. strains, MRS10 (black dot) and IBA3 (grey square).

Table 2
Morphologic and demographic parameters of two asexual *Brachionus* sp. strains, including lorica length at first reproduction (μm, excluding spines), age at first (α, days) and last reproduction (ω, days), duration of juvenile period (JP, hours), reproductive period (RP, days), post-reproductive period (PP, days), mean life-span (ML, days), hatching rate (HR, %), gross reproductive rate (GRR, eggs produced female^{-1}), net reproductive rate (R_0, offspring female^{-1}), generation time (Ŧ, days), and intrinsic rate of population growth (r, day^{-1}), all expressed in mean ± SD.

Parameter	MRS10	IBA3
Lorica length (μm)	147.65 ± 11.78	167.04 ± 13.59*
α (d)	1.53 ± 0.12	1.62 ± 0.45
ω (d)	4.48 ± 0.59	4.88 ± 1.26
JP (h)	21.38 ± 1.01	21.38 ± 2.55
RP (d)	3.22 ± 0.64	3.47 ± 1.29
PP (d)	2.31 ± 1.80	3.70 ± 2.36*
ML (d)	6.34 ± 1.96	7.98 ± 2.69*
HR (%)	87.64 ± 11.39	84.78 ± 26.39
GRR (eggs female^{-1})	20.44 ± 0.84	22.16 ± 4.46
R_0 (offspring female^{-1})	19.46 ± 2.51	20.50 ± 3.16
Ŧ (d)	3.14 ± 0.04	3.40 ± 0.44
r **(d^{-1})**	0.94 ± 0.05	0.89 ± 0.09

* indicates statistically significant differences between strains ($p < 0.05$)

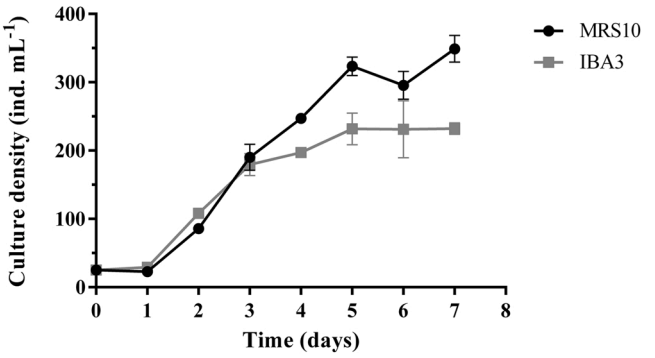

Fig. 3. Population growth of MRS10 and IBA3 cultures, fed with 10^5 cells mL^{-1} of *Tetraselmis* sp. daily for one week. Mean ± SD is shown based on three replicates.

species complex and that, due to its importance for the aquaculture industry, it was essential to better understand its systematics and characteristics (Dooms et al., 2007). As emphasized by Dooms et al. (2007), exchanging rotifers between hatcheries only based on their similar morphological characteristics can result in low production levels and explain some of the reported crashes (great mortality) if different lineages were not able to adapt to the hatchery conditions to where they were transferred.

Restriction fragment length polymorphism (RFLP) typing (Papakostas et al., 2005) and Denaturing Gradient Gel Electrophoresis (PCR-DGGE) techniques based on polymorphisms within the mitochondrial 16S rRNA gene (Dooms et al., 2007) proved to be successful to unambiguously detect the different *Brachionus* species and biotypes. Advances in molecular technologies have allowed to identify more than fifteen genetically different lineages (Guerrero-Jiménez et al., 2019; López et al., 2019; Mills et al., 2017), which have also been divided in three main phylogenetic clades, according to body size (L, SM, SS) (Serra and Fontaneto, 2017). Studies on ecology, reproductive behaviour, and morphology have supported the molecular results for species boundaries (Campillo et al., 2011; Ciros-Pérez et al., 2001; Fontaneto et al., 2007; Papakostas et al., 2013).

The strain MRS was previously classified as *B. plicatilis* sp. 'Cayman' by Papakostas et al. (2006) and Dooms et al. (2007). *Brachionus* sp. 'Cayman' was clustered with *B. ibericus* (Gómez et al., 2002), corresponding to the SM morphotype. *B. koreanus* was formally described later by Hwang et al. (2013) and, in that same year, Han et al. (2013) stated that *B. koreanus* was the formerly *B. ibericus*. Strain IBA does not appear in the literature and, unfortunately, the ARC laboratory has no information on its origin. Sequencing results of the present study showed that MRS10 and IBA3 sequences had better correspondences with *B. koreanus*, although *B.* 'Cayman' also showed good matching results. Morphologically, the strains used in this work correspond to the SM morphotype, described by Ciros-Pérez et al. (2001): three pair of antero-dorsal spines with similar length, medium spine shaped like an equilateral triangle, lorica length of adult females of 175–220 μm. Length and shape of the lorica and spines are characteristics that allow to discriminate morphotypes (Dhert, 1996).

Strains MRS10 and IBA3 were isolated and have been maintained under the exact same environmental and handling conditions for more than a decade and, apparently, are both unable to produce cysts under laboratory conditions. Monogononta rotifers reproduce via cyclical parthenogenesis where, under environmental cues, asexual females produce sexual females. Laboratory cultures can be constituted by both types of females and the mixis ratio (ratio of sexual to asexual females) can indicate if a culture is under stress conditions (Gómez et al., 1997). In the specific case of MRS10 and IBA3, it seems these rotifers became unresponsive to typical environmental cues, such as high density and photoperiod, probably due to the long period under optimal/near-optimal conditions. However, despite this fact and the above results that showed that both strains are the same biotype, some differences could be observed when analysing life history characteristics. These differences have been observed for years while handling the organisms and were now corroborated by empirical evidence.

Moha-León et al. (2015) studied life history parameters of *Brachionus* sp. 'Alvarado', strongly related to *B.* 'Cayman' and included in the SM clade of the species complex. For same light and temperature conditions, and close salinity values, the net reproductive rate (19.58 offspring female^{-1}) was very similar to the one observed for MRS10 in this study. They found that continuous light, as used in the present study, had a positive influence in the reproduction of *B.* 'Alvarado'. The age at fist reproduction and duration of reproductive period was also concordant with the results presented (Table 2). However, reported mean generation time was more than double the time observed for MRS10 and IBA3,

resulting in an intrinsic growth rate of approximately half (0.43 day^{-1}) of what was observed in the present study. Also, *B.* 'Alvarado' appears to be larger than MRS10 and IBA3, since the lorica length of 0–30 min old neonates (147.7 μm) was similar to the length of MRS10 rotifers at first reproduction (147.65 μm).

A significantly higher mean lifespan (ML) was observed for IBA3 (7.98 days) comparing to MRS10 (6.34 days), being concordant with the 7.2 days observed for *B. koreanus* by Lee et al. (2018) but lower than the 8.9 days observed by Kim et al. (2016). The net reproductive rate obtained for IBA3 (R_0 = 20.50) was very similar to the 20.9 offspring $female^{-1}$ described by Lee et al. (2018), and about one descendant more per female comparatively to MRS10 in the present study. However, Kim et al. (2016) observed a much lower value for this parameter (12.7 offspring $female^{-1}$), despite the similar levels of population growth when comparing with the MRS10 culture at day 7 (period studied in our work). It is important to note that not all conditions of these previous life table works were the same as ours, meaning that although the temperature and feed conditions were similar, previous works tested lower salinities (15 psu) and a different photoperiod regime (12:12 h), which may influence the life history parameters.

Several similar studies were performed with other species of *Brachionus* genus, such as *B. calyciflorus* (Ma et al., 2010; Pan et al., 2016; Xiang et al., 2016), *B. havanaensis* (González-Pérez et al., 2018), and *B. angularis* (Ogello et al., 2016). Xiang et al. (2016) reported significant differences in several life history parameters between two clones of the same potential cryptic species from two climatic zones. In addition, the authors observed that temperature significantly affected most of the parameters studied. Although the *B. calyciflorus* clones were from individuals collected in the wild and not strains under laboratory conditions, this could be an indication that the differences observed for MRS10 and IBA3 in the present study may be due to eventual differences in their origin sites, namely temperature and/or salinity which play a major role in the characteristics of the strains.

Main significant differences between MRS10 and IBA3 were observed in length at first reproduction, duration of post-reproductive period, and mean lifespan, which were all parameters with higher values in IBA3 than MRS10. Differences in size but not in duration of juvenile period and in overall fecundity, may indicate IBA3 invests more in growth than in reproduction (Burgess and Bueno, 2021). Also, there was a larger difference between the rate of eggs produced and neonates born per day for this strain, meaning IBA3 tended to produce more non-viable eggs. Sun et al. (2017) showed rotifers confronting stressful environments favoured extended lifespan with lower fecundity. Results presented in this work may indicate this trade-off between reproduction and lifespan occurred in IBA3, as it presented higher lifespan than MRS10, with no better reproduction performance. In addition, differences in length can also be one factor affecting the rate of population increase, since as MRS10 individuals are smaller, more organisms are needed to reach the same biomass density as in IBA3 cultures (Fig. 3). Note that the differences in length at first reproduction may not have influence in the feeding process of larvae, since both strains correspond to the SM morphotype. On the other hand, as ectotherms, when growing in cold conditions, rotifers may grow slower and mature later, presenting larger sizes at maturation, comparing to those growing in warm conditions (Arendt, 2011). Overall, all these factors may indicate the culture conditions at which IBA3 is being maintained for years are under-optimal and this strain may benefit from higher temperatures. Considering this, we hypothesise the origin site of IBA3 may be located in lower latitudes than MRS10, which is from Northern Italy.

On a last note, given the importance and relevance of rotifers from *Brachionus* genus in the aquaculture context, results reported in this study can also be used to make extrapolations into this industry. Hagiwara et al. (2007) reviewed the importance of some parameters to select the most suitable rotifers to be used for aquaculture purposes. High growth rate is one of the most important characteristics for a stable production of food for fish larvae. Small size is especially important for marine fish larvae production, as they require very small prey due to their mouth size. Also, the lack of cysts production poses an advantage, since males are not useful for aquaculture as they: 1) lack a functional digestive system, not allowing enrichment of their nutritional value for larvae; and 2) have a short lifespan (Gilbert, 2020; Moha-León et al., 2015). Therefore, given the higher growth rates, smaller size, and lower occurrence of culture crashes (personal observation), for the culture conditions specified in this study, MRS10 presents more advantages than IBA3 to be successfully reared in hatcheries. This study confirmed that similar morphological characteristics do not mean similar performance, for the same culture conditions.

5. Conclusion

The purpose of this work was to compare two strains of *Brachionus* sp., highlighting the importance of genetically identify aquaculture strains. Despite belonging to the same biotype, MRS10 and IBA3 showed significant differences in important life history characteristics, and this will be a valuable knowledge for future ecotoxicological and molecular studies with these strains, as well as for aquaculture purposes. Based on the results obtained here for the population growth rate, as well as organisms' reproduction rate and duration of reproductive period, especially under culturing conditions of increasing densities and limiting food, and considering the environmental conditions used in the experiments, MRS10 seems to be more suitable to be reared in aquaculture as live food. To increase the suitability of IBA3, further life table experiments with a range of temperatures and salinities should be performed to optimise culture conditions. This work highlights the importance of life table studies in the optimization of cultivation process, essential to avoid culture crashes that, consequently, lead to economic losses in an aquaculture context, especially in the case of cryptic species.

CRediT authorship contribution statement

Luana Granada: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Marco F.L. Lemos:** Resources, Writing – review & editing, Funding acquisition. **Peter Bossier:** Resources, Writing – review & editing, Supervision. **Sara C. Novais:** Conceptualization, Methodology, Resources, Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

This study had the support of Fundação para a Ciência e a Tecnologia (FCT) through the Strategic Project granted to MARE – Marine and Environmental Sciences Centre (UIDB/04292/2020 and UIDP/04292/2020) and the project granted to the Associate Laboratory ARNET (LA/P/0069/2020). Luana Granada also wish to acknowledge the financial support given by FCT (SFRH/BD/102036/2014). Sara Novais is funded by national funds through FCT, in the scope of the framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of 29 August, changed by Law 57/2017, of 19 July.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.aqrep.2022.101309.

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