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Patterns of differentiation in the life history and demography of four recently described species of the *Brachionus calyciflorus* cryptic species complex

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Abstract

1. *Brachionus calyciflorus* is arguably the most studied freshwater monogonont rotifer. Although it has been recognised as a cryptic species complex for more than a decade, a formal (re-)description of the four species known so far (*B. calyciflorus*, *Brachionus dorcas*, *Brachionus elevatus*, and *Brachionus fernandoi*) has only recently been made. Information on the ecology of these species is very scant and fragmented. The aim of this study was to test for ecological divergence between these four species, specifically their life history strategy and population demography.
2. We conducted a life history experiment using 12–16 genotypes per species. For each species, genotypes were extracted from at least three different natural populations. In addition, we performed population-level culture experiments with the aim to compare population growth rates and demographic structure of experimental populations among species. Finally, we searched the literature for life history studies with molecular data allowing retrospective species identification.
3. We found pronounced differences in life history traits between *B. fernandoi* and the other three species. *B. fernandoi* had higher egg and juvenile development times and a lower egg production rate and mictic ratio. We detected no significant life history differences among *B. calyciflorus*, *B. elevatus*, and *B. dorcas*.
4. Population growth rates of *B. fernandoi* and *B. calyciflorus* were higher than those of *B. elevatus* and *B. dorcas*. Life history divergence resulted in marked differences in the demographic structure of populations. Populations of *B. fernandoi* contained larger fractions of pre-reproductive females and lower fractions of adult females with sexual eggs than populations of *B. calyciflorus*, *B. elevatus*, and *B. dorcas*. Mortality was found to be highest in *B. elevatus* and lowest in *B. calyciflorus* populations.
5. Our results show that a reverse taxonomy approach is powerful in revealing sources of variation in ecologically relevant traits of cryptic species, such as life

history and demographic structure. Explicit consideration of this variation is crucial for future studies of their dynamics in natural communities.

KEYWORDS
ecological divergence, integrative taxonomy, monogonont rotifer, reverse taxonomy, sibling species

1 | INTRODUCTION

Cryptic species are two or more morphologically similar species under one species name (Bickford et al., 2007). They are believed to be evenly distributed among major metazoan taxa and biogeographical regions (Pfenninger & Schwenk, 2007). The inability of researchers to distinguish between cryptic species poses serious problems with the estimation, protection, and management of biodiversity (Besansky, 1999; Liu, Möller, Gao, Zhang, & Li, 2011; Martinez-Takeshita et al., 2015) and may also lead to incorrect inferences in studies of species coexistence, speciation and biogeography (Chen, Zhong, Dai, Fan, & He, 2017; Hebert, Penton, Burns, Janzen, & Hallwachs, 2004; Razgour et al., 2011). In the last 2 decades, the increase of molecular phylogenetic studies has resulted in the discovery of unexpectedly high numbers of cryptic species complexes (Crespo & Lumbsch, 2010; Haine, Martin, & Cook, 2006; Janzen et al., 2017). Morphological, physiological, and ecological validation of species delineations suggested by molecular analysis remains necessary.

In Rotifera, new cryptic species complexes are being discovered at a rapid pace (Fontaneto, Kaya, Herniou, & Barraclough, 2009; Gómez, Serra, Carvalho, & Lunt, 2002; Kimpel, Gockel, Gerlach, & Bininda-Emonds, 2015; Obertegger, Flaim, & Fontaneto, 2014). From this phylum at least 42 cryptic species complexes have so far been reported (Fontaneto, 2014; Kimpel et al., 2015; Obertegger, Flaim, & Fontaneto, 2014). The most well-known example is the halophile *Brachionus plicatilis* species complex, for which divergence among species has been supported by phylogenetic (Gómez et al., 2002; Mills et al., 2017; Suatoni, Vicario, Rice, Snell, & Caccone, 2006), morphological (Ciros-Pérez, Gómez, & Serra, 2001; Fu, Hirayama, & Natsukari, 1991; Michaloudi et al., 2016), and ecological evidence (Baer, Langdon, Mills, Schulz, & Hamre, 2008; Ciros-Pérez, Carmona, & Serra, 2001; Lapesa, Snell, Fields, & Serra, 2002; Ortells, Gómez, & Serra, 2003) as well as cross-fertilisation experiments (Berrieman, Lunt, & Gómez, 2005; Gribble & Welch, 2012; Snell & Stelzer, 2005).

Although commonly studied and cosmopolitan, much less is known of the freshwater *Brachionus calyciflorus* species complex. *Brachionus calyciflorus* has long been considered as one species until it was suggested to be a cryptic species complex by Gilbert and Walsh (2005). Since then, several studies have contributed with new insights regarding this species complex (Table 1). Several molecular phylogenetic studies have been performed, suggesting a great genetic divergence among specific clades (Cheng, Xi, & Li, 2008; García-Morales & Elías-Gutiérrez, 2013; Xiang, Xi, & Hu, 2007; Xiang, Xi, Zhu, & Xu, 2017), some of which were also found to be reproductively isolated (Gilbert & Walsh, 2005; Li et al., 2008; Xiang et al., 2011a, 2011b) or differing in size or phenology (Gilbert & Walsh, 2005; Li, Niu, & Ma, 2010; Wen, Xi, Zhang, Xue, & Xiang, 2016; Xiang et al., 2007). In addition, phylogeographical studies have revealed different distribution patterns among phylogenetic clades within China, possibly reflecting historic events of range expansion, long distance dispersal, and secondary contact (Xiang, Xi, Wen, Zhang, & Ma, 2010a, 2010b; Xiang et al., 2011a, 2011b). A comprehensive overview of the *B. calyciflorus* species complex was provided by Papakostas et al. (2016) who suggested the existence of four cryptic species based on a phylogenetic analysis of all publicly available sequence information. In addition, using an integrative taxonomy approach, Papakostas et al. (2016) and Michaloudi et al. (2018) demonstrated subtle but consistent morphometric differences among these species. This work formed the basis for the recent (re-)description of four species, *B. calyciflorus*, *Brachionus dorcas*, *Brachionus elevatus*, and *Brachionus fernandoi* (Michaloudi et al., 2018). Several of these cryptic species have been found to coexist (Xiang et al., 2011b; Zhang et al., 2018). There is hardly any information available about how they differ ecologically.

Life history strategy strongly determines the ecology of a species, e.g. by affecting the growth potential and demographic structure of its populations. The comparative analysis of life history strategies of cryptic sibling species is therefore an informative approach to explore potential ecological divergence. Monogonont rotifers are characterised by a cyclical parthenogenetic reproduction mode (Serra, García-Roger, Ortells, & Carmona, 2019) where asexual, clonal propagation is combined with sexual reproduction. During clonal reproduction, asexual (*amictic*) females produce diploid, subitaneous eggs that develop into genetically identical female offspring. Sexual reproduction may be induced by specific environmental cues (e.g. crowding, change of photoperiod, temperature) that initiate the production of sexual (*mictic*) females. These females are morphologically similar to amictic females but produce haploid eggs through meiosis. Upon fertilisation, they will develop into genetically unique diapausing eggs, which are very resistant to adverse conditions. When conditions turn favourable, diapausing eggs will hatch and give raise to amictic females. The exponential population growth rate of parthenogenetically reproducing clones may be considered as short-term fitness and is strongly determined by life history traits such as individual growth and development rate, fecundity, and survival. Mictic ratio, i.e. the proportion of mictic females produced by amictic females,

TABLE 1 Overview of studies that investigated phylogenetically divergent lineages within the *Brachionus calyciflorus* species complex using molecular markers

Topic	Origin	Marker	ITS1	COI	Reference
Taxonomy and cross mating	America and Australia	ITS and COI	*B. dorcas*, *B. calyciflorus*, and *B. fernandoi*	3, 9 and 12	Gilbert and Walsh (2005)
Sympatric speciation and cross mating	China	ITS and COI	NA[a]	6, 8 and 11	Li et al. (2008)
Taxonomy	China	ITS	*B. dorcas*	NA	Zhang, Xi, Ma, and Xiang (2010)
Phylogeography	China	ITS	*B. dorcas*, *B. elevatus*, and *B. calyciflorus*	NA	Xiang et al. (2010a)
Phylogeography	China	COI	NA	1, 6, 7, 8 and 11	Xiang et al. (2010b)
Phylogeography and cross mating	China	COI	NA	1, 7, 8, 9, 11, 13, 14 and 15	Xiang et al. (2011a)
Phylogeography and cross mating	China	ITS	*B. dorcas*, *B. calyciflorus*, and *B. fernandoi*	NA	Xiang et al. (2011b)
Taxonomy	Mexico	COI	NA	4	García-Morales and Elías-Gutiérrez (2013)
Population growth rate	China	COI	NA	7 and 11	Xiang et al. (2015)
Coexistence	China	COI	NA	14 and 15	Wen et al. (2016)
Taxonomy, morphology and competition	Netherlands, America, China, Mexico and Italy	ITS1, COI, 18S, 28S, microsatellite	*B. dorcas*, *B. elevatus*, *B. calyciflorus*, and *B. fernandoi*	1 to 15	Papakostas et al. (2016)
Population genetic structure	China	16S and ITS	*B. dorcas*, *B. elevatus*, and *B. fernandoi*	NA	Xiang et al. (2017)

Note: Marker: type of molecular markers used by the studies; ITS1: species as described by Michaloudi et al. (2018) according to internal transcribed spacer 1 data provided by the study; COI: COI clades as delimited by Papakostas et al. (2016) using COI data provided by the study. NA indicates data not provided.

[a]ITS1 data not available.

is another critical trait. High mictic ratios may reduce short-term fitness (Stelzer, 2011) because males and sexual eggs do not contribute to immediate exponential population growth. The production of sexual eggs is, nevertheless, an important determinant of long-term fitness across growing seasons (García-Roger, Serra, & Carmona, 2014; Tarazona, García-Roger, & Carmona, 2017). So far, information on life history divergence among species of the *B. calyciflorus* species complex has been scant and fragmented. While many studies have investigated the life history response of *B. calyciflorus* to specific ecological factors, mainly temperature and food quantity (Li, Xi, & Cheng, 2009; Ma, Xi, Zhang, Wen, & Xiang, 2010; Xiang, Xi, Zhang, Ma, & Wen, 2010c; Xiang et al., 2015) molecular information on the phylogenetic position of the investigated genotypes is seldom provided.

Many studies have documented substantial intraspecific clonal variation for life history traits in zooplankton (Gilbert & Schröder, 2007; Michels & De Meester, 2004). As a result, genetic divergence among populations within species may result from drift or adaptation to local (Campillo, García-Roger, Carmona, & Serra, 2011; De Meester, Gómez, Okamura, & Schwenk, 2002; Declerck et al., 2015) or regional conditions (Ma et al., 2010). Such genetic differences potentially bias comparisons among species if based on one or just a few genotypes or populations. To ensure that observed differences reflect fundamental differences among species rather than being contingent on the identity of the genotypes, populations, or region under study, the analysis of ecological divergence between sibling species requires a robust experimental design including numerous genotypes from multiple populations, preferentially from the same geographic region. Species also need to be clearly defined and based on a strong taxonomic foundation, preferentially supported by a comprehensive phylogenetic analysis and additional information on morphology and cross-fertilisation data.

The aims of the present study were: (1) to provide a comprehensive comparison for the existence of systematic life history divergences among the four *cryptic* species described for the *B. calyciflorus* complex by Michaloudi et al. (2018); and (2) to illustrate the potential impact of such differences for population growth rate and demography. For each species, we used genotypes from multiple populations in The Netherlands to combine a life history experiment with a demographic analysis of exponentially growing laboratory cultures. In addition, we searched the literature for life history studies comparing phylogenetically divergent clades of the *B. calyciflorus* species complex and traced these clades back to the four species

described by Michaloudi et al. (2018) using the molecular information provided by these studies.

2 | METHODS

2.1 | Diapausing egg collection, clone establishment, and stock culture maintenance

Sediment samples were collected from seven sites in The Netherlands in April 2016 (Table 2). *Brachionus calyciflorus* diapausing eggs were isolated from the sediments using the sugar flotation method described by Gómez and Carvalho (2000) and hatched under continuous light in petri dishes using distilled water at room temperature (23 ± 1°C). Dishes were checked at 12-hr intervals and hatched *B. calyciflorus* females were transferred individually to wells of 24-well plates filled with 1 ml of chemostat cultured *Chlamydomonas reinhardtii* (1,000 μmol C/L, molar C:P ratio of approximately 200:1) resuspended in WC medium (Guillard, 1975). After population sizes had grown larger than five individuals, they were transferred to 20-ml plastic containers with 8 ml of food suspension and further maintained at room temperature (23 ± 1°C) under continuous light. Every other day, 10–15 females carrying parthenogenetic eggs were transferred to new containers with fresh food.

2.2 | DNA extraction, internal transcribed spacer 1 polymerase chain reaction, restriction fragment length polymorphisms, and microsatellite genotyping

Species were identified using restriction fragment length polymorphisms (RFLPs) together with microsatellite genotyping. DNA was extracted from single rotifer individuals using the HotSHOT method described by Montero-Pau, Gómez, and Muñoz (2008). Internal transcribed spacer 1 (ITS1, between 296 bp and 313 bp) were amplified using the primers III: 5′-CACACCGCCCGTCGCTACTACCGATTG-3′ and VIII: 5′-GTGCGTTCGAAGTGTCGATGATCAA-3′ (Hwang, Dahms, Park, & Lee, 2013). For RFLP analysis, 3 μl of ITS1 polymerase chain reaction products were digested by 1 μl *Alu*I and *Dra*I (New England Biolabs, Inc.) separately and incubated overnight at 37°C. The species were identified by examining the pattern of DNA bands produced on 1% agarose gel as described in Papakostas et al. (2016). The species *B. elevatus* and *B. calyciflorus* have been shown to form hybrids readily (Papakostas et al., 2016). Because interspecific hybrids of *B. elevatus* and *B. calyciflorus* cannot reliably be identified by RFLPs we tested for the occurrence of such hybrid genotypes using microsatellite analysis. Twelve microsatellite loci of *B. elevatus* and *B. calyciflorus* were amplified according to Declerck et al. (2015) and analysed with an ABI Prism 3130 DNA Analyzer (Applied Biosystems, CA, U.S.A.). Hybrids were identified using loci 'A9' and 'A15' as diagnostic loci (Papakostas et al., 2016).

2.3 | Experimental design

Our original design consisted of 16 genotypes originating from four natural populations per species (i.e. 4 species × 4 populations × 4 clones, totalling 64 clones). Unfortunately, four genotypes of both *B. elevatus* and of *B. calyciflorus* had to be omitted from the design because posterior microsatellite analysis showed them to be hybrids (see Table 2 for a full representation of the experimental design). As a result, *B. elevatus* was represented by only three populations and the four populations of *B. calyciflorus* were represented by 2–4 genotypes. Life history and population growth rate experiments were conducted in an incubator at 24°C under continuous darkness to prevent growth of algae. Food suspensions were identical to those of the stock cultures. Culture volumes in life history and population growth rate experiments equalled 1 and 8 ml, respectively. Food suspensions were refreshed on a daily basis.

2.4 | Life history experiment

To minimise maternal effects, we started with a great grandmaternal generation by isolating and individually culturing 10 individuals per clone in 1 ml wells of tissue culture plates. The first neonates of these individuals were then used to start the grandmaternal generation. This procedure was repeated until the animals of the focal generation were born. To avoid pseudoreplication, all individuals were cultured separately across the subsequent generations. In the actual life history experiment we studied three haphazardly chosen replicates of the focal generation per clone (i.e. 56 clones × 3 replicates = 168 units in total).

Life history traits were quantified by monitoring individuals of the focal generation through a major part of their life span. To

TABLE 2 Experimental design of life history and growth rate experiments

Population	Latitude	Longitude	*B. dorcas*	*B. elevatus*	*B. calyciflorus*	*B. fernandoi*
NL7	N 51.854°	E 5.893°	4	4	3	
NL69	N 52.090°	E 4.338°		4	2	
NL101	N 52.025°	E 4.328°		4		
NL128	N 52.640°	E 4.730°	4		3	4
NL134	N 52.734°	E 4.883°	4			4
NL168	N 51.491°	E 4.306°			4	4
NL181	N 51.839°	E 4.144°	4			4

Note: For each species, the number of genotypes and geographic coordinates are given for each of the studied populations. Eight *B. elevatus* and *B. calyciflorus* hybrid clones as identified by microsatellite analysis were excluded from the design.

initiate the experiment the parental generation was inspected every hour in order to isolate focal individuals as soon after their birth as possible. Eight hours after isolation, focal individuals were checked at 2-hr intervals. At each inspection, survival, occurrence of sexual eggs, and number of parthenogenetic eggs and neonates were recorded. Dead or mictic females and newly produced neonates were discarded immediately. When focal individuals produced their second neonates, the focal individuals were preserved in 4% formalin. These samples were used for the estimation of body and egg volume using a stereomicroscope with camera. The life history experiment lasted approximately 8 days.

2.5 | Population growth rate experiment

For each of the 56 clones, a cohort of 10 individuals was isolated from stock cultures and incubated in a single well of a 6-well plate with 8 ml food. Every 24 hr, the total numbers of individuals were counted and 10 females were transferred to a new well with new medium and fresh food to restart the population. Initially, we restarted populations by selecting females in a random fashion. Some clones, however, were characterised by high proportions of mictic females. Because mictic females do not contribute to population growth, this repeatedly resulted in the loss of clones. For this reason, we decided to randomly select and transfer exclusively those individuals without sexual eggs. Populations were considered stable from the day that no temporal trend in population growth could be observed (i.e. absence of a significant correlation between population growth rate and time). From that moment on, during 13 consecutive days, we preserved all individuals that remained after the transfer in 4% formalin and pooled them per clone. Afterwards these samples were counted to study the time-integrated demographic structure of the populations. Individuals were classified as either females with no eggs, females with parthenogenetic eggs, females with sexual eggs, and dead females. Given that experimental animals were fed high concentrations of good-quality food throughout the experiment, females without eggs were all assumed to be pre-reproductive.

2.6 | Literature survey

We conducted a systematic literature survey of all the studies available through Web of Science and Google Scholar. We searched journal articles using "*Brachionus calyciflorus*" and "cryptic species" combined with "life history" or "life table" as keywords and searched from 2005 (the year that *B. calyciflorus* was first suggested to be a species complex) until 2018. From these studies, we only retained those studies that explicitly addressed cryptic species or phylogenetically divergent lineages in *B. calyciflorus* based on the phylogenetic analysis of molecular markers.

2.7 | Data analysis

Life history data of the focal generation were used to calculate mortality, mictic ratio, age at first reproduction (AFR), egg development time of the first egg (EDT1) and second egg (EDT2), egg production rate (EPR), body volume (BV), and average egg volume (AEV). Mortality was estimated as the fraction of the total number of individuals from the focal generation that died before reproducing, whereas the mictic ratio was calculated as the fraction of surviving individuals that were found to produce sexual instead of parthenogenetic eggs. Age at first reproduction referred to the age of the focal individual at which the first egg was produced. Egg production rate was calculated as the total number of eggs produced per hour during a time interval encompassing at least two egg production events per individual. Body volume corresponds to the size of the adult at the time of hatching of its second neonate and was calculated as $V_b = \pi^* L_b^* (W_b/2)^2$ (Zhou, Lemmen, Zhang, & Declerck, 2018), where L_b and W_b are body length and width (in μm), respectively. Average egg volume was calculated as the average volume of all eggs (range 1–4) present in a clutch with the volume of each egg being estimated as an ellipsoid: $V_e = (4/3)^*\pi^*(L_e/2)^*(W_e/2)^2$, where L_e and W_e are egg length and width (in μm), respectively. Daily population counts from the growth rate experiment were used to calculate realised population growth rates as $(\ln N_t - \ln N_0)/t$, where N_0 and N_t represent the population size at the beginning and end of each day interval, respectively.

We applied mixed-effects models to test for differences in life history traits, population growth rates and demographic traits between species. For life history traits, generalised linear mixed-effects models were applied to mortality and mictic ratio data, assuming a binomial distribution with logit link function. The other life history variables were analysed with general linear mixed-effects models assuming a normal distribution. Species were treated as fixed effects by design, the factors *clone* and *population* were specified as random effects in all analyses. The growth rate data from the population growth rate experiment were first averaged over time and these means were subsequently analysed with linear mixed-effects models. Generalised linear mixed-effects models were applied to the mortality data of the growth rate experiment as well as on the fractions of pre-reproductive females, females with parthenogenetic eggs, and females with sexual eggs. In these analyses, species and populations were treated as fixed and random effects, respectively. The significances of individual model terms were assessed by comparing reduced with full models as specified in Tables S1 and S2 using AIC-values and likelihood ratio tests. All statistical analyses were performed in the R software environment 3.3.3 (R Core Team 2017). Generalised and linear mixed-effects models were performed using the lme4 package (Bates, Mächler, Bolker, & Walker, 2014). Tukey post hoc tests were performed with the multcomp package (Hothorn, Bretz, & Westfall, 2008).

3 | RESULTS

3.1 | Life history experiment

Due to the occurrence of mixis and mortality in focal, maternal, and grandmaternal generations, complete life history data of only 75

out of 168 units could be collected. In the focal generation, nine of the 75 individuals died during the experiment. Mortality in the focal generations of *B. dorcas*, *B. elevatus*, *B. calyciflorus*, and *B. fernandoi* equalled 6.3, 5.6, 0, and 8.3%, respectively.

Fifty-one percent of the individuals in the focal generation were mictic. Mictic ratio differed significantly among species ($\chi^2(3) = 29.23$, $p < 0.001$). Species-specific mictic ratios ranged from 15.6% in *B. fernandoi* to 68.8% in *B. dorcas*. Mictic ratio in *B. dorcas*, *B. elevatus*, and *B. calyciflorus* were substantially higher than in *B. fernandoi* (Figure 1a).

Species averages of AFR ranged between 15.4 and 20.1 hr (Figure 1b). Species differed significantly in AFR ($\chi^2(3) = 43.80$, $p < 0.001$). This effect was mainly due to *B. fernandoi* for which AFR was found to be on average 26.9% longer than that of the other three

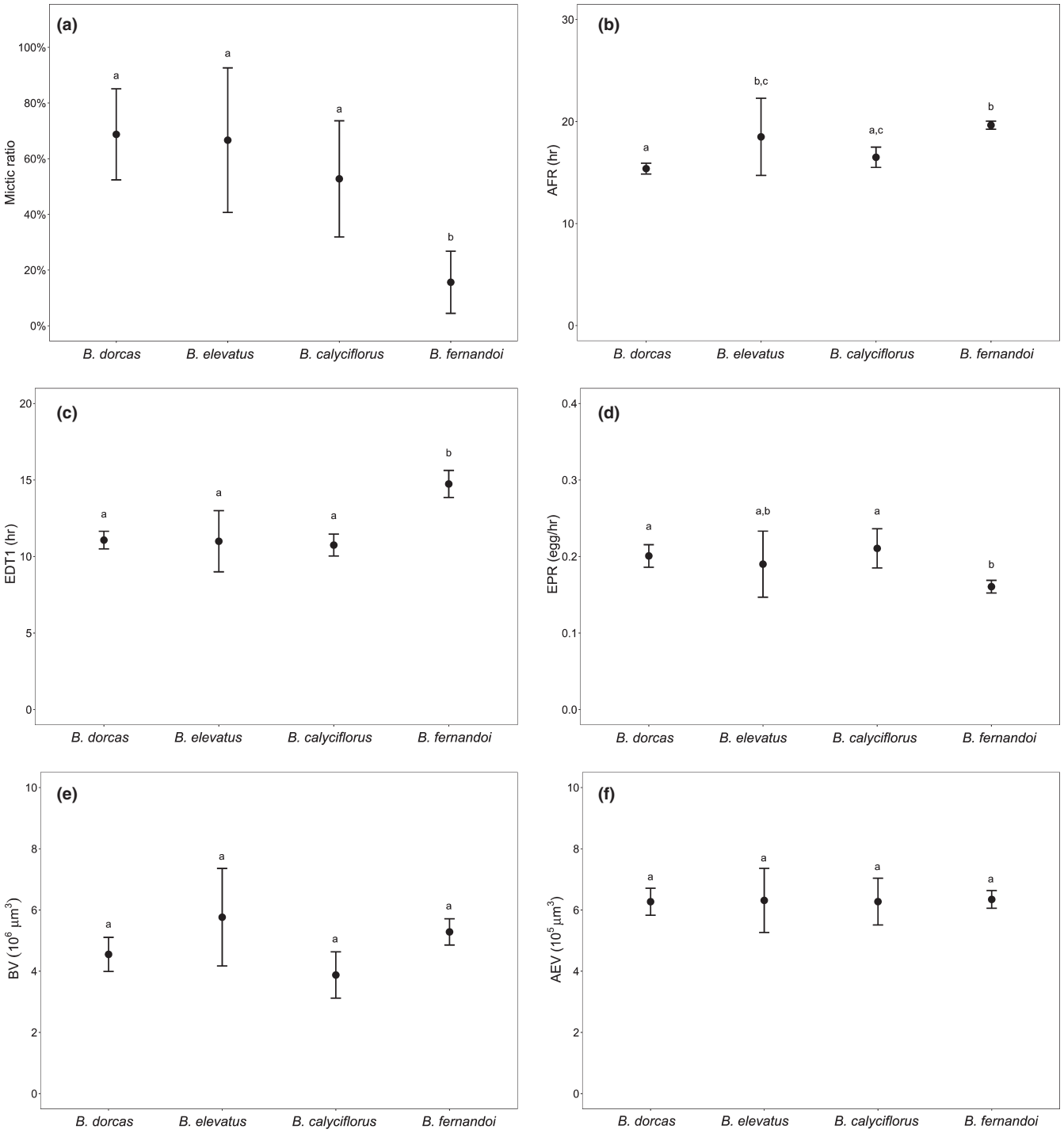

FIGURE 1 Life history traits observed for the four species of the *Brachionus calyciflorus* species complex: (a) mictic ratio; (b) age at first reproduction (AFR); (c) development time of first egg (EDT1); (d) egg production rate (EPR); (e) body volume (BV); (f) average egg volume (AEV). Symbols represent mean values of clones. Error bars reflect variation among clones equalling twice the standard error of the mean. Different letters indicate significant differences according to Tukey post hoc tests

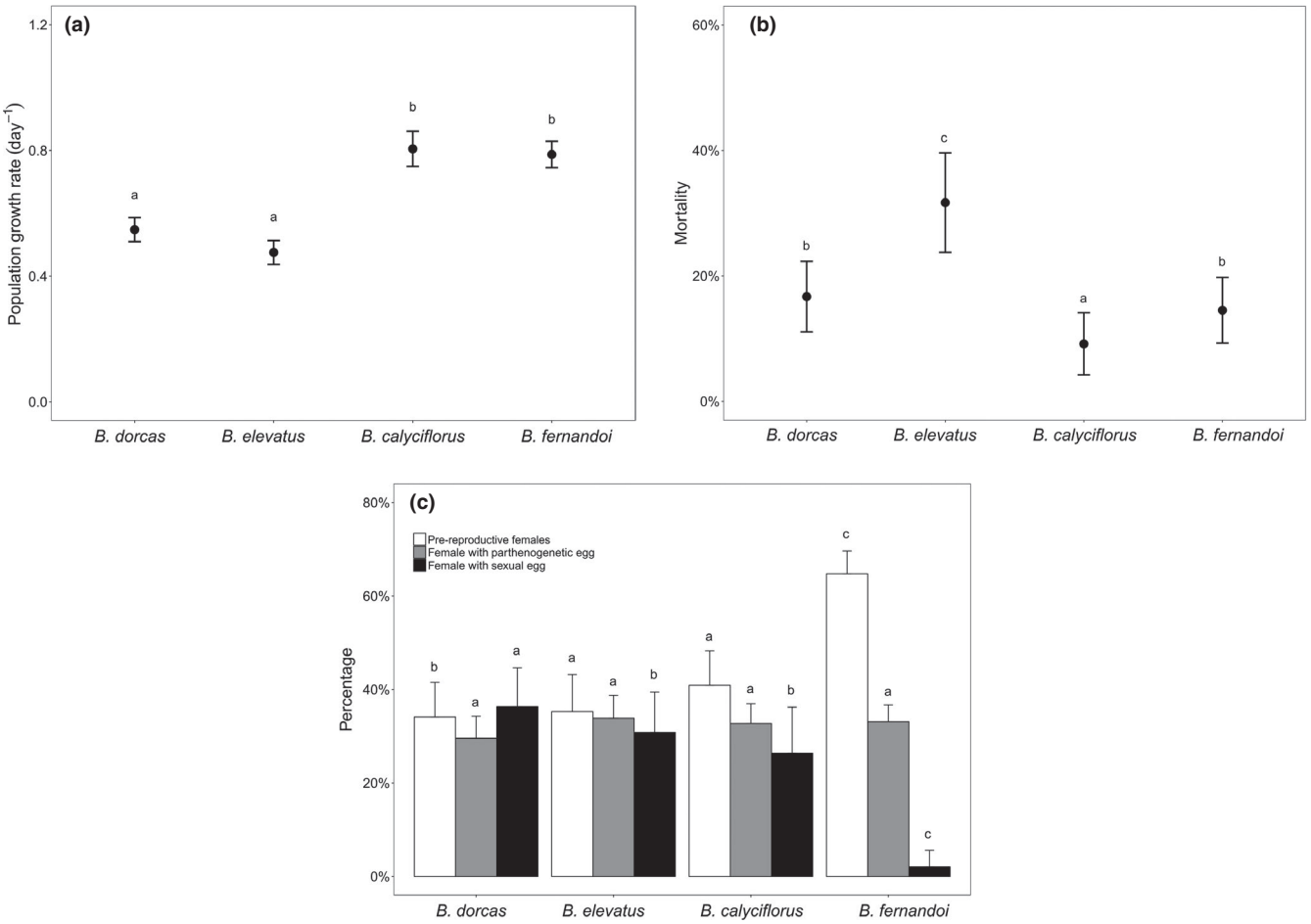

FIGURE 2 Population characteristics of the four studied *Brachionus* species as observed in the population growth rate experiment: (a) population growth rate; (b) mortality expressed as fraction of dead individuals; (c) population demographic structure. Symbols represent mean values across days and clones. Error bars reflect variation among clones and equal twice the standard error of the mean. Different letters indicate significant differences according to Tukey post hoc tests

species. Results were highly similar for the ages at which the second, third, and fourth eggs were produced with differences among species becoming more pronounced (Figure S1).

Development time of the first egg ranged from 10.7 to 14.6 hr and EDT2 ranged from 10.0 to 14.2 hr (Figures 1c and S2) and differed significantly among species (EDT1: $\chi^2(3) = 41.02$, $p < 0.001$; EDT2: $\chi^2(3) = 19.29$, $p < 0.001$). This species effect was due to *B. fernandoi* for which EDT1 and EDT2 (Figure S2) were found to be approximately 35% and 29% longer than that of the average of the other three species, respectively.

Species averages of EPR ranged between 0.16 and 0.21 egg/hr (Figure 1d). Species differed significantly in EPR ($\chi^2(3) = 14.39$, $p = 0.002$). Egg production rate of *B. fernandoi* was on average 22% lower than that of *B. dorcas* and *B. calyciflorus* while *B. elevatus* showed intermediate EPR.

The four species also differed significantly in BV ($\chi^2(3) = 8.01$, $p = 0.046$), although a Tukey post hoc test did not provide evidence for differences among any of the species pairs (Figure 1e). Furthermore, we found no evidence for species differences in AEV (Figure 1f).

The random factor *clone* was found to be significant in the majority of traits, e.g. mictic ratio, AEV, EPR, and BV (Table S3). In contrast, *population* was never found to be significant (Table S3). For a summary of full models, refer to Tables S4 and S5.

3.2 | Population growth rate experiment

Estimated population growth rates varied between 0.48 and 0.81 day^{-1} (Figure 2a) and differed significantly among species ($\chi^2(3) = 33.69$, $p < 0.001$). Growth rates of *B. calyciflorus* and *B. fernandoi* were >50% higher than that of *B. dorcas* and *B. elevatus*. Mortality rates in the populations ranged between 9.2 and 31.7% (Figure 2b) and differed among species ($\chi^2(3) = 227.04$, $p < 0.001$). The fraction of pre-reproductive females in the populations ranged between 34.1 and 64.8% (Figure 2c) and differed among species ($\chi^2(3) = 366.0$, $p < 0.001$). The fraction of pre-reproductive females in *B. fernandoi* was on average 76% higher than that of the other species. The fraction of females that carried parthenogenetic eggs was very similar among species and ranged between 29.6 and 33.9% (Figure 2c). In contrast, the fraction of females carrying sexual eggs

TABLE 3 Overview of life history studies on *Brachionus calyciflorus* that provide the molecular information needed to assign genotypes to taxa

Origin	Marker	ITS1	COI	Population	Genotypes per clade	Replicates per genotype	Factors	Reference
China	COI	NA	9, 11 (6, 15)[a]	1	24	3	3 temperature × 3 food density levels	Li et al. (2010)
China	COI	NA	7 and 11	2	1	48	4 temperature × 4 food density levels	Wang et al. (2014)
China	COI	NA	6 and 7	2	1	48	4 temperature levels	Xiang et al. (2016)
China	ITS and COI	*B. dorcas*, *B. calyciflorus* and *B. fernandoi*	6, 11 and 15	1	4	1	4 temperature × 2 food density levels	Zhang et al. (2018)

Note: ITS1: species as described by Michaloudi et al. (2018) according to internal transcribed spacer 1 data provided by the study; COI: COI clades as delimited by Papakostas et al. (2016) using COI data provided by the study.

[a]Brackets indicate COI clades reported in paper but not studied for life history.

ranged between 2.1 and 36.3% and was substantially lower in *B. fernandoi* than in the other species ($\chi^2(3) = 818.27$, $p < 0.001$).

3.3 | Literature survey

Our literature search yielded 10 and 644 results on Web of Science and Google Scholar, respectively. After application of our additional selection criteria, we retained four studies, which are listed in Table 3. In three of four studies, phylogenetically divergent lineages were identified using COI phylogeny. Li et al. (2010) and Wang et al. (2014) reported life history differentiation between cytochrome c oxidase subunit (COI) lineages under different temperature and food conditions. Xiang, Chen, Han, Wang, and Xi (2016) showed differential responses in life history traits between two COI lineages from different climate zones. Zhang et al. (2018) is the only study that delimited species based on a phylogenetic analysis of ITS1 sequences, thus allowing species identification according to Michaloudi et al. (2018). Using genotypes collected from one single population, they found that *B. dorcas*, *B. calyciflorus*, and *B. fernandoi* showed different responses in their mictic ratio and population growth rates to variation in temperature and food concentrations.

4 | DISCUSSION

Our results demonstrate the existence of substantial life history divergences among the species described by Michaloudi et al. (2018) and illustrate its potential implications for population demography. Along with other studies (Darwell & Cook, 2017; Hebert et al., 2004; Janzen et al., 2017), our study shows that reverse taxonomy is not only a powerful approach for the discovery of new species within cryptic species complexes but that it may also be effective in guiding the exploration of sources of variation in ecologically relevant traits. Life history traits are generally regarded as evolutionary versatile as rapid adaptive responses of such traits to local selection pressures have been demonstrated for a variety of organisms (Jensen et al., 2008; Kelehear, Brown, & Shine, 2012). However, with a design based on genotypes from multiple population origins, our study observed robust differences between species. This illustrates that the correct identification of species is a requirement for the study of ecological dynamics in communities with *cryptic* species.

In our study, the most pronounced divergence was found between *B. fernandoi* and the other three species. Interestingly, this pattern of divergence is concordant with the relative phylogenetic distances recorded by Papakostas et al. (2016) for the nuclear ITS1 marker. According to the life history results, *B. fernandoi* was characterised by a lower degree of mixis, longer juvenile and egg development times, and a lower egg production rate. Proportions of females carrying sexual eggs were also much lower in the *B. fernandoi* cultures of the growth rate experiment than in the populations of *B. dorcas*, *B. elevatus*, and *B. calyciflorus*. Life history theory predicts that long development times and low rates of reproductive output have a negative impact on population growth rates.

A high propensity for sexual reproduction is also known to trade off with rapid clonal population growth in rotifers (Gabaldón, Carmona, Montero-Pau, & Serra, 2015; Gilbert, 2010; Smith & Snell, 2014; Stelzer, 2011).

Brachionus fernandoi realised higher population growth rates than *B. dorcas* and *B. elevatus* but not *B. calyciflorus*. The high relative performance observed for *B. fernandoi* is best explained by its relatively low investment in sexual reproduction. Apparently, the low allocation of reproductive investment of this species towards sexual eggs fully compensated the negative impact of longer development times and lower reproductive output. It should also be remembered that, in an attempt to generate positive population growth, we selected against females with sexual propagules at the daily population transfers in this experiment. Given that sexual females are unable to contribute directly to clonal population growth, selection against sexual females in *B. dorcas*, *B. elevatus*, and *B. calyciflorus* must have resulted in a relative overestimation of their population growth compared to *B. fernandoi*. However, the impact of this selective transfer should also not be overestimated. The fact that sexual eggs were still found in relatively high numbers in *B. dorcas*, *B. elevatus*, and *B. calyciflorus* populations despite their removal at 24-hr intervals, suggests that the populations of these species still consisted of large fractions of non-gravid mictic females that remained unnoticed during the daily transfers. Overall, the results of the population growth rate experiment, nevertheless, suggest a higher capacity for clonal population growth of *B. fernandoi* compared to the other three species.

The strong divergence in life history traits between *B. fernandoi* and the other species was also clearly reflected in the demographic structure of the populations from the population growth rate experiment. Whereas the proportions of pre-reproductive females, and parthenogenetically and sexually reproducing adults in the populations of *B. dorcas*, *B. elevatus*, and *B. calyciflorus* were very similar, the demographic structure of *B. fernandoi* deviated substantially from that of the other species (Figure 2c). The combination of lower per capita fractions of sexually reproducing adults with a higher age at maturity probably resulted in higher proportions of pre-reproductive females, despite longer egg development times. In our experiment, populations were allowed to grow exponentially under food saturating conditions. Although these conditions may only be representative for specific conditions in the field, our results nevertheless illustrate how strong the impact can be of life history divergence on the population demographic structure of cryptic sibling species.

Given their very recent description, not much information is available about ecological differentiation among the species of the *B. calyciflorus* species complex. Papakostas et al. (2016) provided evidence for strong differences in the competitive ability among two of the four species. Using replicate populations composed of varying clonal composition, they demonstrated the consistent exclusion of *B. elevatus* by *B. calyciflorus* under six different culture conditions. These observations were obtained in semi-continuous laboratory culture conditions. Although they are not necessarily very representative for the much more complex conditions in natural populations, they strongly support the idea that species differ in their ecology.

A number of studies (Cheng et al., 2008; Li et al., 2010; Wen et al., 2016; Xiang et al., 2007) have documented temporal dynamics of coexisting phylogenetically divergent lineages within natural communities. The few that provide molecular data suitable for species identification in accordance with Michaloudi et al. (2018) suggest seasonal specialisation between *B. fernandoi* and the other species, probably driven by differences in thermal optima and tolerances. In an ITS-based phylogeographic survey of a 2,300 km north–south gradient in eastern China, Xiang et al. (2011b) exclusively found *B. fernandoi* ("BcI-W" in Xiang et al., 2011b) during the winter. In contrast, *B. dorcas* and *B. calyciflorus* ("BcII-S" and "Bc-SW") were primarily found during summer and only during winter in the subtropical and tropical zone. This finding was further corroborated by the detailed study of Zhang et al. (2018) who performed a full-year monitoring of the zooplankton community in subtropical Lake Tingtang. During summer, *B. dorcas* ("BcII-S" in Zhang et al., 2018) and *B. calyciflorus* ("Bc-SW") were found to coexist until July. After a 3-month disappearance (August–October) these species were found to be entirely replaced by *B. fernandoi* ("BcI-W") throughout the subsequent winter and spring. Additional life history experiments suggested temperature as an important seasonal clue to these phenological differences by showing higher induction of mixis in *B. fernandoi* than in the other species at summer temperatures and the opposite pattern at autumn temperatures (Zhang et al., 2018). These results align well with the recent findings of Paraskevopoulou, Tiedemann, and Weithoff (2018) who demonstrated a consistently higher tolerance to heat stress in *B. calyciflorus* than in *B. fernandoi*.

Several studies have documented life history differences among phylogenetically divergent clades of the *B. calyciflorus* complex. The majority of these studies have based their species delimitation on COI. Mitonuclear discordances within *B. calyciflorus* species complex have been shown to occur, probably as a result of hybridisation, and ITS1-based species delimitations were found to be more accurate predictors of morphological variation than COI-based species delimitations (Papakostas et al., 2016). Thus, COI-based clade delimitations can therefore not be unequivocally traced back to the species described by Michaloudi et al. (2018). To the best of our knowledge, Zhang et al. (2018) is the only study documenting divergence of life history traits among ITS1-delimited clades (Zhang et al., 2018) with each of the clades being represented by multiple replicated genotypes. While in our study *B. fernandoi* was clearly different from the other species by its low degree of mixis at 24°C, Zhang et al. (2018) found the opposite, i.e. within the range of 20–25°C mictic ratios of *B. fernandoi* were considerable higher than for the other species in this study. A direct comparison of the results between studies is of course difficult because experiments were performed under different conditions. Nevertheless, in both studies the species were compared in a common garden environment under standard laboratory conditions, which should still allow comparison of patterns of species divergence across studies. Given that our populations originated from a temperate climate zone and the population studied by Zhang et al. (2018) was subtropical, the observed discrepancies may reflect

species-specific adaptations to climatic conditions. Interspecific life history comparisons between more populations from subtropical lakes would be needed to determine the generality of this pattern. The results of Zhang et al. (2018), nevertheless, confirm that clear life history differences among the species exist, especially between *B. fernandoi* on one hand and *B. dorcas* and *B. calyciflorus* on the other hand, but also indicate that the divergences observed in our study, although robust within The Netherlands, are not necessarily representative for other regions or climate zones in the world.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Baer, A., Langdon, C., Mills, S., Schulz, C., & Hamre, K. (2008). Particle size preference, gut filling and evacuation rates of the rotifer *Brachionus* "Cayman" using polystyrene latex beads. *Aquaculture*, *282*(1), 75–82. https://doi.org/10.1016/j.aquaculture.2008.06.020

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *ArXiv*, 1406.5823. arXiv preprint. https://doi.org/10.18637/jss.v067.i01

Berrieman, H. K., Lunt, D. H., & Gómez, A. (2005). Behavioural reproductive isolation in a rotifer hybrid zone. *Hydrobiologia*, *546*(1), 125–134. https://doi.org/10.1007/1-4020-4408-9_11

Besansky, N. (1999). Complexities in the analysis of cryptic taxa within the genus *Anopheles*. *Parassitologia*, *41*(1–3), 97–100.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Campillo, S., García-Roger, E. M., Carmona, M. J., & Serra, M. (2011). Local adaptation in rotifer populations. *Evolutionary Ecology*, *25*(4), 933–947. https://doi.org/10.1007/s10682-010-9447-5

Chen, W., Zhong, Z., Dai, W., Fan, Q., & He, S. (2017). Phylogeographic structure, cryptic speciation and demographic history of the sharpbelly (*Hemiculter leucisculus*), a freshwater habitat generalist from southern China. *BMC Evolutionary Biology*, *17*(1), 216. https://doi.org/10.1186/s12862-017-1058-0

Cheng, X., Xi, Y., & Li, H. (2008). Seasonal changes in the genetic structure of a *Brachionus calyciflorus* population in Lake Liantang based on ITS sequences. *Acta Zoologica Sinica*, *54*(2), 245–255.

Ciros-Pérez, J., Carmona, M. J., & Serra, M. (2001). Resource competition between sympatric sibling rotifer species. *Limnology and Oceanography*, *46*(6), 1511–1523. https://doi.org/10.4319/lo.2001.46.6.1511

Ciros-Pérez, J., Gómez, A., & Serra, M. (2001). On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research*, *23*(12), 1311–1328. https://doi.org/10.1093/plankt/23.12.1311

Crespo, A., & Lumbsch, H. T. (2010). Cryptic species in lichen-forming fungi. *IMA Fungus*, *1*(2), 167–170. https://doi.org/10.5598/imafungus.2010.01.02.09

Darwell, C. T., & Cook, J. M. (2017). Cryptic diversity in a fig wasp community—morphologically differentiated species are sympatric but cryptic species are parapatric. *Molecular Ecology*, *26*(3), 937–950. https://doi.org/10.1111/mec.13985

De Meester, L., Gómez, A., Okamura, B., & Schwenk, K. (2002). The Monopolization Hypothesis and the dispersal–gene flow paradox in aquatic organisms. *Acta Oecologica*, *23*(3), 121–135. https://doi.org/10.1016/s1146-609x(02)01145-1

Declerck, S. A. J., Malo, A. R., Diehl, S., Waasdorp, D., Lemmen, K. D., Proios, K., & Papakostas, S. (2015). Rapid adaptation of herbivore consumers to nutrient limitation: Eco-evolutionary feedbacks to population demography and resource control. *Ecology Letters*, *18*(6), 553–562. https://doi.org/10.1111/ele.12436

Fontaneto, D. (2014). Molecular phylogenies as a tool to understand diversity in rotifers. *International Review of Hydrobiology*, *99*(1–2), 178–187. https://doi.org/10.1002/iroh.201301719

Fontaneto, D., Kaya, M., Herniou, E. A., & Barraclough, T. G. (2009). Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, *53*(1), 182–189. https://doi.org/10.1016/j.ympev.2009.04.011

Fu, Y., Hirayama, K., & Natsukari, Y. (1991). Morphological differences between two types of the rotifer *Brachionus plicatilis* O.F. Müller. *Journal of Experimental Marine Biology and Ecology*, *151*(1), 29–41. https://doi.org/10.1016/0022-0981(91)90013-m

Gabaldón, C., Carmona, M. J., Montero-Pau, J., & Serra, M. (2015). Long-term competitive dynamics of two cryptic rotifer species: Diapause and fluctuating conditions. *PLoS One*, *10*(4), e0124406. https://doi.org/10.1371/journal.pone.0124406

García-Morales, A. E., & Elías-Gutiérrez, M. (2013). DNA barcoding of freshwater Rotifera in Mexico: Evidence of cryptic speciation in common rotifers. *Molecular Ecology Resources*, *13*(6), 1097–1107. https://doi.org/10.1111/1755-0998.12080

García-Roger, E. M., Serra, M., & Carmona, M. J. (2014). Bet-hedging in diapausing egg hatching of temporary rotifer populations - A review of models and new insights. *International Review of Hydrobiology*, *99*(1–2), 96–106. https://doi.org/10.1002/iroh.201301708

Gilbert, J. J. (2010). Effect of food concentration on the production and viability of resting eggs of the rotifer *Brachionus*: Implications for the timing of sexual reproduction. *Freshwater Biology*, *55*(12), 2437–2446. https://doi.org/10.1111/j.1365-2427.2010.02455.x

Gilbert, J. J., & Schröder, T. (2007). Intraclonal variation in propensity for mixis in several rotifers: Variation among females and with maternal age. *Hydrobiologia*, *593*(1), 121–128. https://doi.org/10.1007/s10750-007-9040-2

Gilbert, J. J., & Walsh, E. J. (2005). *Brachionus calyciflorus* is a species complex: Mating behavior and genetic differentiation among four geographically isolated strains. *Hydrobiologia*, *546*(1), 257–265. https://doi.org/10.1007/s10750-005-4205-3

Gómez, A., & Carvalho, G. (2000). Sex, parthenogenesis and genetic structure of rotifers: Microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology*, *9*(2), 203–214. https://doi.org/10.1046/j.1365-294x.2000.00849.x

Gómez, A., Serra, M., Carvalho, G. R., & Lunt, D. H. (2002). Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, *56*(7), 1431–1444. https://doi.org/10.1111/j.0014-3820.2002.tb01455.x

Gribble, K. E., & Welch, D. B. M. (2012). The mate recognition protein gene mediates reproductive isolation and speciation in the *Brachionus plicatilis* cryptic species complex. *BMC Evolutionary Biology*, *12*(1), 134. https://doi.org/10.1186/1471-2148-12-134

Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In W. L. Smith & M. H. Chanley (Eds.), *Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport* (pp. 29–60). Boston, MA: Springer.

Haine, E. R., Martin, J., & Cook, J. M. (2006). Deep mtDNA divergences indicate cryptic species in a fig-pollinating wasp. *BMC Evolutionary Biology*, *6*(1), 83. https://doi.org/10.1186/1471-2148-6-83

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(41), 14812–14817. https://doi.org/10.1073/pnas.0406166101

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hwang, D.-S., Dahms, H.-U., Park, H. G., & Lee, J.-S. (2013). A new intertidal *Brachionus* and intrageneric phylogenetic relationships among *Brachionus* as revealed by allometry and CO1-ITS1 gene analysis. *Zoological Studies*, *52*(1), 13. https://doi.org/10.1186/1810-522x-52-13

Janzen, D. H., Burns, J. M., Cong, Q., Hallwachs, W., Dapkey, T., Manjunath, R., … Grishin, N. V. (2017). Nuclear genomes distinguish cryptic species suggested by their DNA barcodes and ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(31), 8313. https://doi.org/10.1073/pnas.1621504114

Jensen, L. F., Hansen, M. M., Pertoldi, C., Holdensgaard, G., Mensberg, K. L. D., & Loeschcke, V. (2008). Local adaptation in brown trout early life-history traits: Implications for climate change adaptability. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1653), 2859–2868. https://doi.org/10.1098/rspb.2008.0870

Kelehear, C., Brown, G. P., & Shine, R. (2012). Rapid evolution of parasite life history traits on an expanding range-edge. *Ecology Letters*, *15*(4), 329–337. https://doi.org/10.1111/j.1461-0248.2012.01742.x

Kimpel, D., Gockel, J., Gerlach, G., & Bininda-Emonds, O. R. P. (2015). Population structuring in the monogonont rotifer *Synchaeta pectinata*: High genetic divergence on a small geographical scale. *Freshwater Biology*, *60*(7), 1364–1378. https://doi.org/10.1111/fwb.12574

Lapesa, S., Snell, T., Fields, D., & Serra, M. (2002). Predatory interactions between a cyclopoid copepod and three sibling rotifer species. *Freshwater Biology*, *47*(9), 1685–1695. https://doi.org/10.1046/j.1365-2427.2002.00926.x

Li, L., Niu, C., & Ma, R. (2010). Rapid temporal succession identified by COI of the rotifer *Brachionus calyciflorus* Pallas in Xihai Pond, Beijing, China, in relation to ecological traits. *Journal of Plankton Research*, *32*(6), 951–959. https://doi.org/10.1093/plankt/fbq014

Li, H., Xi, Y., & Cheng, X. (2009). Comparative studies on life history characteristics of three sibling species in *Brachionus calyciflorus* complex. *Acta Ecologica Sinica*, *29*, 581–588.

Li, H., Xi, Y., Cheng, X., Xiang, X., Hu, C., & Tao, L. (2008). Sympatric speciation in rotifers: Evidence from molecular phylogenetic relationships and reproductive isolation among *Brachionus calyciflorus* clones. *Acta Entomologica Sinica*, *51*(11), 1099–1128.

Liu, J., Möller, M., Gao, L.-M., Zhang, D.-Q., & Li, D.-Z. (2011). DNA barcoding for the discrimination of Eurasian yews (*Taxus* L., Taxaceae) and the discovery of cryptic species. *Molecular Ecology Resources*, *11*(1), 89–100. https://doi.org/10.1111/j.1755-0998.2010.02907.x

Ma, Q., Xi, Y., Zhang, J., Wen, X., & Xiang, X. (2010). Differences in life table demography among eight geographic populations of *Brachionus calyciflorus* (Rotifera) from China. *Limnologica-Ecology and Management of Inland Waters*, *40*(1), 16–22. https://doi.org/10.1016/j.limno.2009.05.002

Martinez-Takeshita, N., Purcell, C. M., Chabot, C. L., Craig, M. T., Paterson, C. N., Hyde, J. R., & Allen, L. G. (2015). A tale of three tails: Cryptic speciation in a globally distributed marine fish of the genus *Seriola*. *Copeia*, *103*(2), 357–368. https://doi.org/10.1643/ci-124-224

Michaloudi, E., Mills, S., Papakostas, S., Stelzer, C.-P., Triantafyllidis, A., Kappas, I., … Abatzopoulos, T. J. (2016). Morphological and taxonomic demarcation of *Brachionus asplanchnoidis* Charin within the *Brachionus plicatilis* cryptic species complex (Rotifera, Monogononta). *Hydrobiologia*, *796*(1), 19–37. https://doi.org/10.1007/s10750-016-2924-2

Michaloudi, E., Papakostas, S., Stamou, G., Neděla, V., Tihlaříková, E., Zhang, W., & Declerck, S. A. J. (2018). Reverse taxonomy applied to the *Brachionus calyciflorus* cryptic species complex: Morphometric analysis confirms species delimitations revealed by molecular phylogenetic analysis and allows the (re)description of four species. *PLoS One*, *13*(9), e0203168. https://doi.org/10.1371/journal.pone.0203168

Michels, E., & De Meester, L. (2004). Inter-clonal variation in phototactic behaviour and key life-history traits in a metapopulation of the cyclical parthenogen *Daphnia ambigua*: The effect of fish kairomones. *Hydrobiologia*, *522*(1), 221–233. https://doi.org/10.1023/b:hydr.0000029988.02195.35

Mills, S., Alcántara-Rodríguez, J. A., Ciros-Pérez, J., Gómez, A., Hagiwara, A., Galindo, K. H., … Walsh, E. J. (2017). Fifteen species in one: Deciphering the *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy. *Hydrobiologia*, *796*(1), 39–58. https://doi.org/10.1007/s10750-016-2725-7

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*(6), 218–222. https://doi.org/10.4319/lom.2008.6.218

Obertegger, U., Flaim, G., & Fontaneto, D. (2014). Cryptic diversity within the rotifer *Polyarthra dolichoptera* along an altitudinal gradient. *Freshwater Biology*, *59*(11), 2413–2427. https://doi.org/10.1111/fwb.12447

Ortells, R., Gómez, A., & Serra, M. (2003). Coexistence of cryptic rotifer species: Ecological and genetic characterisation of *Brachionus plicatilis*. *Freshwater Biology*, *48*(12), 2194–2202. https://doi.org/10.1046/j.1365-2427.2003.01159.x

Papakostas, S., Michaloudi, E., Proios, K., Brehm, M., Verhage, L., Rota, J., … Fontaneto, D. (2016). Integrative taxonomy recognizes evolutionary units despite widespread mitonuclear discordance: Evidence from a Rotifer cryptic species complex. *Systematic Biology*, *65*(3), 508–524. https://doi.org/10.1093/sysbio/syw016

Paraskevopoulou, S., Tiedemann, R., & Weithoff, G. (2018). Differential response to heat stress among evolutionary lineages of an aquatic invertebrate species complex. *Biology Letters*, *14*(11), 20180498. https://doi.org/10.1098/rsbl.2018.0498

Pfenninger, M., & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, *7*(1), 121. https://doi.org/10.1186/1471-2148-7-121

R Core Team (2017). *R: A language and environment for statistical com-puting*. Vienna, Austria: R Foundation for Statistical Computing.

Razgour, O., Clare, E. L., Zeale, M. R. K., Hanmer, J., Schnell, I. B., Rasmussen, M., … Jones, G. (2011). High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and Evolution*, *1*(4), 556–570. https://doi.org/10.1002/ece3.49

Serra, M., García-Roger, E. M., Ortells, R., & Carmona, M. J. (2019). Cyclically parthenogenetic rotifers and the theories of population and evolutionary ecology. *Limnetica*, *38*(1), 67–93. https://doi.org/10.23818/limn.38.13

Smith, H. A., & Snell, T. W. (2014). Differential evolution of lifespan and fecundity between asexual and sexual females in a benign environment. *International Review of Hydrobiology*, *99*(1–2), 117–124. https://doi.org/10.1002/iroh.201301711

Snell, T. W., & Stelzer, C.-P. (2005). Removal of surface glycoproteins and transfer among *Brachionus* species. *Hydrobiologia*, *546*(1), 267–274. https://doi.org/10.1007/1-4020-4408-9_27

Stelzer, C.-P. (2011). The cost of sex and competition between cyclical and obligate parthenogenetic rotifers. *The American Naturalist*, *177*(2), E43–E53. https://doi.org/10.1086/657685

Suatoni, E., Vicario, S., Rice, S., Snell, T., & Caccone, A. (2006). An analysis of species boundaries and biogeographic patterns in a cryptic species complex: The rotifer—*Brachionus plicatilis*. *Molecular Phylogenetics and Evolution*, *41*(1), 86–98. https://doi.org/10.1016/j.ympev.2006.04.025

Tarazona, E., García-Roger, E. M., & Carmona, M. J. (2017). Experimental evolution of bet hedging in rotifer diapause traits as a response to environmental unpredictability. *Oikos*, *126*(8), 1162–1172. https://doi.org/10.1111/oik.04186

Wang, X., Xiang, X., Xia, M., Han, Y., Huang, L., & Xi, Y. (2014). Differences in life history characteristics between two sibling species in *Brachionus calyciflorus* complex from tropical shallow lakes. *Annales de Limnologie - International Journal of Limnology*, *50*(4), 289–298. https://doi.org/10.1051/limn/2014024

Wen, X., Xi, Y., Zhang, G., Xue, Y., & Xiang, X. (2016). Coexistence of cryptic *Brachionus calyciflorus* (Rotifera) species: Roles of environmental variables. *Journal of Plankton Research*, *38*(3), 478–489. https://doi.org/10.1093/plankt/fbw006

Xiang, X., Chen, Y., Han, Y., Wang, X., & Xi, Y. (2016). Comparative studies on the life history characteristics of two *Brachionus calyciflorus* strains belonging to the same cryptic species. *Biochemical Systematics and Ecology*, *69*, 138–144. https://doi.org/10.1016/j.bse.2016.09.003

Xiang, X., Wang, X., Tao, Y., Jiang, R., Ke, L., & Xi, Y. (2015). Comparative studies on population growth of two *Brachionus calyciflorus* sibling species. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *87*(2), 369–376. https://doi.org/10.1007/s40011-015-0613-y

Xiang, X., Xi, Y., & Hu, H. (2007). Seasonal changes in the genetic diversity of *Brachionus calyciflorus* population in Lake Jinghu. *Acta Ecologica Sinica*, *27*(6), 2443.

Xiang, X., Xi, Y., Wen, X., Zhang, J., & Ma, Q. (2010a). Spatial patterns of genetic differentiation in *Brachionus calyciflorus* species complex. *Zoological Research*, *31*(3), 205–220. https://doi.org/hdl.handle.net/1807/64490

Xiang, X., Xi, Y., Wen, X., Zhang, J., & Ma, Q. (2010b). Spatial patterns of genetic differentiation in *Brachionus calyciflorus* species complex collected from East China in summer. *Hydrobiologia*, *638*(1), 67–83. https://doi.org/10.1007/s10750-009-0010-8

Xiang, X., Xi, Y., Wen, X., Zhang, G., Wang, J., & Hu, K. (2011a). Genetic differentiation and phylogeographical structure of the *Brachionus calyciflorus* complex in eastern China. *Molecular Ecology*, *20*(14), 3027–3044. https://doi.org/10.1111/j.1365-294x.2011.05147.x

Xiang, X., Xi, Y., Wen, X., Zhang, G., Wang, J., & Hu, K. (2011b). Patterns and processes in the genetic differentiation of the *Brachionus calyciflorus* complex, a passively dispersing freshwater zooplankton. *Molecular Phylogenetics and Evolution*, *59*(2), 386–398. https://doi.org/10.1016/j.ympev.2011.02.011

Xiang, X., Xi, Y., Zhang, J., Ma, Q., & Wen, X. (2010c). Effects of temperature on survival, reproduction, and morphotype in offspring of two *Brachionus calyciflorus* (Rotifera) morphotypes. *Journal of Freshwater Ecology*, *25*(1), 9–18. https://doi.org/10.1080/02705060.2010.9664352

Xiang, X., Xi, Y., Zhu, L., & Xu, Q. (2017). Comparative studies of the population genetic structure of the *Brachionus calyciflorus* species complex from four inland lakes in Wuhu, China. *Biochemical Systematics and Ecology*, *71*, 69–77. https://doi.org/10.1016/j.bse.2017.01.015

Zhang, J., Xi, Y., Ma, Q., & Xiang, X. (2010). Taxonomical status of two *Brachionus calyciforus* morphotypes in Lake Liantang based on ITS sequence. *Acta Hydrobiologica Sinica*, *34*, 935–942.

Zhang, Y., Zhou, A., Xi, Y., Sun, Q., Ning, L., Xie, P., … Xiang, X. (2018). Temporal patterns and processes of genetic differentiation of the *Brachionus calyciflorus* (Rotifera) complex in a subtropical shallow lake. *Hydrobiologia*, *807*(1), 313–331. https://doi.org/10.1007/s10750-017-3407-9

Zhou, L., Lemmen, K. D., Zhang, W., & Declerck, S. A. J. (2018). Direct and indirect effects of resource P-limitation differentially impact population growth, life history and body elemental composition of a Zooplankton consumer. *Frontiers in Microbiology*, *9*, 172. https://doi.org/10.3389/fmicb.2018.00172

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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