

DNA barcodes reveals that the monogonont rotifer *Brachionus quadridentatus* Hermann, 1783 is a species complex

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Abstract

Previous DNA barcoding works have revealed the presence of cryptic species in several rotifer taxa. Thus demonstrating that diversity in rotifers still needs to be investigated. *Brachionus quadridentatus* is a cyclical parthenogen rotifer, which inhabit fresh and brackish water worldwide. This species exhibits a wide intraspecific morphological variation associated with the spine development, and in consequence different forms and varieties were described in this taxon. However, taxonomic status of these variants need to be comprehensively analyzed. Barcodes were obtained from 176 individuals collected from Mexico. Our phylogenetic analyses discriminated seven distinct lineages (BqI-BqVII), which are highly congruent with seven morphotypes discriminated in this study. Genetic divergencies among the seven lineages ranged from 12 to 18%. Our results are evidence of the underestimated diversity in *B. quadridentatus*, and indicate that this taxon represent a species complex. However, more sampling is needed in areas between distant locations and even out Mexico in order to reveal the extent of genetic differentiation and diversity within this taxon.

INTRODUCTION

Compared with other zooplankters as microcrustaceans, the taxonomy in rotifers is unclear (Fontaneto et al., 2009). In monogonont rotifers discriminate between species on the basis of morphological features is limited because of the morphological stasis present in some species (Campillo et al., 2005), and also by the high phenotypic variation observed in other, which results from phenomena as cyclomorphosis and inducible defenses (Gilbert, 2001). Because of this, different morphological variants have been described as different subspecies, forms and varieties (Segers, 2007).

Brachionus quadridentatus is a cosmopolitan species reported in all continents including Antarctica (Dumont, 1983). It exhibits broad intraspecific morphological variation associated with spine development, which is reflected in different forms and varieties. Two of these forms are *Brachionus quadridentatus* f. *brevispinus* (short-spined) and *Brachionus quadridentatus* f. *cluniorbicularis* (no spined) (Koste & Shiel, 1987). However these variants are of doubtful taxonomic validity (Segers, 2007).

In the present study we used sequence variation of the COI gene to explored the diversity of *B. quadridentatus* from inland waterbodies of Mexico. Also we examined if different morphotypes in this taxon may represent putative cryptic species.

MATERIALS AND METHODS

The studied area consisted of 17 locations along Mexico. Samples were collected using a 50 µm mesh size plankton net and preserved in 96% ethanol. Specimens were identified morphologically using Koste & Shiel (1987) taxonomic key. Some specimens were illustrated by using a camera lucida. We used DNA extraction and PCR protocols described in García-Morales & Elías-Gutiérrez (2013). COI sequences were aligned in MEGA with default settings.

Bayesian inference (BI) and maximum-likelihood (ML) analyses were used to infer phylogenetic relationships among the populations of *B. quadridentatus*. The model of molecular evolution that best fit the mtDNA was TVM+G. Genetic distances (uncorrected *p*-values) within and among the lineages discriminated by the phylogenetic analyses were calculated in MEGA.

Table 1 Average percent of uncorrected genetic distances of COI gene. In diagonal genetic distances within the seven *B. quadridentatus* lineages and below the diagonal genetic distances among the seven lineages

Lineage	BqI	BqII	BqIII	BqIV	BqV	BqVI	BqVII
BqI	0.0						
BqII	14.8	2.0					
BqIII	15.6	16.4	0.5				
BqIV	16.4	18.3	14.8	1.5			
BqV	17.3	16.5	17.2	18.1	0.0		
BqVI	13.4	16.6	16.5	16.8	15.2	0.2	
BqVII	15.3	17.0	16.8	18.1	16.2	12.4	3.1

References
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RESULTS

The final alignment for COI had 635 bp, with 242 polymorphic sites and 233 parsimony informative sites, defining 38 unique haplotypes from 176 sequences. Most haplotypes were found in single populations, but two haplotypes were shared between two populations (COLVEG1 and VERCH1-1) (Fig. 1).

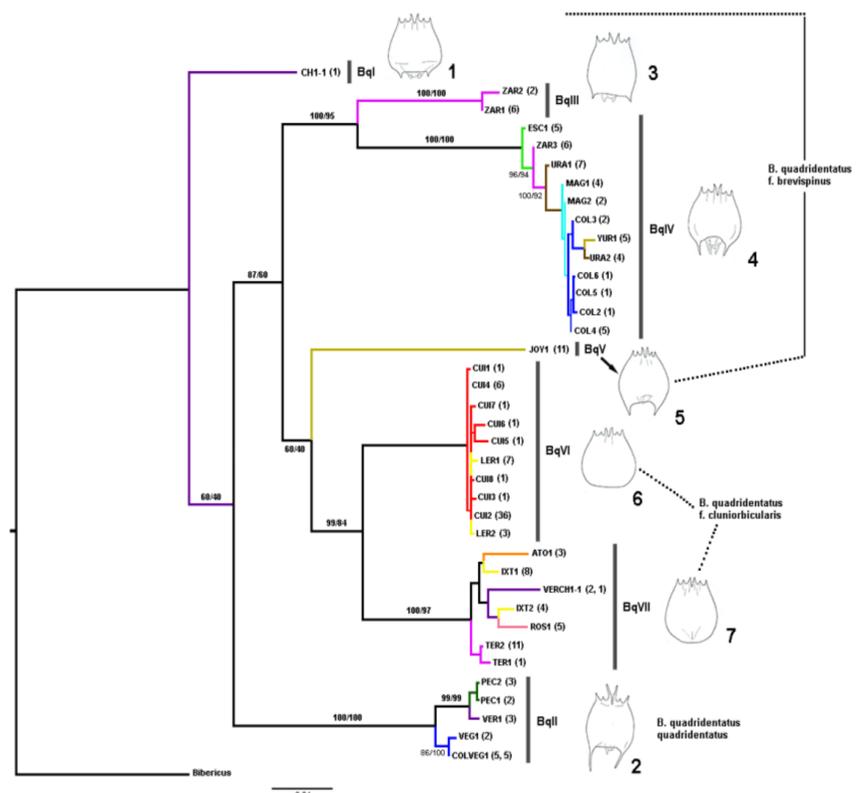


Fig. 1 Bayesian Inference phylogram of the mtDNA showing the relationship of the 38 *B. quadridentatus* haplotypes. Haplotype names are indicated by the acronym of the water body in which they were collected. Different morphotypes are indicated by arabic numbers (1 to 7)

RESULTS

The trees produced by BI and ML were identical and retrieved seven major lineages (BqI-BqVII). Uncorrected *p* distances within these seven lineages ranged from 0 to 3.1%, whereas, distances among them ranged from 12 to 18% (Table 1). We found seven morphotypes, which exhibit clear morphological differences. Four morphotypes were identified as the *B. quadridentatus brevispinus* form, two as the *B. quadridentatus cluniorbicularis* form and one was identified as *B. quadridentatus quadridentatus*.

Besides these seven morphotypes (1-7) correlates with the seven lineages discriminated in phylogenetic analysis (Fig. 1). The high genetic distances among the seven lineages also indicate that these divergent lineages may represent cryptic species. The “*brevispinus*” and “*cluniorbicularis*” forms need an exhaustive taxonomic revision as we found that these forms can represent more than one putative species. An integrative taxonomic framework, including more markers, morphological, reproductive, and ecological analyses will be needed in order to understand diversity within *B. quadridentatus*.

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