DNA barcodes reveals that the monogenont rotifer Brachionus quadridentatus Hermann, 1783 is a species complex
Alma E. Garcia-Morales and Omar Dominguez-Dominguez

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 parthenogenetic 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 divergences 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 brachionidae ‘Brachionus quadridentatus’ as a cosmopolitan species is 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 & Shielp, 1987). However these variants are of doubtful taxonomic validity (Segers, 2007).

In the present study we used sequence variation of the COI gene to explore the diversity of B. quadridentatus from inland waterbodies of Mexico. 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 & Shielp (1987) taxonomic key. Some specimens were illustrated by using a camera lucida. We used DNA extraction and PCR protocols described in Garcia-Morales & Elias-Gutierrez (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 TAMU+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

<table>
<thead>
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<th>Lineage</th>
<th>BqI</th>
<th>BqII</th>
<th>BqIII</th>
<th>BqIV</th>
<th>BqVI</th>
<th>BqVII</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>BqII</td>
<td>14.8</td>
<td>2.0</td>
<td></td>
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<tr>
<td>BqIII</td>
<td>15.6</td>
<td>16.4</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BqIV</td>
<td>16.4</td>
<td>18.3</td>
<td>14.8</td>
<td>1.5</td>
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<tr>
<td>BqV</td>
<td>17.3</td>
<td>16.5</td>
<td>17.2</td>
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<td>BqVI</td>
<td>13.4</td>
<td>16.6</td>
<td>16.5</td>
<td>16.8</td>
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<tr>
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<td>17.0</td>
<td>16.8</td>
<td>18.1</td>
<td>12.2</td>
<td>3.1</td>
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</tbody>
</table>

RESULTS
The final alignment for COI had 635 bp, with 242 polymorphic sites and 232 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 (CDUVEG1 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)

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.

REFERENCES


References continued


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