

## Introduction

Rotifers are organisms with high levels of cryptic speciation (Fontaneto *et al.* 2009), therefore genetics analysis are necessary to clarify the status of the species. The *Brachionus plicatilis* complex group is composed by three groups. Large group "L" with *B. asplachnoidis* (Michaluidi *et al.* 20017), *B. plicatilis* s.s. (Muller 1786), *B. manjavacas* (Fontaneto *et al.* 2007); the medium size group "SM" with *B. ibericus* (Ciros-Pérez *et al.* 2001) and *B. koreanus* (Hwang *et al.* 2013); and the small size group "S" with *B. rotundiformis* (Tschugunoff 1921). However, Mills *et al.* (2016) determined the possible presence of 15 species within the *B. plicatilis* group. After genetics analysis we are contributing to clarify the status of one specie in the group.

## Methods

Water samples from the volcanic lake Rincon de Parangueo, Guanajuato, Mexico were collected and fixed with 4% formaldehyde for taxonomy and other sample was fixed with 96% ethanol for genetics.

### Taxonomy

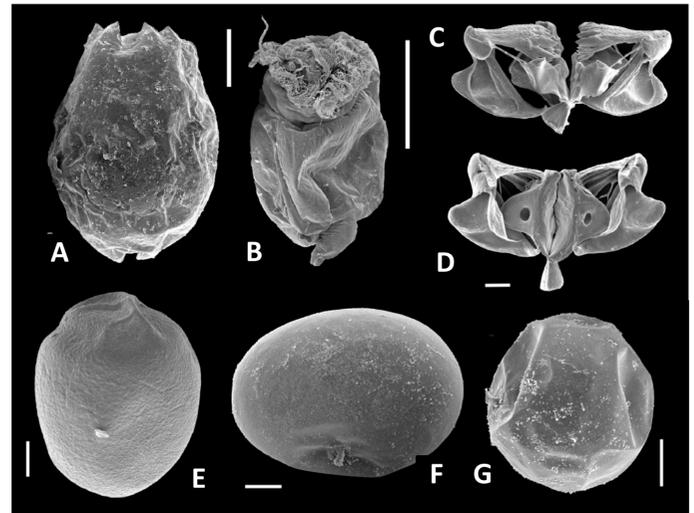
Pictures of females, males, different kind of eggs, and trophi were taken by SEM. Details of every stage were analyzed to determine its characters which define the specie.

### DNA extraction

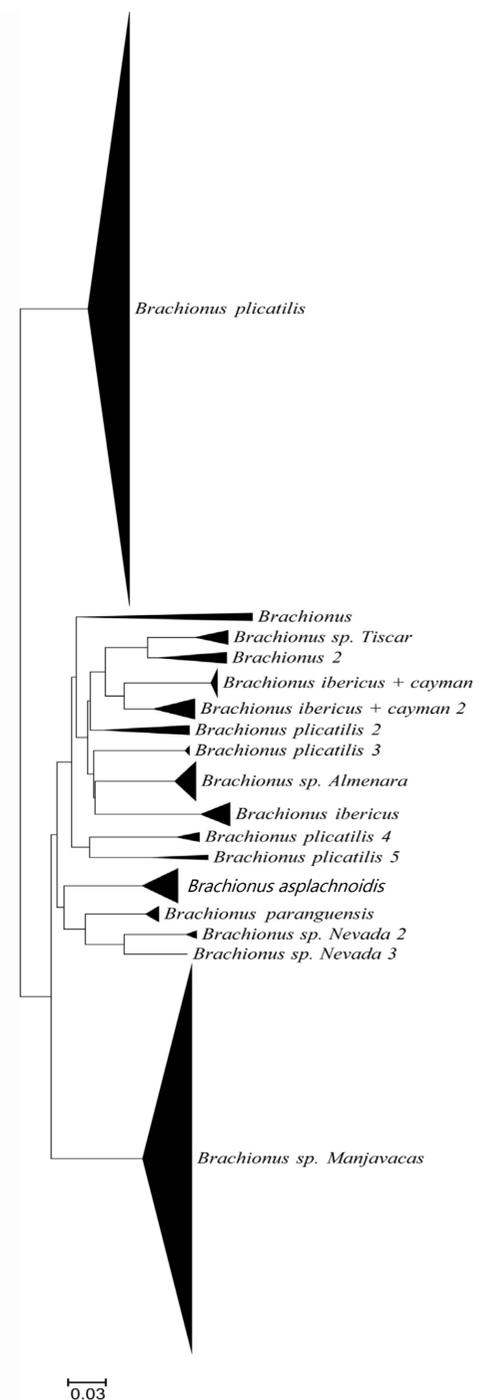
Genomic DNA was extracted using the method of glass fiber membrane in 2µm Pall plates. The primers used were the proposed for zooplankton by Prosser *et al.* (2013). The sequences obtained were uploaded to the Barcode of Life Database ([www.boldsystems.org](http://www.boldsystems.org)) and then compared with the data set previously used by Mills *et al.* (2016). The ABGD method was used for the delimitation of the putative species. To compare results, we constructed a Neighbor Joining (NJ) ID tree with the divergences calculated by the Kimura method (K2P) using all members of the *B. plicatilis* group available in the BOLD system. A simplified tree was constructed with Mega 7.0 program (Kumar *et al.* 2016) from the NJ tree obtained.

## Results and discussion

Results shows that in *B. plicatilis* complex group has three sub-clades named "Nevada", where "B. Paranguensis", "Nevada 2", and "Nevada 3" are related. However, according with DNA barcode analysis, this three groups have high divergence between them (see Figure 2). Furthermore, pictures of females, males, different kind of eggs, and trophi are provide as an addition information to support genetics (see Figure 1). In this context, we would not add more controversy to this group, we are trying to solve the status of the "B. Paranguensis" in the *B. plicatilis* complex group.



**Figure 1.** *Brachionus paranguensis*: female (A), male (B) scale bar 50 µm; trophi ventral view (C) and dorsal view (D) scale bar 10 µm; resting egg (E), partenogenetic egg (F), unfertilized sexual egg (G) scale bar 20 µm.



**Figure 2.** Simplified NJ tree of the 776 specimens of the *B. plicatilis* group. Each major clade was named after the most common name found in BOLD database. The size of the black triangle is accordingly with the number of sequences for each clade.