

Phylogenetic Assessment of New Isolates of *Brachionus plicatilis*

from WHEELBASE, contributed by Terry Snell

Introduction

This protocol is designed to enable you to quickly assess the phylogenetic affinities of a new *Brachionus plicatilis*-type rotifer using COI sequence data, assuming you do not have local access to alignment and phylogenetic software. Definitive molecular characterization may require more sophisticated phylogenetic analysis methods, and molecular analyses should be supported by morphometric criteria. The taxonomic framework is that described by Gomez *et al.*(2002) with additional sequences from Derry *et al.* (2003). If you are not working online, you will need to download the following alignments from WHEELBASE: BpInitialCluster, Bpss, Bp, Br, and Bi.

Procedure

1. Obtain COI sequence for the new rotifer isolate, following, for example, the procedure of Gomez *et al.* 2002. PCR-based direct sequencing should employ primers flanking the region of the gene used by Gomez *et al.* 2002 and Derry, *et al.* 2003. See the Alignments section of WheelBase for details.

2. Determine the general phylogenetic affinity of the isolate:

Open BpInitialCluster. This contains published COI sequences from GenBank of selected species of the *B. plicatilis* group, in FASTA format . These taxa represent the major clades, so grouping of the new strain with one of these will provide guidance for selecting a subgroup for more detailed analysis. Copy and paste the COI sequences in this file onto a new page in your word processor. Copy and paste the COI sequence of the isolate onto the end of this new page. The sequence should be in exactly the same format as the others, with a title line followed by continuous sequence without spaces or numbers.

3. Copy the entire file and open one of the following ClustalW websites:

- a. <http://www.ebi.ac.uk/clustalw/index.html>
- b. <http://clustalw.genome.ad.jp>

4. Paste the sequences into the window provided. Click **Run**.

ClustalW will determine an alignment of the 9 reference COI sequences and the sequence of the new isolate (these sites have a 10 sequence limit). The length of each sequence is listed at the top. Scrolling down reveals the alignment scores and the multiple sequence alignment. At the bottom, you will see a tree. Click on it to

reveal a menu. Choose **format** and then **phylogram**.

Click on the tree again and choose **distances**, then **show tree distances**. The new isolate should fall within or near one of the four clades, Bpss - (*Brachionus plicatilis sensu strictu*), Bp - *B. plicatilis* (other species Nevada, Austria, Manjavacas), Br - *B. rotundiformis*, or Bi - *B. ibericus*.

5. Determine the specific phylogenetic affinity of the isolate:

Choose the subgroup file that is most appropriate for the new isolate (Bpss, Bp, Br, or Bi). Copy the COI sequences in this file onto a new page in your word processor and then copy and paste the COI sequence of the new isolate onto the end of this file as in Step 2. Copy the file and open ClustalW. Follow the steps as in Step 3. The resulting tree should show how the new isolate is related to other taxa within the clade.

This phylogenetic analysis should only be considered preliminary. Publishable phylogenetic analysis may require more sophisticated methods for calculating trees.

References

- Gomez, A., M. Serra, G.R. Carvahlo, & D.H. Lunt, 2002. Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution* 56(7):1431-1444.
- Derry, A., P.D.N. Hebert, & E.E. Prepas, 2003. Evolution of rotifers in saline and subsaline lakes: A molecular genetic approach. *Limnol. & Oceanogr.* 48:675-685.