

## Phylogenetic Position of Eurihaline Rotifer *Brachionus* sp. Originated From Tumpa'an Waters, South Minahasa, North Sulawesi

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### ABSTRACT

This study aims to determine the phylogenetic position of rotifer *Brachionus* sp. sample originated from Tumpa'an coastal water, North Sulawesi in the phylogenetic tree of *Brachionus* spp. from 27 samples of rotifer selected from 100 samples available in NCBI Genbank. The phylogeny tree was constructed on the basis of COI (Cytochrome Oxidase I) gene sequences using MEGA7 software and the neighbour-joining method. Results show that the phylogeny tree was divided into 2 clades, in the group (closely related group) and out-group (distantly related group). It is confirmed that the position of the rotifer originating from Tumpa'an Coastal Water is within in group, which has closely related to the *B. plicatilis* complex BUS06 indicated by a bootstrap value of 80%. The group belongs to SS-type rotifers with an average body size of  $149 \pm 1.3 \mu\text{m}$ . This rotifer group has been known as live prey for fish larvae with a mouth-opening size of  $150 \mu\text{m}$ .

Keywords: Rotifer; Tumpa'an Coastal Water; North Sulawesi; Gen COI; Phylogeny Tree

### INTRODUCTION

Rotifer is a zooplanktonic group belongs to Phylum Rotifera Cuvier 1817 which is divided into 2 classes, Monogononta, Bdelloida, and Seisonidea (Wallace et al., 2019). *Brachionus* spp. are the most famous monogonont rotifers due to their ecological role as secondary producers for aquatic larvae (Rumengan, 1990; Rumengan et al., 1998). They are highly diverse cosmopolitan zooplankton, especially the eurihaline rotifer *Brachionus* spp., with formerly known single species, *Brachionus plicatilis*, but distinguished into two morphotypes, L- and S-type rotifers (Fu et al., 1995; Hagiwara et al., 1995).

The karyotypic evidence that their chromosome numbers are different (Rumengan et al., 1991; 1993), and the reproductive isolation between the two morphotypes confirms them as different species, namely *B. plicatilis* and *B. rotundiformis* for L- and S-type, respectively (Hagiwara et al., 1995; Segers, 1995). Within the S-type rotifers, morphological variation is still investigated as a phenomenon of polymorphism. More recent findings have described the S-type rotifer as a cryptic species (Wullur, 2017).

They are not a single species but probably consist of two or more distinct species (Bickford et al., 2007). Based on the COI gene sequences, by constructing their phylogenetic tree, the distinct morphotypes could be then confirmed. In such a molecular approach, the phylogenetic position of rotifers originated from Tumpa'an water, South Minahasa, North Sulawesi could be determined.

## METHODS

This bioinformatic analysis was conducted using COI gene sequence data provided by Sahari et al. (2017), who collected rotifer samples from Tumpaan Waters, South Minahasa, North Sulawesi for molecular identification. Data sources for nucleotide sequences are obtained from NCBI (<http://www.ncbi.nlm.nih.gov>).

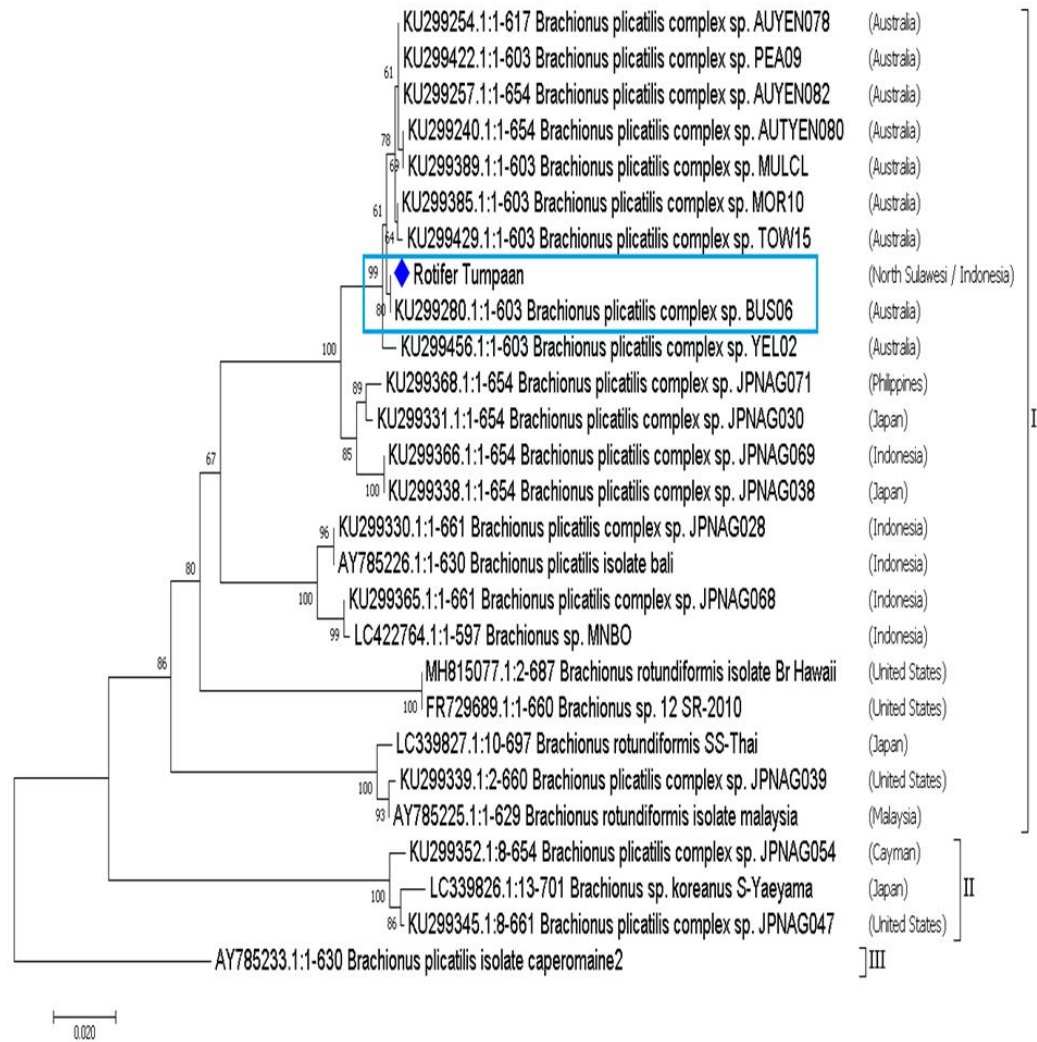
The data used are nucleotide sequences of 27 rotifer samples (9 samples of Australia, 5 samples of United States, 5 samples of Indonesia, 4 samples of Japan, 1 sample of each Malaysia and Cayman) and 1 original sample of Tumpaan Waters, North Sulawesi. The selected sequences are downloaded in fasta form. The downloaded nucleotide sequences were analysed using MEGA7 software, and the alignment of the sequences was performed by the Clustal-W tool.

The phylogenetic tree was then constructed from the different clustering algorithms available in MEGA 7 with the neighbor joining method on 1000 bootstraps using the 2-parameter Kimura model. Analysis of generated phylogenetic trees for relationships interpretations of rotifer species was allowed to determine the phylogenetic position of rotifer *Brachionus* sp. sample originated from Tumpaan coastal water, North Sulawesi of the 100 samples available in the genbank.

## RESULTS AND DISCUSSION

Results of the phylogenetic analysis of COI gene sequences show that *B. plicatilis* complex sp originates from the same ancestor and forms three clades, each clade assembled by the similarities in sequence characters (**Figure 1**). The clades I and II have close relationship, considered as in groups, and the clade III is an outgroup, a distant related group. In clade I, the grouping *B. plicatilis* complex sp originating from Australia, North Sulawesi, Indonesia, the Philippines, Japan, the United States, and Malaysia, which is determined as SS-type, and classified as *B. rotundiformis*, the same as for the S-type rotifer. However, SS-type rotifers have a smaller body size, with an average lorica length of  $149 \pm 1.3 \mu\text{m}$ , and their morphological distinct feature, having 3 pairs of spines with different sizes, and the tip of the spines is longer than other types of rotifers (Wullur, 2017). Clade II consists of the grouping *B. plicatilis* complex sp originating from Cayman, Japan, and the United States, which is classified as SM-type (Small Medium) with lorica length of  $193 \pm 1.4 \mu\text{m}$ . This phylogenetic tree shows the two distinct S-type rotifers, namely SS- and SM-type. It is different from the morphotype, L-type rotifer.

The phylogenetic position is determined based on the magnitude of the bootstrap value, the location of the parallel line and strengthened by the calculated genetic distance (pairwise distance). A bootstrap value in the 70-100% range indicates reliable clustering. Lower bootstrap values may reflect unreliable clustering based on nucleotide sequences (Rosidiani et al., 2013). The Tumpaan rotifer sample is in the same clade as *B. plicatilis* complex sp. BUS06 from Australia shows a close relationship with a bootstrap value of 80%. As indicated in **Table 1**, this closeness is consistent with the genetic distance being 0.000 between the two samples, while the genetic distance ranged from 0.000 to 0.198. The lower the value of genetic distance, the closer genetic relationship between species (Darmayanti, 2011).



**Figure 1.** The phylogenetic tree of rotifer samples was created using the Neighbor joining method on Mega 7 software.

**Table 1.** Genetic distance between *Brachionus* complex based on COI gene sequences of phylogenetic analysis

Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1 Rotifer Tumpa																										
2 B. plicatilis complex sp. AUYEN082	0.005																									
3 B. plicatilis complex sp. AUYEN080	0.007	0.002																								
4 B. plicatilis complex sp. JPNAG071	0.028	0.033	0.031																							
5 B. plicatilis complex sp. JPNAG030	0.024	0.030	0.031	0.007																						
6 B. plicatilis complex sp. JPNAG069	0.028	0.033	0.035	0.017	0.014																					
7 B. plicatilis complex sp. JPNAG038	0.028	0.033	0.035	0.017	0.014	0.000																				
8 B. plicatilis complex sp. AUYEN078	0.005	0.000	0.002	0.033	0.030	0.033	0.033																			
9 B. plicatilis complex sp. BUS06	0.000	0.005	0.007	0.028	0.024	0.028	0.028	0.005																		
10 B. plicatilis complex sp. MOR10	0.003	0.002	0.003	0.031	0.028	0.031	0.031	0.002	0.003																	
11 B. plicatilis complex sp. TOW15	0.005	0.003	0.005	0.033	0.030	0.033	0.033	0.003	0.005	0.002																
12 B. plicatilis complex sp. PEA09	0.005	0.000	0.002	0.033	0.030	0.033	0.033	0.000	0.005	0.002	0.003															
13 B. plicatilis complex sp. YEL02	0.007	0.009	0.010	0.031	0.028	0.031	0.031	0.009	0.007	0.010	0.012	0.009														
14 B. plicatilis complex sp. MULCL	0.007	0.002	0.000	0.031	0.031	0.035	0.035	0.002	0.007	0.003	0.005	0.002	0.010													
15 B. plicatilis complex sp. JPNAG028	0.092	0.092	0.094	0.082	0.082	0.090	0.090	0.092	0.092	0.094	0.096	0.092	0.092	0.094												
16 B. plicatilis complex sp. JPNAG068	0.096	0.096	0.098	0.086	0.086	0.094	0.094	0.096	0.096	0.098	0.100	0.096	0.096	0.098	0.014											
17 B. plicatilis isolate bali	0.092	0.092	0.094	0.082	0.082	0.090	0.090	0.092	0.092	0.094	0.096	0.092	0.092	0.094	0.000	0.014										
18 B. rotundiformis isolate Br Hawaii	0.133	0.135	0.137	0.134	0.126	0.126	0.126	0.135	0.133	0.137	0.139	0.135	0.128	0.137	0.113	0.109	0.113									
19 B. rotundiformis SS-Thai	0.142	0.142	0.139	0.144	0.144	0.138	0.138	0.142	0.142	0.142	0.144	0.142	0.148	0.139	0.124	0.131	0.124	0.152								
20 Brachionus sp. 12 SR-2010	0.133	0.135	0.137	0.134	0.126	0.126	0.126	0.135	0.133	0.137	0.139	0.135	0.128	0.137	0.113	0.109	0.113	0.000	0.152							
21 Brachionus sp. MNBO	0.098	0.098	0.100	0.088	0.088	0.096	0.096	0.098	0.098	0.100	0.102	0.098	0.098	0.100	0.015	0.002	0.015	0.111	0.133	0.111						
22 B. plicatilis complex sp. JPNAG039	0.142	0.142	0.140	0.144	0.144	0.138	0.138	0.142	0.142	0.142	0.144	0.142	0.148	0.140	0.129	0.135	0.129	0.152	0.010	0.152	0.137					
23 B. rotundiformis isolate malaysia	0.140	0.140	0.138	0.142	0.142	0.136	0.136	0.140	0.140	0.140	0.142	0.140	0.146	0.138	0.127	0.133	0.127	0.150	0.009	0.150	0.135	0.002				
24 Brachionus sp. koreanus S-Yaeyama	0.194	0.194	0.194	0.195	0.194	0.185	0.185	0.194	0.194	0.194	0.197	0.194	0.201	0.194	0.179	0.179	0.179	0.203	0.174	0.203	0.181	0.178	0.176			
25 B. plicatilis complex sp. JPNAG054	0.188	0.188	0.188	0.183	0.183	0.188	0.188	0.188	0.188	0.188	0.190	0.188	0.194	0.188	0.168	0.168	0.168	0.196	0.172	0.196	0.170	0.176	0.174	0.017		
26 B. plicatilis complex sp. JPNAG047	0.188	0.188	0.188	0.183	0.183	0.183	0.183	0.188	0.188	0.188	0.190	0.188	0.194	0.188	0.168	0.168	0.168	0.196	0.172	0.196	0.170	0.176	0.174	0.009	0.009	
27 B. plicatilis isolate caperomaine2	0.181	0.181	0.181	0.181	0.181	0.185	0.185	0.181	0.181	0.181	0.183	0.181	0.188	0.181	0.176	0.170	0.176	0.192	0.198	0.192	0.172	0.198	0.196	0.192	0.190	0.187

The size of the SS-type rotifer is still an important concern in aquaculture, especially at the hatchery stage, because some commercially important fish hatcheries such as grouper and napoleon require SS-type rotifers. According to Arif (2016), observations of cultivated napoleon larvae have a mouth opening range of 100-150  $\mu\text{m}$  while grouper larvae observations have a mouth opening range of 113-150  $\mu\text{m}$  (Sudewi et al., 2020). SS-type rotifers contain higher unsaturated fatty acids than S and L-types (Priyono, 2017), so they are needed at the larval growth stage.

## CONCLUSION

COI gene sequences based phylogenetic analysis of rotifer samples indicate the position of Tumpaan rotifer is closed to *Brachionus plicatilis* complex sp. BUS06 from Australia with a bootstrap value of 80% and a genetic distance of 0.000. The local rotifers belong to SS-type group, which is popularly utilized as live food for small-mouth fish larvae in aquaculture. Investigation on morphometric characteristics of this rotifer is still required for confirmation. More molecular identification of various tropical rotifers is necessary to confirm speciation in *Brachionus* complex rotifers.

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