

# **Diversity and Phylogeny of the Order Arthrotardigrada (Tardigrada: Heterotardigrada)**

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## **DIGEST**

### **Introduction**

Tardigrades are microscopic ecdysozoans with four pairs of legs assigned to their own phylum Tardigrada closely related to Arthropoda and Onychophora. They are distributed from the highest mountains to the deep sea and more than one thousand species are known. Among the terrestrial, freshwater and marine species, the marine representatives of the order Arthrotardigrada (class Heterotardigrada) display the highest morphological diversity harboring approximately 170 species of 42 genera, nine subfamilies and seven families. Genuine study of this group only began in the latter half of the twentieth century and its research on diversity and phylogeny is at an immature stage with many undescribed taxa expected and higher phylogeny unresolved. However, this group has been traditionally regarded as the most ancestral group of the phylum based on morphology, thus understanding the diversity and phylogeny of the order Arthrotardigrada is crucial in elucidating the evolution of the whole phylum.

To have a deeper understanding of arthrotardigrade diversity and to elucidate its phylogenetic relationships, I focused on arthrotardigrades from the Japanese coast, Northwest Pacific where high diversity is implied by previous studies but taxonomic position of many taxa remain to be studied. Therefore, I conducted a series of formal taxonomic studies of the order from this region to understand the order's diversity. In addition, joint with the collection effort in Florida, U.S.A., I conducted molecular phylogenetic analyses of the order.

### **Materials and Methods**

The taxonomic study was based on specimens collected from Tateyama Bay, Boso Peninsula and Tanabe Bay, Kii Peninsula, Honshu island, Shimabara Bay, Kyushu island and Ryukyu Archipelago, Japan, Northwest Pacific. The morphology of these specimens was studied by differential interference microscopy, phase contrast microscopy and scanning electron microscopy. The molecular phylogenetic study based on nucleotide sequences of nuclear 18S (for some OTUs) and 28S (for all OTUs) ribosomal RNA genes was conducted using specimens collected during the taxonomic study, specimens collected in Florida, U.S.A. and data available from GenBank. The dataset was analysed with the maximum likelihood and the Bayesian inference methods. The present study used a far more comprehensive dataset including longer sequences and more intensive taxon sampling covering six out of the seven known families, compared to the preceding molecular study

of arthrotardigrades.

## **Results**

### Taxonomic Study of the Order Arthrotradrigrada from Japan, Northwest Pacific

The present taxonomic study focusing on taxa from the Japanese coast, Northwest Pacific resulted in collection of 22 species of 17 genera, eight subfamilies and six families. Most of these taxa were undescribed. I described a portion of these taxa with interesting morphology as new taxa at species-level, genus-level and subfamily-level at the time.

### Higher Phylogeny of the Order Arthrotardigrada Based on Molecular Evidence

The topology recovered by the molecular phylogenetic analyses confirmed the monophylies of the two classes and the paraphyly of Arthrotardigrada within the class Heterotardigrada as indicated by the previous molecular phylogenetic studies. In addition, the comprehensive dataset of arthrotardigrades revealed new phylogenetic relationships of arthrotardigrade families and subfamilies. For the largest arthrotardigrade family, Halechiniscidae, its polyphyly was indicated.

## **Discussion**

The taxonomic study advanced our understanding of the order's morphological diversity and the order's species diversity in the Northwest Pacific. Adding the number of named species obtained in this study and that of the previous reports, the Northwest Pacific is now the most species rich region in the Pacific Ocean. Considering the many species not identified to species in this study and previous studies and many localities, environments and substrates not surveyed, future taxonomic studies would most likely increase the number of species in this region with description of many new species, genera and even higher taxa. Based on the topology recovered by the molecular phylogenetic study, I presented new hypotheses on the ancestral morphology of taxa, suggested a new interpretation of a morphological character, amended the taxonomic status of taxa and also presented an overview of the evolutionary history of the phylum. To further understand the evolution of the order and moreover the phylum Tardigrada, continuous effort in collecting arthrotardigrade specimens, reporting new records and describing species are of primary importance. This effort joint with molecular phylogenetic studies using more molecular markers would provide phylogenetic relationships with higher resolution. In addition, fossil records and more information on the cryptobiotic abilities would be preferred to understand the evolutionary history of the order and the phylum.