

The University of Shiga Prefecture

**Phylogeography of parasitic nematodes recovered from *Bufo* species  
in mainland Japan**

A Dissertation

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## Summary

In order to fully assess the diversity of these parasites in *Bufo* spp. in Japan, we performed an integrated approach: morphological and molecular analysis. Morphological analysis was performed through light microscopy and scanning electron microscopy (SEM), while molecular analysis was done by comparing DNA sequence divergence using the partial nuclear small ribosomal DNA (18S and 28S) and partial mitochondrial DNA cytochrome *c* oxidase subunit 1 (COI) target regions through phylogenetic trees and pairwise distance. First, I identified through morphological analyses the lungworm species, *Rhabdias incerta* which appeared to be specific to the genus *Bufo*. Molecularly, this species is subdivided into two or three phylogroups based on the subspecies divisions and biogeographic of their hosts. In this study, it also revealed the presence of undescribed and cryptic *Rhabdias* species, which morphologically resembles the species *R. tokyoensis*.

Second, I identified and redescribed the *Cosmocercoides pulcher* found commonly in the gastrointestinal tract of the toads. Modern morphological techniques revealed additional morphological characteristics absent from the original description. Additionally, I also molecularly characterized this species which revealed two possible new species from the same host *Bufo japonicus* from Kyushu and from other amphibians in Hokkaido. However, we haven't examined morphologically the voucher specimens from these localities. Therefore, we cannot describe them as two new species. For their description, we need to obtain new samples for morphological studies in the future.

This study was the first to molecularly characterize the species *Rhabdias incerta* and *Cosmocercoides pulcher*, both are originally described from *Bufo japonicus* in Japan. Through this,

it will help biodiversity study and will be useful for species delimitation and differentiation since both species, morphologically, are difficult to distinguish from their congeneric species. This study suggests the importance of scanning electron microscopy (SEM) in identifying and describing nematodes that morphologically resembles each other. With the help of it, I was able to describe and confirm the characteristics that were not mentioned in the original description of the species, *Cosmocercoides pulcher*. The phylogenetic evidence from the present study was able to determine that the rhabdiasids follow their hosts resources instead of their host phylogenies, in this case the host segregation, geographical distances, and isolation of the Japanese toads. Lastly, molecular approach not only helps us with the identification of species, but also reveals cryptic species that are not able to distinguish from their related species by morphology.