

# Demographic history and coevolution study of millipede associated nematodes from Western Caucasus

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## ● Introduction

Nematodes *Severianoia pachyiuli* (Oxyuridomorpha: Thelastomatoidea) inhabit the hind gut of spirobolid millipedes *Pachyiulus krivolutskiy* Golovatch, 1977 (Diplopoda: Julidae) which is an endemic species in the Western Caucasus. All studied millipedes are infected with a single species that is common for the thelastomatids and makes them attractive in the light of population study. Specimens of millipedes were collected from nine localities situated on the Southern and Northern slopes of the Caucasus Range.

## ● Methods

Nematode specimens were fixed in 95% ethanol, the DNA was extracted with the aid of QiAamp Micro Kit (Qiagen®, Germantown, USA) according to manufacturer's instructions. PCR reactions were performed using the Encyclo Plus PCR kit (Evrogen®, Moscow, Russia). DNA sequences were obtained for two markers for millipedes (COI and D2D3 segment 28S rDNA) and for three markers for nematodes (COI, EF1 and D2D3 segment 28S rDNA). The BEAST software package was used to perform a median estimate of the effective population size. Study of nematode eggs was carried out with the aid of scanning electron microscope using special slides coated with polylysine (Poly-L-Lysine Coated German Glass Cover Slips).

## ● Results

Millipede sequencing results have shown that the COI gene in this species is extremely conservative. Thus, populations located on the eastern side of the Greater Caucasus Mountain Range show almost no differences, while differences between eastern and western side populations on this gene are insignificant.

According to the D2D3 fragment, the differences are more pronounced; on the diagrams and phylogenetic trees, one can see that there are even differences between the populations of millipedes located in neighboring but isolated gorges.

The nematode COI gene has proven to be more variable and suitable for intraspecific phylogenetic studies. Using the BEAST software package, we performed a median estimate of the effective population size based on the obtained sequences. For millipedes, *Pachyiulus krivolutskii* it is 6841 specimens, for nematodes *Severianoia pachyiuli* - 8342 specimens. The number is stable for about 3,000 and 3,500 generations, respectively

SEM photographs of females of *Severianoia pachyiuli*. A: front end of the body; B: posterior end of the body; C, D: head capsule; D: vulva.

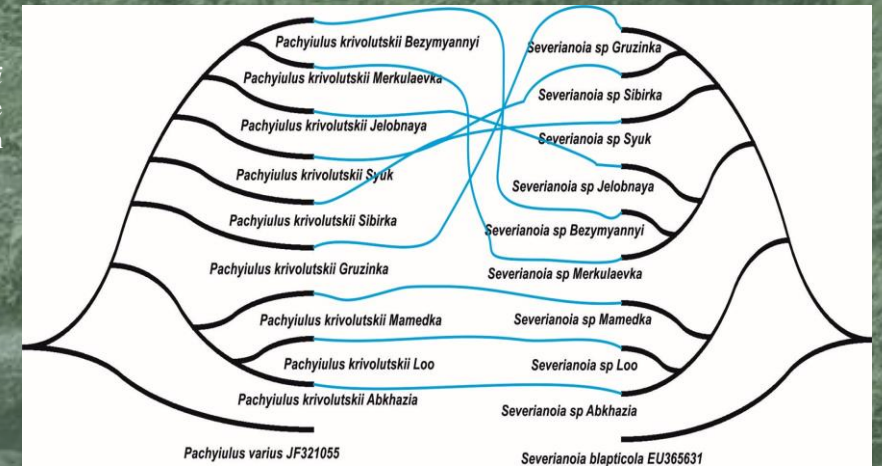
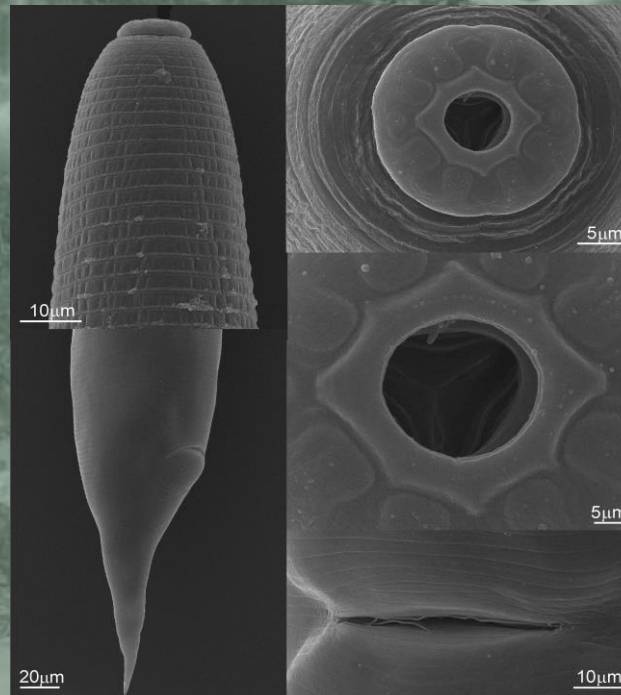


Illustration of coevolution relationships between populations of millipedes and nematodes inferred from the analysis of COI mtDNA.



SEM photographs of eggs of *Severianoia pachyiuli*.