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GENERAL BIOLOGY

# Viable Nematodes from Late Pleistocene Permafrost of the Kolyma River Lowland

**A. V. Shatilovich[a, *], A. V. Tchesunov[b], T. V. Neretina[c], I. P. Grabarnik[d], S. V. Gubin[a], T. A. Vishnivetskaya[a], T. C. Onstott[e], and E. M. Rivkina[a]**

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**Abstract**—We have obtained the first data demonstrating the capability of multicellular organisms for long-term cryobiosis in permafrost deposits of the Arctic. The viable soil nematodes *Panagrolaimus* aff. *detritophagus* (Rhabditida) and *Plectus* aff. *parvus* (Plectida) were isolated from the samples of Pleistocene permafrost deposits of the Kolyma River Lowland. The duration of natural cryopreservation of the nematodes corresponds to the age of the deposits, 30 000–40 000 years.

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The Arctic permafrost is a unique cryobank of genetic resources. Permafrost sediments contain a considerable taxonomic diversity of unicellular organisms remaining viable after the tens and hundreds of thousands of years in cryobiosis. Aerobic and anaerobic bacteria, cyanobacteria, actinomycetes, unicellular green algae, yeasts, mixomycetes, naked amoebas, heterotrophic flagellates, infusorians, moss spores, and the seeds of higher plants capable of germinating after long-term natural cryopreservation have been found in the permafrost [1].

In the present study, the first viable multicellular organisms, namely, soil nematodes, have been isolated from permafrost deposits.

We analyzed more than 300 samples of permafrost deposits of different ages and origins, buried soils and fossil rodent burrows. Two samples were shown to contain viable nematodes. The nematodes were isolated from the material of the buried ground squirrel burrow (burrow P-1320) taken from the permafrost wall of the Duvanny Yar outcrop in the lower reaches of the Kolyma River (68°37′ N, 159°08′ E) in 2002. The fossil burrow consisting of a shaft and a large chamber (up to 25 cm in diameter) was at a depth of about 30 m below the contemporary day surface in the layer of permafrost deposits of a glacial complex. A series of such burrows with a radiocarbon age of about 32 000 years had been found in this layer previously [2]. The chamber stuff contained well-preserved crushed remains of herbaceous and fruticulose plants and large amounts of seeds of the higher plants.

The nematodes were also found in the permafrost sample from glacial deposits obtained by core drilling in the vicinity of the Alazeya River (69°20′ N, 154°60′ E) in 2015. The sample was taken from a core at a depth of 3.5 m (bore AL3-15) and contained weakly decomposed plant remains. The age of permafrost deposits, where nematodes were isolated from, was 41 700 ± 1400 years according to radiocarbon dating (AA109003, AMS Laboratory, University of Arizona, United States).

The proper temperature and sterility during sampling and transportation were maintained according to the techniques approved by the Laboratory of Soil Cryology, Institute of Physico-Chemical and Biological Problems of Soil Science, Russian Academy of Sciences, in the microbiological studies of permafrost sediments [3]. In the laboratory, the samples were stored at –20°C. Viable nematodes were isolated from permafrost by the method of enrichment culture. Permafrost samples (1–2 g) were placed into Petri dishes with the Prescott–James medium and cultivated at 20°C for several weeks [4]. The clonal cultures of nematodes were obtained from the enrichment culture. Further cultivation was carried out in agar and liquid

[a] *Institute of Physico-Chemical and Biological Problems of Soil Science, Russian Academy of Sciences, Pushchino, Moscow oblast, 142290 Russia*

[b] *Moscow State University, Moscow, 119991 Russia*

[c] *Pertsov White Sea Biological Station, Moscow State University, Moscow, 119991 Russia*

[d] *Higher School of Economics, Pushchino, Moscow oblast, 142290 Russia*

[e] *Department of Geosciences, Princeton University, 08544-1013 Princeton, NJ, United States*

* *e-mail: nastya.shat@rambler.ru*

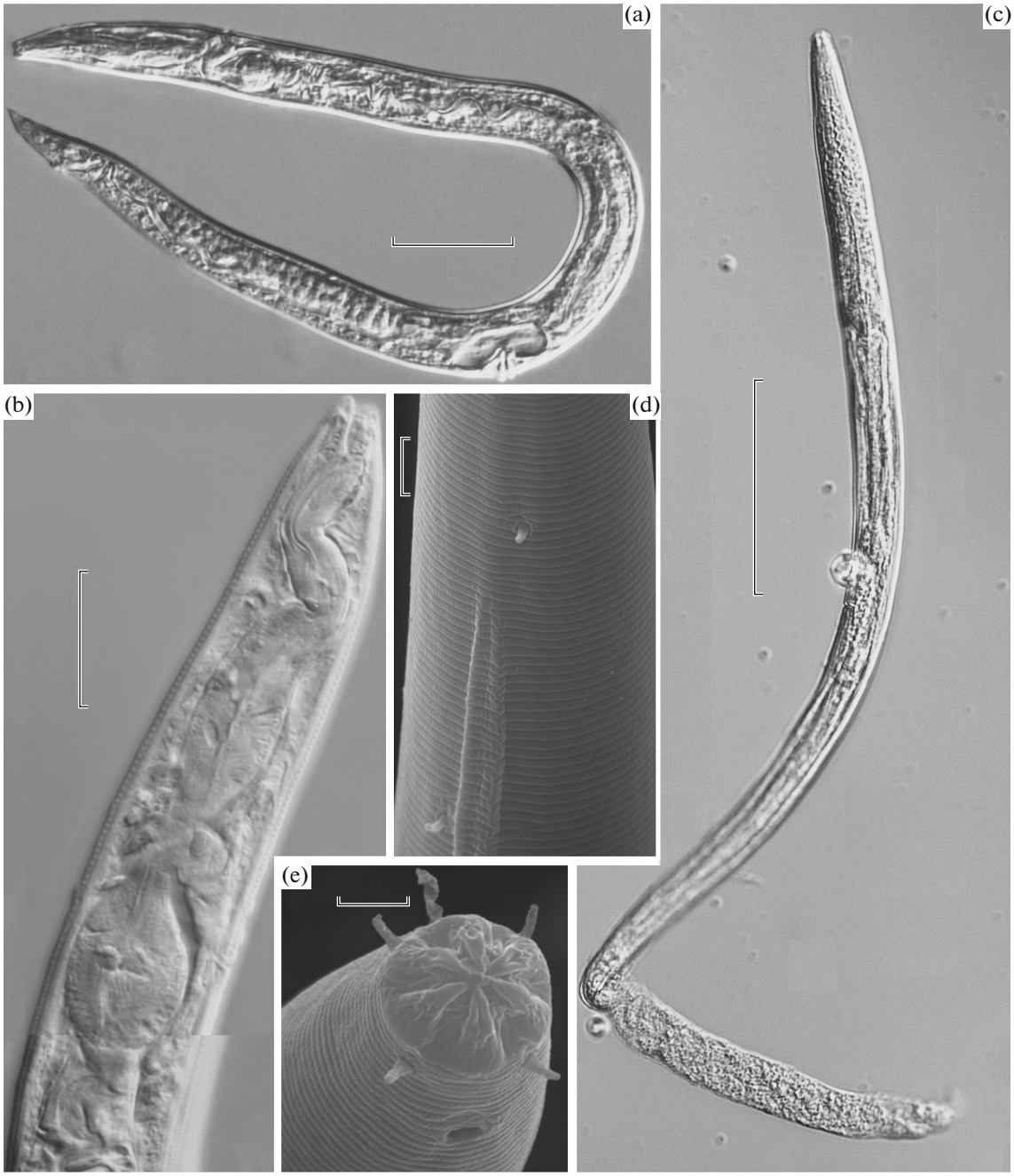


**Fig. 1.** The nematodes isolated from Pleistocene permafrost deposits of the Kolyma River Lowland. *Panagrolaimus* aff. *detritophagus*: (a) the overall view of a female; (b) the pharyngeal part of the body. *Plectus* aff. *parvus* : (c) the overall view of a female with the remainder of exuvial cuticle near the tail; (d) the photograph obtained by scanning electron microscopy (SEM) of the lateral surface of body at the mid-pharynx level, showing the lateral crest and somatic setae); (e) the SEM photograph of the head end. Scale bars, μm: (a) 50; (b) 20; (c) 100; (d) 3; (e) 3.

Prescott–James media with the addition of *Escherichia coli* bacteria as a food.

The taxonomic affiliations of discovered nematodes were determined by microscopic examination of morphological and morphometric characteristics in permanent preparations obtained by the standard procedure [5].

Additionally, the 18S rRNA genes have been studied. For this study, three overlapping fragments of the 18S rRNA gene were obtained by PCR. The primers and PCR conditions are described in the article [6]. The resultant fragments were sequenced according to Sanger. Phylogenetic reconstructions were based on the sequences obtained in this study and a set of

47 sequences of the nematode 18S RNA gene from the GenBank presented in the GenBank database (www.ncbi.nih.gov).

The phylogenetic analysis has shown that the nematodes from buried burrow stuff comprise a clade within the genus *Panagrolaimus*, while the nematodes from permafrost deposits of the Alazeya River belong to the clade within the genus *Plectus*. The discovered nematodes correspond by morphometric and structural characteristics to the species *Panagrolaimus detritophagus* Fuchs, 1930 (Rhabditida, Panagrolaimidae) and *Plectus parvus* Bastian, 1865 (Plectida, Plectidae). Though males are known for both species [7–9], only females were present in the cultures under study (obviously, both species are characterized by facultative parthenogenesis). Since females do not have a plenitude of diagnostic characteristics, our identification is yet somehow conventional.

Some factors prevent the opportunity for nematodes to penetrate into permafrost strata many meters deep from the superposed modern tundra soil. The depth of seasonal thawing in the regions under study is up to 80 cm and was no more than 1.5 m even about 9000 years ago, during the Holocene Thermal Maximum. Below this level, in permafrost deposits of the Late Pleistocene glacial complex firmly cemented by ice, the influence of external factors is drastically limited. Thermal diffusion and migration of nematodes with unfrozen water films with a thickness of no more than several nanometers is impossible. The presence of thick cavern-load ices is evidence that the enclosing rocks are syncryogenic (i.e., sedimentation and frost penetration occurred here simultaneously) and have never been thawed. The age of biota in syncryogenic strata corresponds to the age of sedimentary rocks [10].

It is known that some nematodes can sustain long-term exposure to unfavorable conditions, including negative temperature (cryobiosis), both in natural habitats (soils and ice in the polar regions) and under laboratory conditions, by combining different survival strategies [11]. The nematodes *Tylenchus polyhypnus* maintained viability in a herbarium specimen for 39 years [12]. The Antarctic nematode *Plectus murrayi* remained viable for 25.5 years in the moss samples stored at –20°C [13].

The nematodes of the families Panagrolaimidae and Plectidae, which the species found in permafrost deposits belong to, inhabit soil and freshwater biotopes, are widespread on all continents (including the Antarctic) and highly resistant to drying and freezing [14].

Thus, our data demonstrate the ability of multicellular organisms to survive long-term (tens of thousands of years) cryobiosis under the conditions of natural cryoconservation. It is obvious that this ability suggests that the Pleistocene nematodes have some adaptive mechanisms that may be of scientific and practical importance for the related fields of science, such as cryomedicine, cryobiology, and astrobiology.

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