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GENERAL BIOLOGY

Viable Nematodes from Late Pleistocene Permafrost of the Kolyma River Lowland

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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

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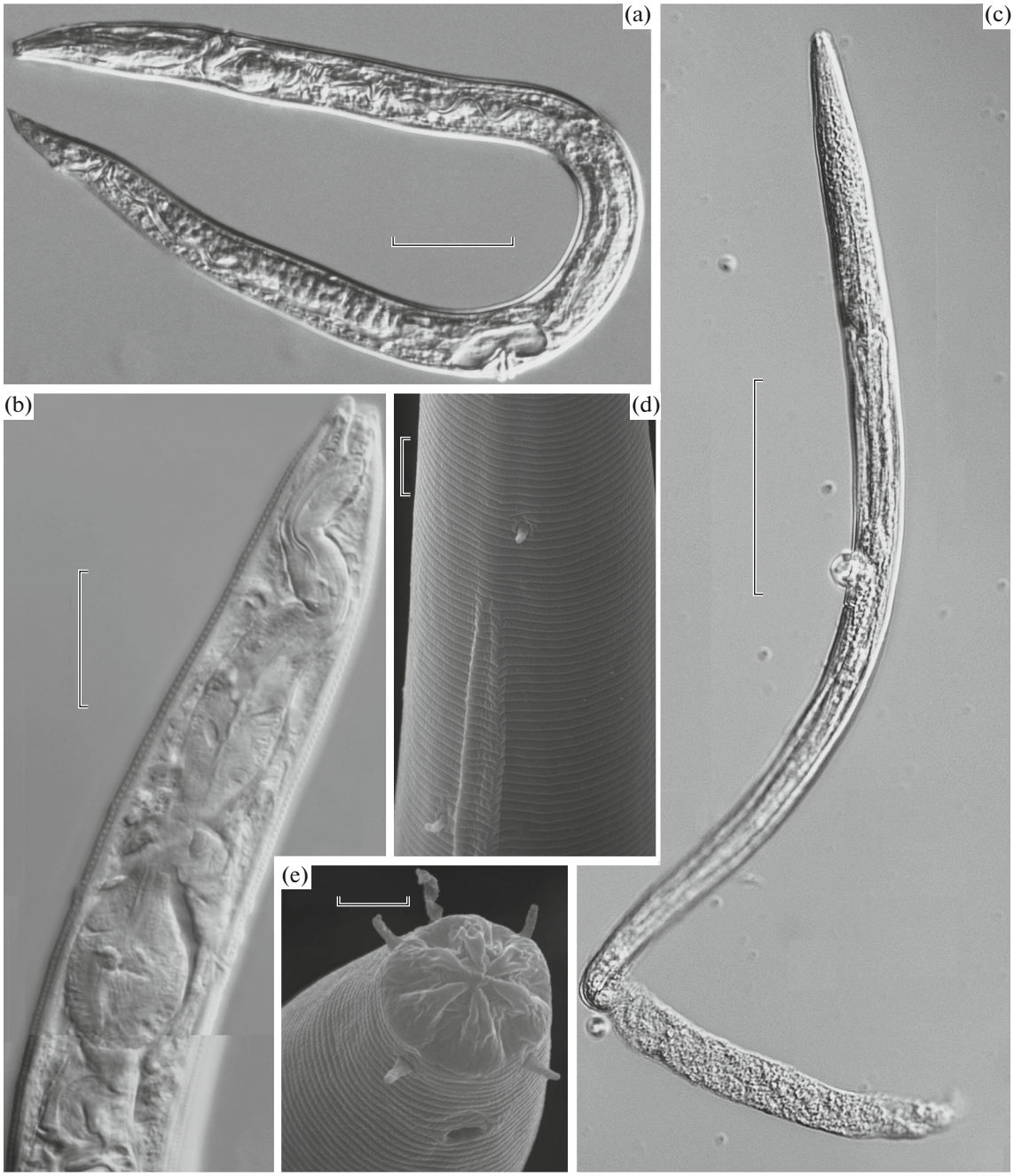

Fig. 1. The nematodes isolated from Pleistocene permafrost deposits of the Kolyma River Lowland. *Panagrolaimus* aff. *detritophagus*: (a) the overall view of a female; (b) the pharyngeal part of the body. *Plectus* aff. *parvus* : (c) the overall view of a female with the remainder of exuvial cuticle 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