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GENERAL BIOLOGY

# Viable Protozoa in Late Pleistocene and Holocene Permafrost Sediments

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It has been established that viable prokaryotic and eukaryotic organisms may remain viable for a long time under natural conditions at constant low temperatures. For example, viable anaerobic and aerobic bacteria [1–4], cyanobacteria, unicellular green algae [5, 6], yeast [7], and mycelial fungi [8] have been found in permafrost sediments dated to several hundred million to several thousand million years ago. In addition, spores of mosses and seeds of higher plants capable of germination after long-term cryopreservation have been found [9].

The study of the communities of viable paleoorganisms offers the unique possibility to make substantial progress in understanding the preservation of life in the cryosphere during geologically significant periods of time and analyzing cryoanabiosis and psychrophily, as well as the ecological and evolutionary characteristics of organisms that lived in previous geological epochs. Obviously, these studies would be incomplete without studying various lower eukaryotes belonging to Protozoa; however, there were no reliable data on the presence of viable protozoa in permafrost layers until this study. On the other hand, various forms of cryptobiosis [10] are known to be widespread among modern representatives of most macrotaxa of protozoa [11, 12].

The purpose of this study was to find viable forms of protozoa in syngenetically frozen late Pleistocene and Holocene sediments and isolate these organisms.

The age of the biota in syncryogenic layers corresponds to the age of the sediments forming these layers, which can be reliably dated by the radiocarbon method in the case of late Pleistocene sediments. The possibility to determine the age of the biota determined the choice of late Pleistocene syncryogenic sediments in the eastern sector of the Arctic region, including the Kolyma Lowland and the Laptev Sea coast (the Bykovskii Peninsula and Cape Chukochii) as the object of the study.

We studied late Pleistocene sediments from a 28 000- to 35 000-year-old glacial complex and the soils and material of fossil rodent burrows that were buried in it in the Stanchikovskii Yar outcrop on the Malyi Anyui River. The glacial complex (cryopedolith) was represented by powdered loam containing sparse thin roots of grassy plants, fine plant detritus, and humus compounds; the loam was transformed by processes involved in synlithogenic soil formation. Its ice content was as large as 40–80% due to thick ice veins and texture-forming ice. The buried soils were represented by peat and a profile of humus–peat gley soil, and the material of fossil burrows, by loam intermixed with powdered remains of grassy plants, seeds of higher plants, rodent feces, and hair of large animals. The burrows were located within the layers of sediments at a depth of 30–40 m; their ages were 28 000 and 32 000 years, according to the results of radiocarbon analysis [13].

In addition to the glacial complex (l-al $Q_{III}$) represented by medium powdered loam with an ice content as high as 80–90% because of ice veins that was saturated with slightly decayed organic matter, we studied lake–bog Holocene ($lQ_{IV}$) sediments with a layer thickness of 1–5 m represented by light powdered loam with predominantly layered cryogenic textures and an ice content of 40–50% on the Bykovskii Peninsula and Cape Chukochii [14].

Samples of permafrost sediments and soils buried in them were taken from cores obtained by exploratory drilling or from holes in fresh frozen walls of outcrops under sterile conditions. The samples were stored under field conditions at the same temperatures as in the natural deposit. When transporting the samples to the laboratory and storing them, we strictly observed all the necessary requirements concerning temperature conditions and sterility [2, 3]. All subsequent manipulations with soil samples and inoculation of organisms to Petri dishes were performed in a microbiological box. The sterility of media and laboratory equipment was constantly controlled in the course of experiments.

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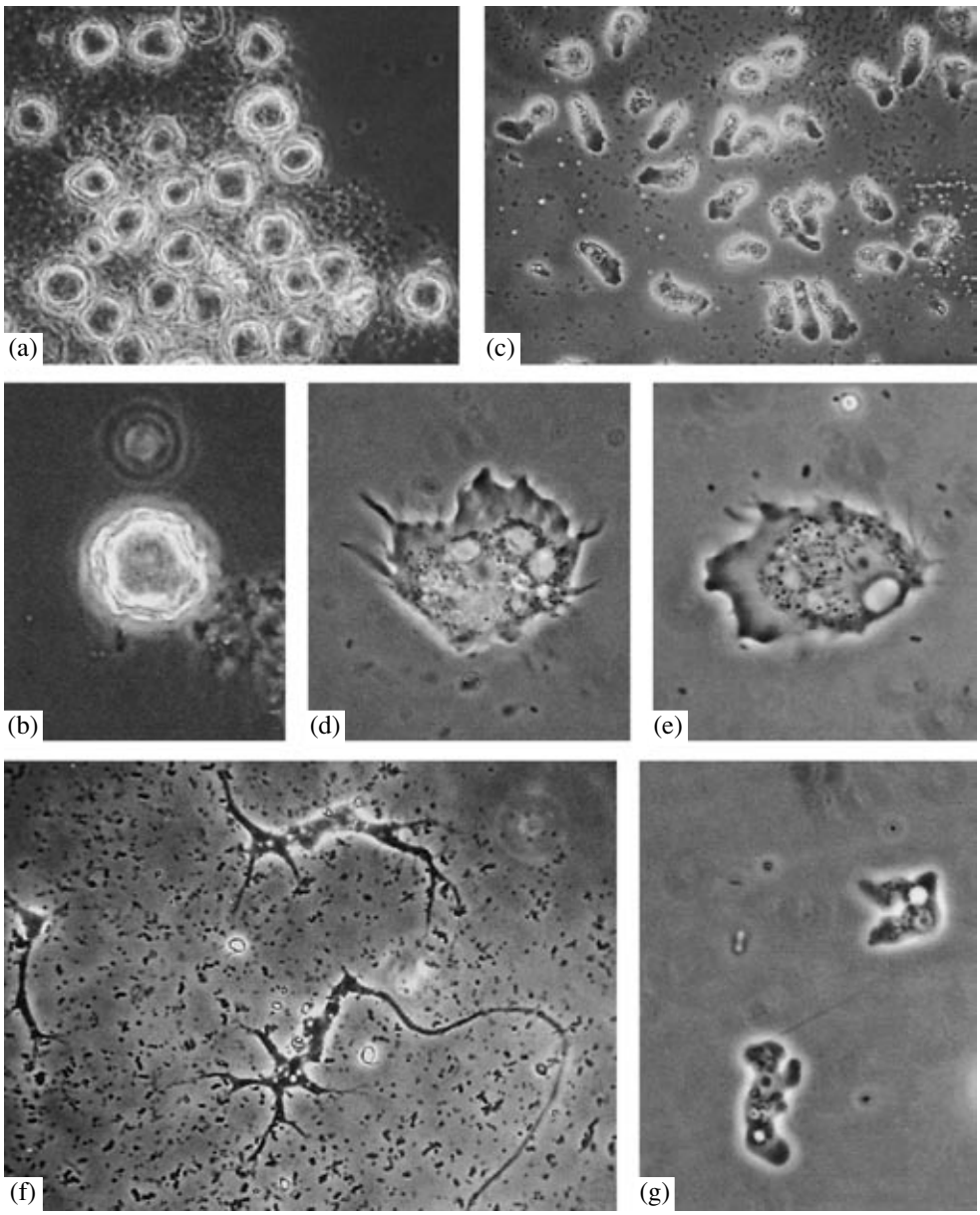


**Fig. 1.** Some species of amoeboid protozoa from late Pleistocene and Holocene permafrost sediments and soils buried in them: (a, c) cysts; (b, d–g) trophozoites. A phase-contrast microscope.

At the initial stages of the study, we used accumulation cultures on mineral and nutrient (containing cerophyl) media [15]. Petri dishes containing inoculated samples were kept at room temperature and daylight illumination or at 5°C in the dark. The organisms identified were sampled under sterile conditions and cultured on liquid and agar media containing various food objects (e.g., the bacteria *Escherichia coli* and *Klebsiella aerogenes*).

During these studies, we found viable representatives of almost all main groups of Protozoa: various nude amoebas (Fig. 1), heterotrophic flagellates, and ciliates. Protozoa were found in 19 samples of late Pleistocene and Holocene permafrost sediments taken from eight holes at depths as large as 19 m and in 22 samples of cryopedolith, buried soils, and fossil rodent burrows sampled from late Pleistocene sediment of the glacial complex of the Kolyma Lowland. Flagellates were found in 78% of "populated" samples of permafrost sediments, and amoebas and ciliates, in 46 and 20% of the samples, respectively. All protozoa isolated from the samples were relatively small and were capable of forming cryptobiotic stages of their life cycle (so-called resting-cysts) extremely resistant to various adverse environmental factors. The viable cysts of amoebas (Figs. 1a, 1c) and ciliates found in the samples had a thick, complex protective envelope consisting of several layers. Note that, of all free-living protozoa, the

capacity for forming resting-cysts is most typical of representatives of soil fauna [11].

The observed general trend of increase in the numbers and species diversity of viable protozoa in samples rich in plant debris may have been explained by more favorable cryopreservation conditions and the originally rich fauna.

We often found viable protozoa within the first meters from the day surface (in layers as thin as 2.5 m). The thawing depth in the regions studied is 30–50 cm; however, this boundary may be at a depth of 1 m in abnormally warm years or even somewhat deeper under especially favorable conditions. Therefore, the age of protozoa in the roof of permafrost is no older than several tens or hundreds of years. However, in the layers strongly cemented with ice that are located below this level, the effect of external factors is drastically restricted; aquifers and infiltration are absent. The thermal diffusion and migration of protozoa along with films of unfrozen water are also practically impossible, because their sizes are incomparably larger than the thickness of these films, which is about $10^{-3}$ μm. The presence of thick ice veins is a direct evidence that the rocks containing them have never thawed; hence, the biota found there cannot have penetrated into these layers during thawing periods. It also cannot have been brought in the course of drilling, because the method of sterile core sampling were tested earlier in microbiological studies on permafrost layers [2, 3]. Therefore, we may conclude that viable representatives of various species of Protozoa that we found in permafrost layers were present there *in situ.*

Thus, our study has yielded the first data that protozoa belonging to different macrotaxa may remain viable for periods of time as long as several tens of thousands of years in permafrost sediments, soils buried in them, and other paleoecological objects. Moreover, preliminary results allow us to expect that further studies will reveal considerable species diversity in this important group of lower eukaryotes.

In addition to its importance for paleoecology and evolution of organisms, the study of viable protozoa at the cellular level is obviously of theoretical and practical interest for understanding the adaptive mechanisms of the prolongation of viability on the geological time scale and preservation of biodiversity on Earth and, probably, outside it.

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