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EXPERIMENTAL ARTICLES

Microbiological Characterization of the Accreted Ice of Subglacial Lake Vostok, Antarctica

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Abstract—The accreted ice of subglacial Lake Vostok extends upward from the lake water level (a depth of 3750 m) to the bottom surface of the overlying Antarctic ice sheet. All of the accreted ice samples, taken from depths between 3541 and 3611 m, were found to contain pro- and eukaryotic microorganisms, whose number and diversity varied in different ice horizons and correlated, to a certain degree, with the occurrence of organic and inorganic impurities in a given horizon. Some biological objects found in the accreted lake ice, including bacteria, microalgae, and the pollen of higher plants, were morphologically similar to those found earlier in the glacier ice bulk. The others were not. It is suggested that the microorganisms found in the lake ice may come from different locations—the bottom layer of the glacier ice, the bedrock underlying the glacier, and the lake water.

Key words: Antarctica, glacier, anabiosis, microorganisms.

Our earlier studies showed that the ice samples taken from different depths of the Antarctic ice sheet contain ancient air bubbles, atmospheric dust particles, some microorganisms, and other microscopic objects, which had been transferred to the ancient glacier surface by winds for hundreds of thousands of years [1–6]. The microscopic examination of these samples made it possible not only to determine the number and to characterize the morphology of the microorganisms detected at depths down to 2750 m but also to reveal a correlation between the concentration of bacterial cells in a given ice horizon and the amount of the ancient atmospheric dust particles that depended on changes in climatic conditions on Earth [3–6].

These data were obtained upon the investigation of the ice cores extracted from well 5G at Vostok station, where the thickness of the ice sheet reaches 3750 m [7]. Presently, the well bottom is at a depth of 3623 m, i.e., about 150 m above the water level of subglacial Lake Vostok, whose existence was predicted by Russian scientists 40 years ago [8, 9] and later confirmed by seismic and radar remote sensing data [10, 11].

Lake Vostok is the largest subglacial lake of Antarctica, extending 250 km in length and 50 km in width. The lake became isolated from the Earth's surface more than one million years ago and presumably has never frozen, in spite of considerable climatic variations [12, 13].

Unlike the waters of many other Antarctic lakes, in which some processes typical of bacterial and cyanobacterial communities were revealed [14], the Lake Vostok water presumably does not contain phototrophic organisms because of the great thickness of the overlying ice. The lake ecosystem is most likely heterotrophic, although probable geothermal processes at the lake bottom [15, 16] may provide reduced chemical compounds necessary for the growth of chemoautotrophic microorganisms.

At present, the obtaining of samples from the lake water and sediments is hardly possible, as it requires special equipment and extraordinary precautions to ensure that the contamination of the established lacustrine ecosystem cannot occur.

However, some idea of the Lake Vostok microflora can be gained from the study of the ice samples that were taken from well 5G at a depth of 3538 m below the upper surface of the overlying glacier and deeper. Jouzel *et al.* [17] found that, beginning from a depth of 3358 m, the ice considerably differs from the overlying aerogenic ice in structure and physicochemical features and represents most likely the so-called accreted ice, which has resulted from the accretion of the lake water to the bottom surface of the glacier.

The aim of the present work was to study the morphological diversity of microorganisms in the accreted ice of Lake Vostok and to compare the data of this investigation with those obtained upon the study of the microflora of the overlying glacier.

MATERIALS AND METHODS

The thickness of the ice sheet at Vostok station is 3750 m [10, 13] and that of the accreted ice of Lake Vostok is about 200 m [17]. We investigated 10 ice samples taken aseptically from the central part of an ice core extracted from depths between 3534 and 3611 m.

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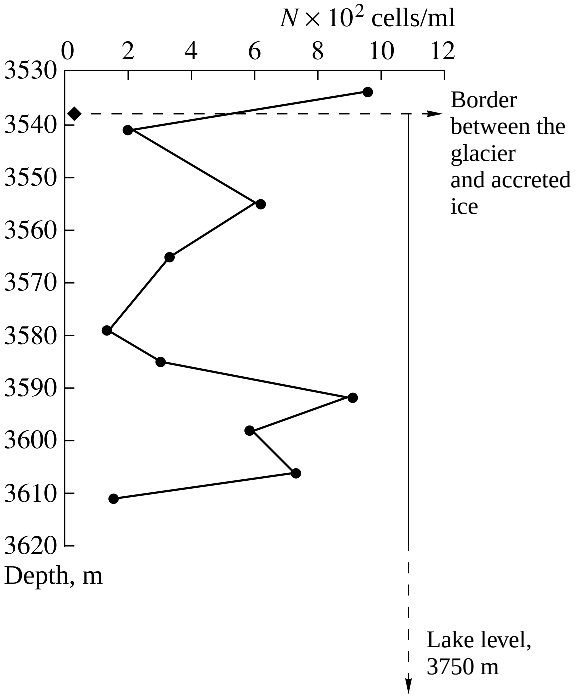

Fig. 1. Total number of bacterial cells (N) in different horizons of the accreted ice.

The first sample from a depth of 3534 m represented a glacier ice of aerogenic origin and the other nine samples, taken from depths of 3541, 3555, 3565, 3579, 3585, 3592, 3598, 3606, and 3611 m, represented an accreted ice. Horizon 3611 m is located 140–150 m above the lake water level.

The ice samples were thawed and the melt water in a volume of 100–150 ml from each of the ice samples was passed through 0.23-μm-pore-size polycarbonate filters, which retain microorganisms and other microscopic objects more than 0.23 μm in size. Filters with a retained material were stained with fluorescamine, a fluorescent dye reacting with proteins and other aminocompounds, and examined under a LYUMAM-I2 luminescence microscope (Russia) to evaluate the morphological diversity of the microorganisms and to determine their number by a direct method as described earlier [18]. We examined 40–80 microscope fields for each of the filters.

RESULTS AND DISCUSSION

As can be seen from the results presented in Fig. 1, there are three peaks in the depth dependence of the total bacterial count. Such a behavior of this dependence can reflect ice nonhomogeneity and the presence of a morainic material at depths below 3538 m [13]. As judged from morphological evidence, the accreted ice contained both pro- and eukaryotic microorganisms. The total number of microorganisms in the ice varied from tens to hundreds of cells in one milliliter of melt water, while, according to our earlier observations [3, 4], it varies from several hundreds to several thousands of cells/ml melt water in the glacier ice.

Along with microorganisms, the sediment on the filters usually contained a fraction of loamy morainic material consisting of heterogeneous particles about 50 μm in size. Such material was absent from the glacier ice sample taken from horizon 3534 m, but was rather abundant in the ice sample from horizon 3541 m (the upper layer of the accreted ice). Morainic material was found in small amounts in the accreted ice samples taken from depths of 3555 and 3579 m, in trace amounts in the ice samples taken from depths of 3565 and 3598 m, and was absent in the ice samples taken from deeper horizons (3606 and 3611 m). The observed nonuniform distribution of morainic material in the accreted ice is in agreement with the data of Lipenkov and Barkov [13] and is probably related to occasional thawing of the bottom glacier ice described by Zotikov in 1961 [19].

Such thawing, as well as the horizontal glacier flow at an annual rate of 3 m [19], favored the entry of microorganisms to the accreted ice from the bulk of the overlying glacier and also from the side horizontal ice layers lying on the glacier bed. The accreted ice was also found to contain lacustrine microorganisms, which appeared there due to the lake water accretion. Therefore, the occurrence of microorganisms in the accreted ice was due to many factors. In light of this, there can hardly be a clear correlation between the amount of particulate material and the number of microbial cells in the accreted ice, as was observed for the glacier ice [3–6].

The ice horizons investigated slightly differed in the morphology and taxonomic composition of bacterial populations. The ice horizons with poor bacterial populations (3541, 3579, and 3611 m) were characterized primarily by the presence of micrococci and short rod-shaped bacteria (Fig. 1). Similar data were obtained for the horizon at 3603 m by American researchers [20]. The poor morphological diversity of microflora in these ice horizons correlated with an insignificant content of organic and morainic materials. The ice horizons containing such materials in greater amounts, had, as a rule, a higher bacterial number and diversity. However, the correlation between these parameters in the accreted ice was less profound than in the glacier ice, in which environmental conditions are more stable.

A comparative analysis of microorganisms from the accreted ice and the lower layer of the glacier showed that they were morphologically similar, confirming the suggestion that microorganisms from the glacier ice may occur in the accreted ice due to the glacier ice thawing. The accreted ice contained many cocci, small rod-shaped cells (Fig. 2a), single and colonial cyanobacteria (Fig. 2b), microalgae, microobjects resembling the pollen of higher plants (Fig. 4), single actinomycete filaments (horizon 3565 m), yeast cells (horizon 3611 m),

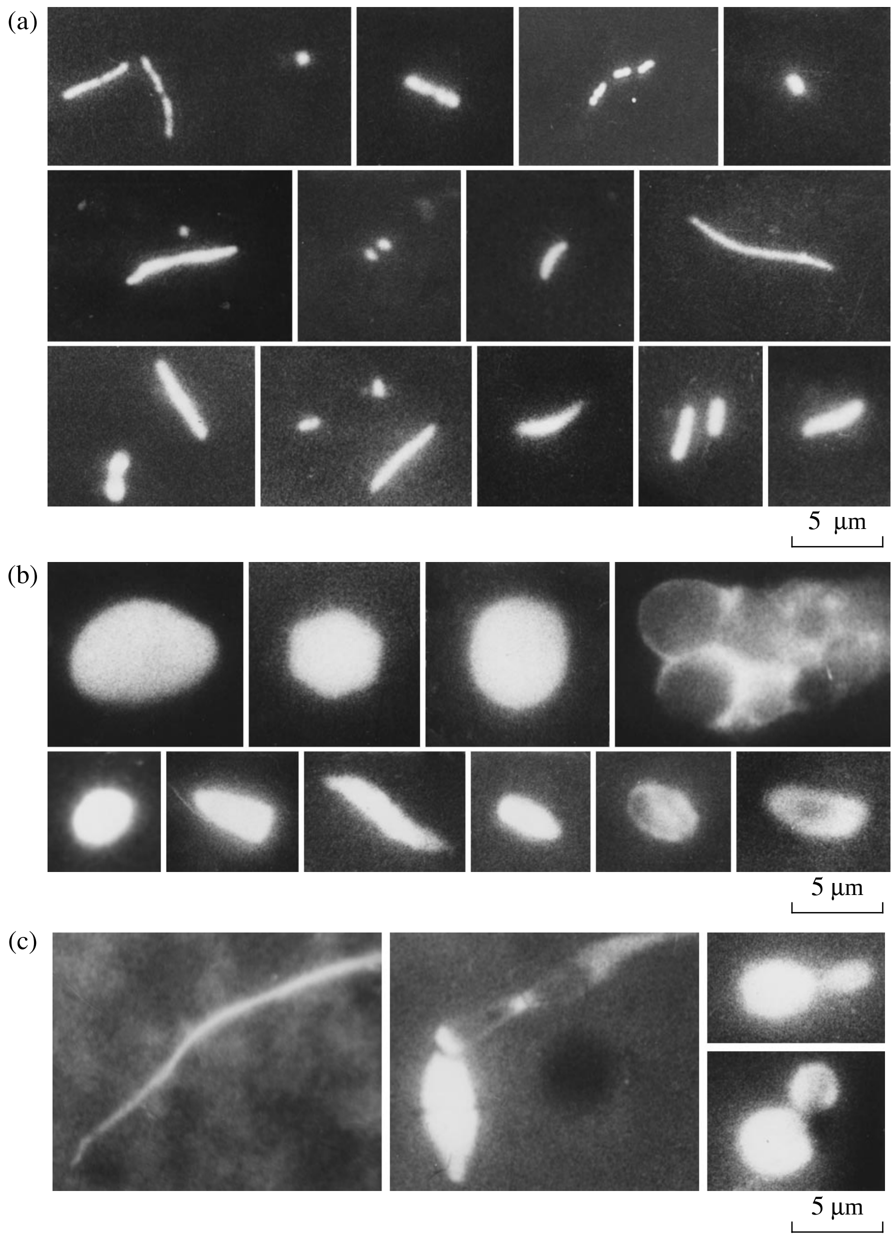

Fig. 2. The fluorescence microscopy of microorganisms detected in both the glacier and accreted ice: (a) the most typical bacteria found in all ice horizons; (b) degraded and undegraded cyanobacterium-like cells; (c) a fragment of an actinomycete filament from horizon 3565 m (on the left), a fragment of a partially lysed fungal hypha with a conidium from horizon 3585 m (on the center), and yeast cells from horizon 3611 m (on the right).

as well as fungal hyphae and conidia (horizon 3585 m) (Fig. 2c). The cells presented in Figs. 2a–2c were assigned to the first arbitrary group of microorganisms present in all of the investigated samples of the glacier and accreted ice.

Some microorganisms, which were assigned to the second arbitrary group of microorganisms, were detected only in the accreted ice. These are bacteria of the genera *Cytophaga* and *Caulobacter* and the group *Pleurocapsa*.

Bacterial cells of the genus *Cytophaga* (Fig. 3a) were detected in the ice sample taken from horizon 3555 m, where they were associated with morainic material, suggesting that these bacteria came to the accreted ice from the bedrock underlying the glacier.

Bacteria of the genus *Caulobacter* (Fig. 3b) occurred in a relatively large amount in the accreted ice horizon 3565 m but were rare in the glacier ice samples. Their occurrence in the accreted ice was most likely due to the horizontal flow of the glacier from the shelf zone of Lake Vostok, to where they could be transferred

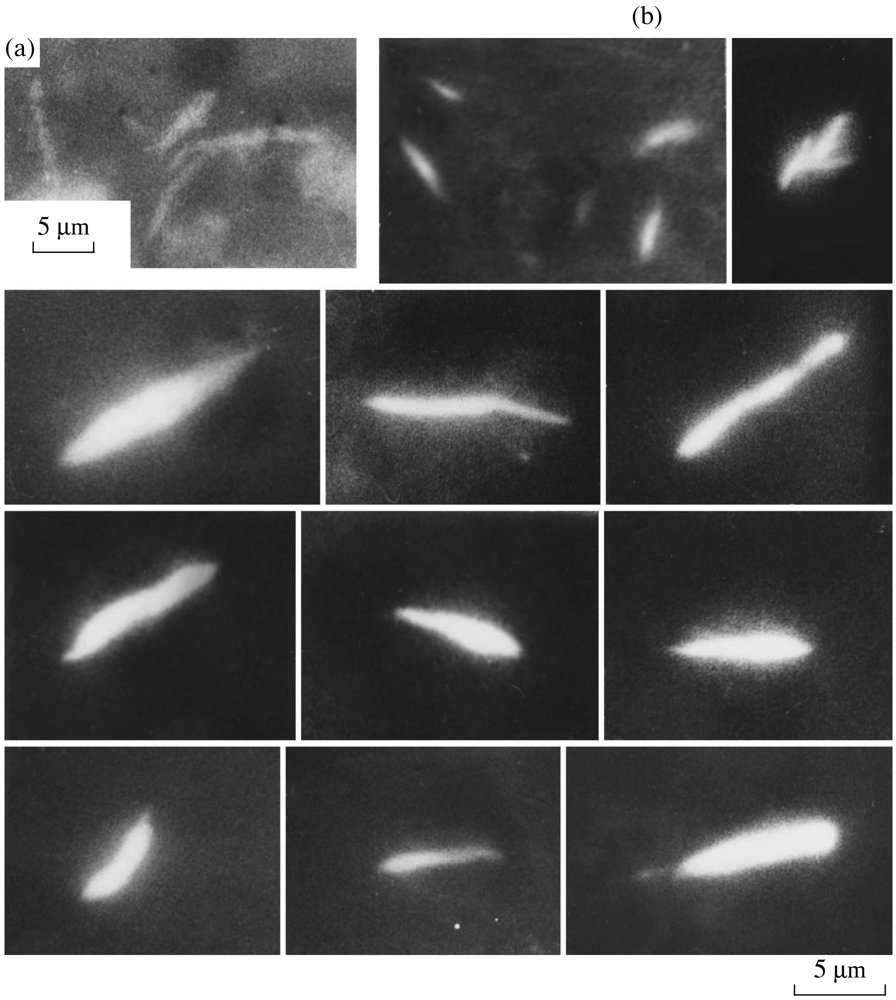

Fig. 3. The fluorescence microscopy of microorganisms detected in the accreted ice: (a) lysed weakly fluorescent bacterial cells resembling bacteria of the genus *Cytophaga* from horizon 3555 m; (b) bacteria of the genus *Caulobacter* from horizon 3565 m; (c) cyanobacterium-like cells of the group *Pleurocapsa* with natural fluorescence and different types of *Baeocytes* cells from horizon 3611 m; and (d) some types of life forms found only in the glacier ice.

with the Lake Vostok water and also from the snow cover surrounding the glacier cap or from neighboring small lakes.

Microorganisms morphologically similar to cyanobacteria of the group *Pleurocapsa* (Fig. 3c) were detected in the accreted ice sample taken from horizon 3611 m. The fluorescence of these microorganisms increased in blue or ultraviolet light. Such a bright fluorescence was not observed during the investigation of a great number of the glacier ice samples taken from different horizons.

The third group of microorganisms included those which were detected only in the glacier ice (horizons 161, 746, 1203, and 3299 m). These are primarily large rod-shaped cells, resembling some soil bacteria in their morphology. Some of these bacteria are shown in Fig. 3d and some were described earlier [21]. These bacteria either have never occurred in the accreted ice or could not survive there over an extended time period.

The microorganisms of aerogenic origin have been conserved in the glacier ice for tens and hundreds of thousands of years. Some of them preserve their integrity and, probably, viability due to their ability to transit to anabiosis [3]. The transfer of anabiotic cells to the accreted ice, which is characterized by cyclic changes of the liquid and solid states of water, did not favor their survival, probably because of their occasional hydration. This suggestion is confirmed by the observation that the relative number of brightly fluorescent fluorescamine-stained cells in the accreted ice is much lower than in the glacier ice. The weak fluorescence of microbial cells is believed to be related to a low protein content of such cells [22]. The relative number of poorly fluorescent cells was 40–50% in the glacier ice and 70–80% or more in the accreted ice. Among such microbial forms, we observed both prokaryotic (cocci, rod-shaped cells, cyanobacteria, and representatives of the genus *Cytophaga*) and eukaryotic (yeasts and

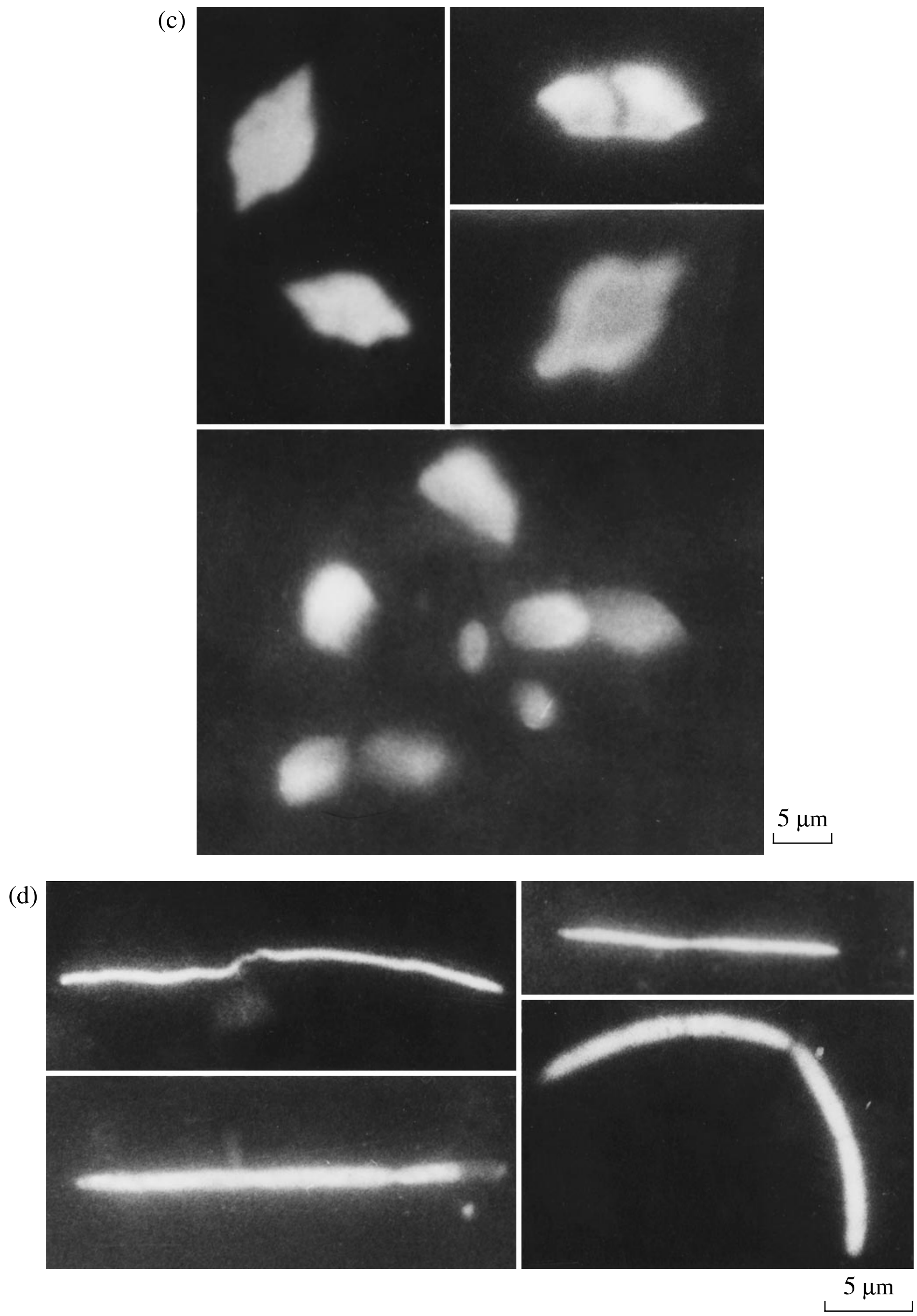

Fig. 3. (Contd.)

hyphal fungi) organisms. Most of the cells that were morphologically similar to cyanobacteria did not show their natural red fluorescence.

All accreted ice samples contained, in addition to microbial cells, unicellular microalgae and microobjects resembling the pollen of higher plants (Figs. 4 and 5). These organisms were either brightly fluorescent whole cells (Fig. 4, left photographs) or partially, and even completely, lysed (Fig. 4, right photographs). Different horizons of the accreted ice differed in the abundance and diversity of these organisms. The microalgae were dominated by diatoms, especially in ice horizons 3541 and 3611 m. The total number of undegraded microalgae in 1 ml of the accreted ice meltwater comprised tens and hundreds of cells, which was one to two orders of magnitude lower than that of bacterial cells. Figure 4 shows the most typical representatives of microalgae found in both the glacier and accreted ice.

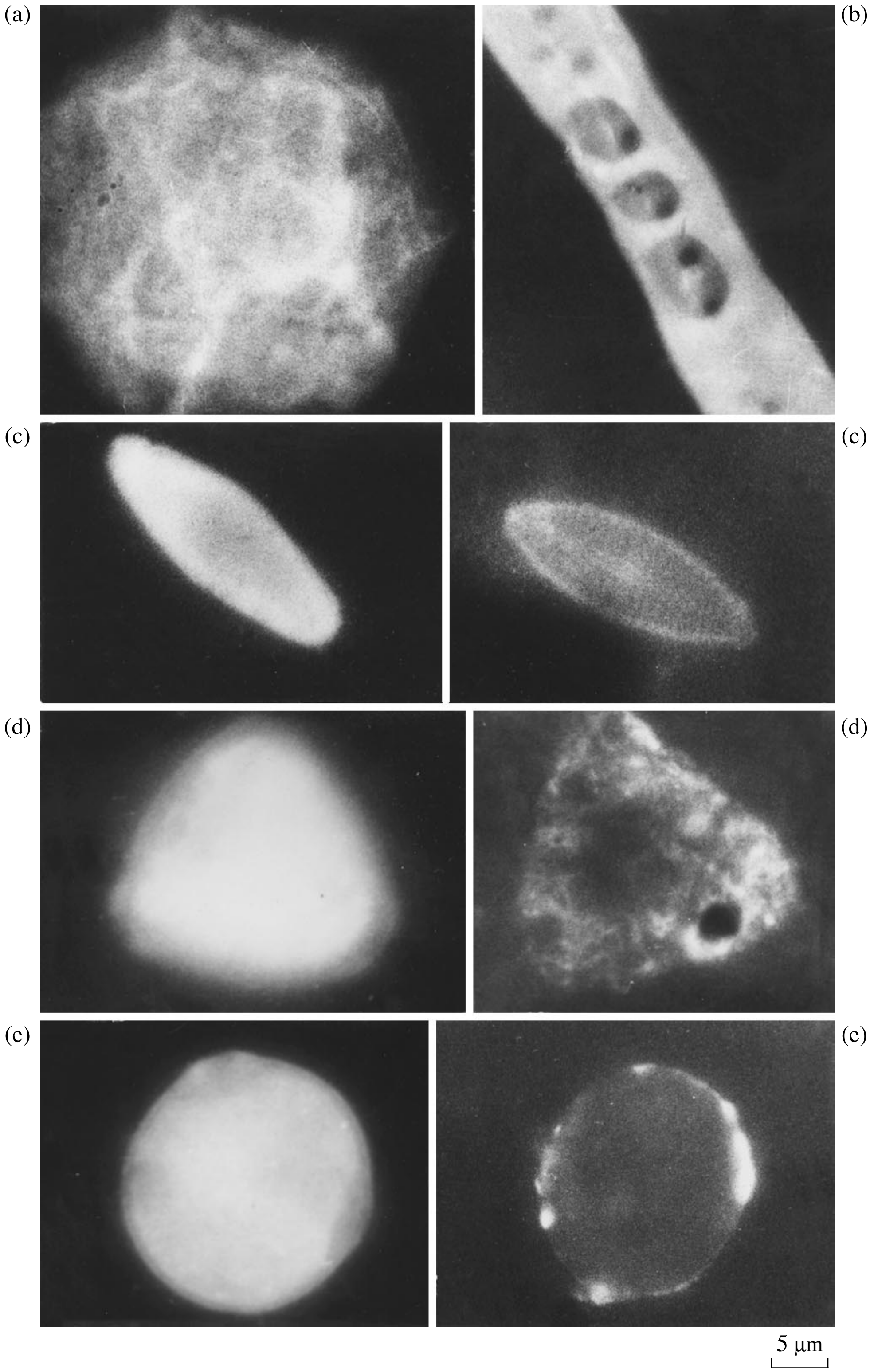

Fig. 4. The fluorescence microscopy of typical eukaryotic cells detected in both the glacier and accreted ice. On the left: brightly fluorescent and, therefore, potentially viable cells. On the right: weakly fluorescent and, therefore, presumably nonviable cells. Micrographs: (a) the microalga *Coccolitophoridae* found in nearly all glacier ice horizons; (b) a fragment of the silicified remains of diatoms detected in horizons 2902 and 3611 m; (c) diatoms detected in horizons 952, 1097, 2874, 2950, 3344, and 3555 m; (d) unicellular organisms resembling the pollen of higher plants detected in horizons 1097, 1203, 2929, 3541, and 3606 m; and (e) pollen-like unicellular organisms detected in horizons 1097, 1203, 1454, 3025, 3325, 3534, 3541, and 3611 m.

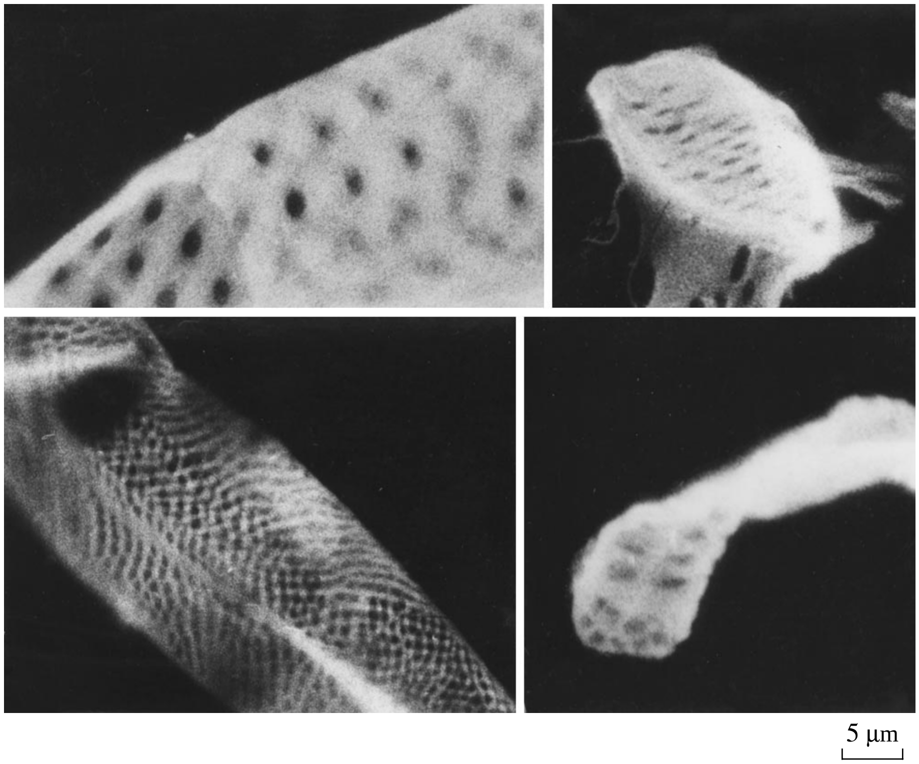

Fig. 5. Silicified remains of diatoms in the accreted ice sample taken from horizon 3541 m.

The most abundant microalgae of the genus *Coccolitophoridae* (Fig. 4a) were detected in almost all horizons of the glacier and accreted ice, especially in horizons 3555, 3579, and 3598 m. Other types of microalgae were detected as single cells (Figs. 4b–4e).

Some microalgae were detected only in the accreted ice. For instance, ice samples from horizon 3541 m contained the silicified remains of diatoms (Fig. 5), which were not detected in the other ice horizons. Taking into account that horizon 3541 m also contained a great amount of morainic material, it can be suggested that the silicified remains of diatoms were transferred to the accreted ice together with the morainic material as a result of the horizontal flow of the glacier.

As follows from the data obtained, variations in the number of bacterial cells in different ice horizons reflect, to a certain degree, the varying conditions of ice formation. The morphological diversity of bacteria detected in the accreted ice is lower than in the glacier ice thickness. There are considerable grounds to believe that the microorganisms found in the accreted ice were transferred from different locations—the bottom layers of the glacier ice of aerogenic origin, morainic material from the coastal zone of Lake Vostok, and the lake water. This suggestion is confirmed by the fact that some life forms found in the accreted ice are very uncommon in the bulk of the glacier ice.

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