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Biological Material in Ice Cores

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Introduction

Snow falling on polar and high altitude regions has formed ~1.6 x10^7 km^2 of glacial ice that provides an invaluable archive of past conditions on Earth. The expansive ice sheets of Greenland and Antarctica cover ~10% (>1.5 x 10^7 km^2) of Earth's terrestrial surface with ice and contain ~70% of the fresh water on the planet (Paterson 1994). Temperate glaciers cover more than 5 x 10^5 km^2 and comprise ~3.5% of the glacial ice on our planet (Table 1). The present volume of the Earth's glacier ice, if totally melted, represents about 80 m in potential sea-level rise with 91%, 8% and 1% represented by the East and West Antarctic Ice Sheets, the Greenland Ice Sheet, and mountain glaciers, respectively (Hambrey and Alean 2004).

<Table 1 near here>

Research on ice cores from polar and temperate glaciers has focused primarily on the reconstruction of the paleoclimate record to determine the mechanisms responsible for ice sheet mass balance, associated sea level change, and the processes leading to the transition between glacial and interglacial periods (e.g., Petit *et al.* 1999, Alley 2002). Ice cores collected from Greenland more than a decade ago provided important evidence showing persistent climate instability over the last glacial cycle. Data from the Antarctic Vostok ice core have shown that over the past 400,000 years, there was a clear correlation between temperature and greenhouse gases, implying that greenhouse gases contributed to the temperature observations during this period. A recent ice core collected as part of the European Project for Ice Coring in Antarctica (EPICA), which has produced a record spanning more than 900,000 years and covers more than eight glacial cycles (EPICA 2004), has extended this record. Information derived from the ice-core record allows predictions of future changes in climate and provides important data to anticipate how these changes will impact future societal issues on our planet.

Atmospheric impurities deposited in glacial ice include aerosols emitted by the oceans and the continents, and anthropogenic activity. Figure 1 shows the process of glacial deposition together with the global distribution of glaciers on our planet. The cold, dry conditions typical of glacial periods reduced the hydrological cycle and precipitation rate. The reduced precipitation increased the residence time of aerosols and dust allowing

them to disperse to great distances. This paradigm is revealed in the dust record from the Vostok ice core, which shows that dust concentrations during glacial periods were ~50 times greater than for interglacial periods (Petit *et al.* 1999; Figure 2). The isotopic (Sr and Nd) composition of dust in the Vostok core further shows that it originated in the Patagonian region of South America (Delmonte *et al.* 2004). Unfortunately, the biogenic nature of the aeolian dust particles in glacial ice has received relatively little attention despite its known role in microbial transport (Griffin *et al.* 2003).

<Figure 1 & 2 near here>

The biological contents of ice cores were first investigated in the 1980's by Abyzov (Abyzov 1993, Abyzov *et al.* 2001), who used microscopic observations and traditional cultivation methods to show that microorganisms were present in the Vostok ice core and were positively correlated with the concentration of dust particles. An example of dust and associated microbiota in the Sajama ice core (Bolivia) is shown in Figure 3. Abyzov's results, although controversial, motivated other microbiologists to examine the biotic content of ice cores collected from many icy regions of our planet (e.g., Priscu *et al.* 1998, 1999, Karl *et al.* 1999, Christner *et al.* 2000, 2001, 2003; Castello *et al.* 2005). Anomalies in the concentration and isotope ratio in biogenic trace gases (e.g., CH_4, N_2O) trapped in temperate and polar glacial ice have led glaciogeochemists to suggest enzymatic alteration within the ice by *in situ* metabolism (Sowers 2001, Campen *et al.* 2003). These trace gas anomalies imply that microorganisms are actually metabolizing in solid ice following deposition. Collectively, these data infer that ice cores represent "ice museums" containing novel records of evolution and habitat variability on our planet. Clearly, biologists should be included in future ice coring efforts if we are to produce a comprehensive record of past conditions on Earth. We present an overview of recent investigations that focus on biogenic matter in ice cores collected from the polar ice sheets (Antarctica and Greenland) and from low latitude mountain glaciers. We conclude by discussing the metabolic and evolutionary potential of ice-bound microorganisms.

<Figure 3 near here>

Biogenic matter in polar ice sheets and temperate mountain glaciers

The study of biogenic material in ice cores is in its infancy and, except for a few reports, was unheard of a decade ago. We now know that microorganisms and associated nonliving organic matter are present in virtually all ice samples examined for biological properties (Figure 4). The past decade has seen biological research in ice cores go from a novelty to a focused area of scientific research.

<Figure 4 near here>

The Vostok Ice Core

Vostok Station is centrally located on the East Antarctic Ice Sheet (78°27'51"S 106°51'57"E; altitude 3448 masl) and sits over ~3743 m of ice. Nearly 20 years after Vostok Station was constructed for the purpose of paleoclimatic ice core research, airborne radio-echo surveys and satellite images revealed that a very large lake (Subglacial Lake Vostok) exists beneath the ice sheet (Kapitsa *et al.* 1996, Studinger *et al.* 2004) (Figure 5). A 3,623 m core (designated 5G) was recovered from the ice sheet by an international drilling team in 1998 before drilling was terminated ~120 m from the ice-water interface to prevent contamination of the lake environment.

<Figure 5 near here>

The upper 3310 m of the Vostok ice core has provided a detailed paleoclimate record spanning the past 420,000 years (Petit *et al.* 1999). However, the deepest portion of the ice core (3,539 to 3,623 m) has a chemistry, isotopic composition, and crystallography distinctly different from the overlying glacial ice (Jouzel *et al.* 1999, Figure 6). Geochemical and physical data from this deep ice indicate that it originated from the freezing (accretion) of subglacial lake water to the underside of the ice sheet. Microbiological studies of the Vostok glacial and accreted ice have indicated that low, but detectable, concentrations of prokaryotic cells (34 to 430 cells ml^{-1}; Christner *et al.* unpublished) and DNA are present (Priscu *et al.* 1999, Christner *et al.* 2001, Bulat *et al.* 2004), and a portion of the entrapped microbial assemblage is metabolically active when melted and exposed to nutrients (Karl *et al.* 1999, Christner *et al.* 2001). Many of the bacterial cells are associated with non-living organic and inorganic particulate matter (Figure 7). Molecular identification of microbes within the accreted ice (by both culturing

and direct 16S rDNA amplification) show close agreement with present day surface microbiota that classify within the Proteobacteria (α, β, and γ), Low G + C Gram Positives, Actinobacteria, and the Cytophaga-Flavobacterium-Bacteroides line of descent (Priscu *et al.* 1999, Christner *et al.* 2001, Bulat *et al.* 2004). Recent data from the Vostok ice core, using electron backscatter scanning electron microscopy, have also shown that up to 40% of the particles in glacial ice from the Vostok core represents non-living biogenic matter; this value reaches 60% for accretion ice (Figure 8; Royston-Bishop, unpublished data). Dissolved organic carbon (DOC) in the Vostok core ranges from 4 $x10^{-7}$ M to $7x10^{-7}$ M in glacial ice and increases to almost $12x10^{-7}$ M in the underlying accretion ice. The dissolved and particulate organic fractions are positively correlated, inferring that these constituents co-varied in the atmosphere when deposited on the ice sheet or were produced within the glacial ice by complimentary *in situ* metabolic activity following deposition. The positive relationship between these fractions in accretion ice could result from complimentary *in situ* metabolism in either the ice itself or within Lake Vostok before the ice accreted to the bottom of the ice sheet.

<Figure 6, 7, & 8 near here>

There has been speculation that geothermal input into Lake Vostok may fuel chemolithoautotrophic microbial communities (Bulat *et al.* 2004). Importantly, it is still possible for a chemolithoautotrophic-based ecosystem to exist without invoking geothermal activity. A range of energy-rich reduced compounds (e.g., HS^-, S^0, and Fe^{2+}) are supplied to the lake by the ice sheet itself, which alone can supply the energy and carbon required to support metabolic activity within the lake. Organisms trapped in the ice sheet can also provide the microbial seed to initiate the lake population (Priscu *et al.* 1999, Karl *et al.* 1999). Although geothermal input is not required to support life within the lake, it can provide an additional energy source for microbial growth in the lake if present. While other views of life in Lake Vostok exist, they all imply that microorganisms are present in this subglacial environment, despite high pressure, constant cold, low nutrient input, and an absence of sunlight (e.g., Bulat *et al.* 2004, Petit *et al.* 2005, Priscu *et al.* 1999, Karl *et al.*. 1999). The exact nature of the biology in Lake Vostok awaits direct sampling of the lake water.

Ice Cores From Greenland

Biological studies of glacial ice cores have focused primarily on Antarctic cores and non-polar, high elevation glaciers (Priscu and Christner 2004 and references within). However, new studies on ice cores drilled in Greenland (i.e., GISP2, Gow *et al.* 1997; NGRIP, Anderson *et al.* 2004) have yielded important data on the nature of biogenic matter within and below the Greenland Ice Sheet. Culturable bacteria were recovered from the "silty" ice in the basal portion of the GISP2 core, with reported total cell concentrations $>10^7$ cells ml^{-1} of melt water (Sheridan *et al.* 2003, Miteva *et al.* 2004). The bacteria entrapped in the deepest sediment-laden portion of the GISP2 core could have originated from both the overlying 120,000-year old glacial ice and the underlying glacial till. Phylogenetic analysis of 16S rRNA gene sequences in the silty ice revealed that 36% of the bacterial isolates obtained were related to genera previously documented in glacial and permanently cold environments (i.e., *Methylobacterium, Rhodococcus, Mycobacterium, Sphingomonas, Arthrobacter,* and *Frigoribacterium*; Miteva *et al.* 2004), providing support for the notion that members of these genera are particularly adept at surviving freezing and persistence under cold and low or non-growth conditions.

Refrozen, sediment-laden ice core samples from the subglacial environment in Greenland were recently obtained from the NorthGRIP borehole when pressurized, "pink"-colored basal water at the base of the ice sheet entered the lower 45 m of the borehole and froze (Anderson *et al.* 2004) (Figure 9). Before this discovery, the existence of large amounts of water at the base of the Greenland ice sheet was not anticipated. Although cell concentrations in this refrozen basal water were 10x greater than in the overlying glacial ice (basal ice=1.6×10^3 cells ml^{-1}, glacial ice= 1.6×10^2 cells ml^{-1}; Christner *et al.*, unpublished), the basal material was extensively contaminated with the hydrocarbon fluid used for ice core drilling, making conclusions about the microbiology of the subglacial environment tentative. This subglacial debris contains minerals (e.g., sulfides and iron, which produced the pinkish color) and organic material from the bedrock, which could fuel microbially-mediated chemical weathering reactions (e.g., Tranter *et al.* 2002) and support a community isolated from direct input from the surface. The data collected so far show that Greenland, like Antarctica, contains microorganisms within glacial ice and in the liquid subsurface environment.

<Figure 9 near here>

Temperate Mountain Glaciers

Glacial ice cores from non-polar, low-latitude glaciers in the Andes, Himalayas, and New Zealand generally contain more recoverable bacteria (i.e., colony forming units) and a greater variety of species than those from polar ice cores (Christner *et al.* 2000, Christner *et al.* 2003, Xiang *et al.* 2005). This geographic difference is consistent with the closer proximity of mountain glaciers to locations with substantial vegetation and exposed soils, which serve as major sources of atmospheric particles. Despite great differences in the environments contributing biological particles to polar and nonpolar glaciers, phylogenetic analysis reveals that many of the bacteria characterized from different geographical locations belong to the same genera and species (Priscu and Christner 2004). One striking result from biological studies of ice cores is that there is no consistent, monotonic decrease in the number of recoverable bacteria with increasing age of the ice core (Christner *et al.* 2000). Rather, the numbers of recoverable bacteria at different depths appear to be a reflection of the prevalent climate and individual events that occurred at the time of deposition.

Cells revived from ice core samples have endured desiccation, high solar irradiation while at the surface, freezing, an extended period of frozen storage, and eventual thawing. Therefore, it is not surprising that a large number of the isolates recovered belong to bacterial groups that form spores (e.g., *Bacillus* and *Actinomyces*) or have thick cell walls and polysaccharide capsules. These structures would help overcome the stresses associated with water loss, namely increased intracellular solute concentrations, decreased cell size, a weakened cell membrane, and physical cell rupture caused by freezing and thawing. The high frequency of pigment production in recovered isolates from temperate ice cores (Christner *et al.* 2000) is consistent with the need to absorb harmful solar irradiation, which can cause lethal DNA damage. Even though the surviving cells may have resistant structures and protective pigments, they must still incur some radiation and chemical damage during extended periods of inactivity. Long periods (30-180 days) of incubation were often necessary before visible colonies appear during culture efforts, yet the vast majority of the isolates could subsequently be

subcultured to the stationary phase in <1 week. This observation indicates that time is required to repair accumulated cellular damage before growth and reproduction could begin.

A significant number of glacial bacterial isolates are phylogenetically related to species recovered from Antarctic lake mats, sea ice, polar ice cores, and other predominantly cold environments (Priscu and Christner 2004). In terms of geographical differences between ice core sites, *Bacillus* and *Paenibacillus* relatives of strains prevalent in soils were most commonly isolated from nonpolar glacial ices, and species of *Sphingomonas*, *Methylobacterium*, *Acinetobacter*, and *Arthrobacter* were ubiquitous and recovered from both polar and nonpolar ice core locations. It is noteworthy that close relatives of the radiation-resistant type strains *Methylobacterium radiotolerans* and *Acinetobacter radioresistens* were also commonly isolated. The recovery of related microorganisms from many geographically diverse, but predominantly frozen environments, argues that these species probably have features that confer resistance to freezing and survival under frozen conditions.

Methods For Measuring Biotic Matter In Ice Ccores

Particles in ice cores are typically measured on discrete core segments (usually at a depth resolution of several meters) using instruments based on the Coulter principle (e.g., Delmonte *et al.* 2004). These investigations focused on the size, concentration and source of the particles but failed to discriminate between biotic and abiotic forms, collectively referring to them as “dust”. Most of the data on the abundance of microorganisms in ice cores are from light microscopy, epifluorescence microscopy and electron microscopy. Although these microscopic methods allow characterizing of the microorganisms both morphologically and physiologically, they are tedious and subject to large errors, particularly when cell density is low. Hence, microscopic analysis does not lend itself to high resolution, routine determinations of biogenic matter in ice cores.

Borehole optical loggers have been used successfully to quantify the *in situ* abundance of dust in several systems and modifications are underway to incorporate filter fluorometers on the loggers to detect biota and biomolecules (Bay *et al.* 2005). Flow cytometers offer another promising method to detect and characterize biogenic matter

rapidly in ice cores retrieved from selected sites. Flow cytometers were developed for use in the medical field to rapidly enumerate, characterize and sort cells from blood and human tissues. More recently, they have been used to characterize viruses, bacteria and phytoplankton in natural marine environments (e.g., Minor and Nallathamby 2004). Flow cytometers examine particles in a focused stream of fluid directed individually in front of an optical beam. Morphologically characterization and enumeration of the particles (biotic and abiotic) can be made by their light scattering properties. The physiological properties of the microbial component of the "dust" can be determined by either autofluorescence or fluorescence induced by the addition of specific fluorescence probes (Bay *et al.* 2005). Autofluorescence or induced fluorescence allows microorganisms to be enumerated separately from abiotic particles, and can provide detailed physiological measurements such as DNA content, cell membrane integrity and metabolic potential.

Our laboratory has begun to use flow cytometry to characterize particulate matter in ice cores from Antarctic and Greenland. Importantly, these ice cores all followed strict preparation and decontamination protocols outlined by Christner *et al.* (2005) before cytometric analyses to ensure samples were not contaminated. A Microcyte™ flow cytometer (www.biodetect.biz) was placed in a class 100 environmental chamber, and calibrated with fluorescent beads of known size and fluorescence quantum yield (Molecular Probes Inc.; www.probes.com). The fluorescent DNA probe Syto 60 (Molecular Probes Inc) was added to melted ice cores and incubated in the dark for 5 minutes before cytometric analysis. Forward light scatter was used to measure the size and abundance of the particles, while fluorescence counts discriminated the biotic from the abiotic particles in the samples. Initial tests on Vostok glacial (1686 and 2334 m) and accretion ice (3612 m) showed that counts of total particles and DNA containing bacterial cell can be successfully determined (Figure 10). Data obtained from these samples by flow cytometry were verified by epifluorescence microscopy of samples stained with the DNA probe SYBR Gold and by scanning electron microscopy (Christner *et al.* 2005). Two previous studies have also employed flow cytometers to enumerate microbes in glacial ice cores. Karl *et al.* (1999) used a flow cytometer to show that there were 500 to 700 cells ml^{-1} in Vostok accretion ice from 3603 m, and Miteva *et al.* (2004) found concentrations of bacterial cells ranging from 6.1-9.1 x 10^7 cells ml^{-1} in ice cores taken

from the GISP2 (Greenland) "silty" ice (~10 m above bedrock). In both of these studies, as well as our own, small cells (~1 µm) dominated the populations found within ice cores.

<Figure 10 near here>

Metabolic and evolutionary potential of ice-bound microorganisms

The question of whether or not the microorganisms in glacial ice are metabolically active has fundamental significance to both glacial microbiologists and geochemists. Microbiologists are most interested in the diversity, evolution, and survival mechanisms of microorganisms, whereas glacial geochemists are concerned that *in situ* microbial activity consumes or liberates trace gases, thereby effecting the absolute concentration and isotopic content of the gases under concern. If the gases within the ice core are significantly altered by biogenic activity, a reassessment of ice core paleo-gas records may be necessary (Campen *et al.* 2003, Sowers 2001).

Priscu and Christner (2004) estimated that the polar ice sheets of Greenland and Antarctica contain a combined total of 9.6×10^{25} prokaryotic cells, which equates to 2.7×10^{-3} petagrams ($1Pg = 10^{15}g$) of organic carbon. This value represents a previously unrecognized carbon pool and approaches the bacterial carbon contained in all surface freshwaters on our planet (Whitman *et al.* 1998). Paleoclimate studies have accurately dated layers within the ice caps of both Antarctica (Petit *et al.* 1999, EPICA 2004) and Greenland (Alley 2002), providing an important stratigraphic age record for these microbial ice repositories. This reservoir of ancient microorganisms, which can include fungi, bacteria, and viruses (Castello and Rogers 2005), provides an unexplored frontier for the study of microbial variability over at least eight glacial cycles (~1 million years). Data collected from selected habitats on our planet shows that microbial cells can be revived after extended entrapment in geological materials (Table 2).

<Table 2 near here>

A global assessment of prokaryotic diversity in glacial ice, based on 16S rRNA gene sequences, revealed that many of the isolates are related to psychrophilic and psychrotolerant species originated from sites ranging from aquatic and marine ecosystems to terrestrial soils, with little in common except that all are permanently cold

or frozen. The distribution of related species in geographically diverse glacial environments implies that members of these bacterial genera evolved under cold circumstances and likely possess similar strategies to survive freezing, and to metabolize at low temperatures. This paradigm supports the contention of others (e.g., Price 2000, Campen *et al.* 2003, Sowers 2001) that the organisms are actually metabolizing in "solid ice", presumably within grain boundaries (Figure 11). If microorganisms are indeed metabolizing and growing within the ice itself, a completely new set of selection pressures must be considered.

<Figure 11 near here>

Rogers *et al.* (2004) postulated that selected pathogenic microbes survive and are recycled through ice in a process they call "genome recycling" (Figure 12). The premise of their contention is that organisms trapped in ice for hundreds of thousands to millions of years are eventually released when glaciers calve and the ice melts and mixes with the genomes of contemporary microbial populations. The mixing of ancient and modern genotypes may affect mutation rates, fitness, survival, pathogenecity and other characteristics through a change in allele proportions in the populations. Genome recycling is dependent on the revival and establishment of organisms once they emerge from the ice sheets and glaciers and transfer their genetic information to extant populations, which are then reincorporated into ice sheets and glaciers via aeolian processes. Rogers *et al.* (2004) estimate that 10^{17} to 10^{21} viable microorganisms are released annually from melting glacial ice.

<Figure 12 near here>

Castello *et al.* (2005) documented the recovery and identification of viable phage from the bacterium *Bacillus subtilis*, and also have amplifed genomic segments of tomato mosaic tobamovirus from ice cores assigned dates exceeding 100,000 years before present. Our laboratory has also observed free viral particles in both meteoric and accretion ice from the Vostok ice core (Figure 13), implying that viruses, like bacteria, are prevalent in glacial ice. These viral data, in concert with the concept of genome recycling proposed by Rogers *et al.* (2004), have led Smith *et al.* (2004) to postulate that glacial ice may provide a reservoir for pathogenic human viruses, particularly caliciviruses, influenza viruses, and enteroviruses. These viral groups occur in great

abundance, are readily transported in the atmosphere, and may participate in ongoing disease cycles once released from the ice. Smith *et al.* (2004) contend that icy reservoirs may explain cyclic calicivirus events and the decades-long disappearances and subsequent reappearances of influenza-A subtypes. Although further research on ice cores is required, these preliminary data infer that viral reservoirs provided by glacial ice should be considered in eradication efforts for pathogenic human viruses.
<Figure 13 near here>

Conclusions

Glacial ice covers more than 15 x 10^6 km^2 of our planet and has a role in driving all processes on Earth. Glacial ice also contains an important reservoir of information on past climatic events, extending back at least one million years. Paleoclimatologists have used this information to determine past climate changes and to predict what changes may occur in the future. Despite the wealth of information trapped in ice cores, little information exists on the microorganisms immured in the ice. Recent discoveries over the past decade have shown that glacial ice contains an important record of microorganisms on our planet that theoretically could be used to assess biogeochemical processes and habitat types that occurred during past glacial and interglacial periods. This record may also contain important information on microbial evolution and physiology, and provide new biomedical information on pathogens. It is important that biologists be included in future ice coring efforts if a comprehensive view of past conditions on Earth is to be obtained. Such information will benefit all sciences involved in deciphering the ice core record and will provide the necessary information in our search for life on other icy worlds.

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Table 1. Aerial coverage by selected glaciated areas on Earth (data from the World Glacier Monitoring Service, 1989).

Region	Surface Area (km^2)	Percent of world total
Polar		
Antarctica	13,593,310	87.5
Greenland	1,726,400	10.88
Polar total	15,319,710	96.58
Temperate		
Africa	10	<0.01
Asia and Eastern Europe	185,211	1.17
Australasia (i.e. New Zealand)	860	0.01
Europe (Western)	53,967	0.34
North America excluding Greenland	276,100	1.74
South America	25,908	0.16
Temperate total	542,056	3.42
World total	15,861,766	

Table 2. Reports of viable microorganisms retrieved from icy habitats representing a range of temporal isolation.

Investigators	**Location**	**Age (years)**
Sheridan *et al.* (2003); Miteva *et al.* (2004)	Glacial ice; GISP2, Greenland	120,000
Abyzov (1993); Christner *et al.* (2005)	Glacial ice: Vostok, Antarctica	420,000
Christner *et al.* (2003)	Glacial ice: Guliya, China	750,000
Shi *et al.* (1997)	Permafrost	3,000,000

List of Figures

York) using RAMP elevation data provided by the National Snow and Ice Data Center (NSIDC).

Figure 6. Shematic of the Vostok ice core showing the region of transition between glacial and accretion ice. The accretion ice is represented by dirty ice containing numerous sediment inclusions and clear ice, which was formed over the lake proper and is relatively free of sediment. The ice below 3,623 m has yet to be samples but it is thought to have formed over the lake proper like the clear ice above it. See Jouzel *et al.* (1999) for details of the transition zone.

Figure 7. Scanning electron (A,B,C) and atomic force (D) microscope images of bacterial cells from Vostok accretion ice showing their close association with organic particles. The large particle in panel "A" was shown to be organic using backscattered electron imaging. The numbers on each panel refers to the depth from which the ice cores were collected. Samples for microscopy were prepared as outlined by Priscu *et al.* (1999).

Figure 8. Scanning electron microscope images showing the relative amounts of particulate mineral and organic matter in a Vostok glacial ice core collected from 2,779 m below the surface. Backscattered electron imaging was used to view the particles in panel B; only mineral particles are detectable using electron backscatter. Organic particles not detected by electron backscatter are denoted by the yellow outlines in panel A. The percentage of organic particles ice core below ~1,600 m, relative to the total particle count, is presented in panel C along with profiles of dissolved organic carbon (DOC). The positive relationship between particulate organic matter and DOC is shown in panel D. DOC and scanning electron microscope methodology are described in Priscu *et al.* (1999).

Figure 9. Images of an ice core collected at a depth of ~3,310 m from the NGRIP (Greenland) drilling site (Anderson *et al.* 2004). The ice core contains both colored frozen basal water and glacial ice (A). Low resolution scanning electron micrographs of particles in glacial ice and adjacent frozen basal water are shown in (B) and (C), respectively.

Figure 10. Preliminary flow cytometer data showing the relative abundance and size distribution of cellular and abiotic particles in Vostok glacial ice (A = 1,686 m; B = 2,334 m) and accretion ice (C = 3,612 m). Cellular particles were characterized by their fluorescence induced with the DNA stain CYTO 60.

Figure 11. Cross-polarized image (A) of a Vostok accretion ice core from 3,590 m below the surface showing the grain boundary between 2 crystals. Panel B shows a microscopic view of the same vein following treatment with an epoxy resin, which highlights the grain boundary. The grain boundaries in polycrystalline ice, particularly where they form triple junctions have been proposed as a site for life in solid ice (Price 2000).

Figure 12. General scheme of genome recycling through glacial ice proposed by Rogers *et al.* (2004). Organisms immured in the ice for millennia are eventually released as the

glaciers calve or melt, releasing them to the environment where there genes can mix with contemporary gene pools. Organisms forming the new gene pools are eventually trapped as dust particles in the ice where they remain isolated until the next cycle begins. Courtesy of S. Rogers, Bowling Green State University.

Figure 13.Viral particles observed in the Vostok ice core from selected depths in the glacial and accretion ice. All images were obtained by transmission electron microscopy. Courtesy of M. Young, Montana State University at Bozeman.

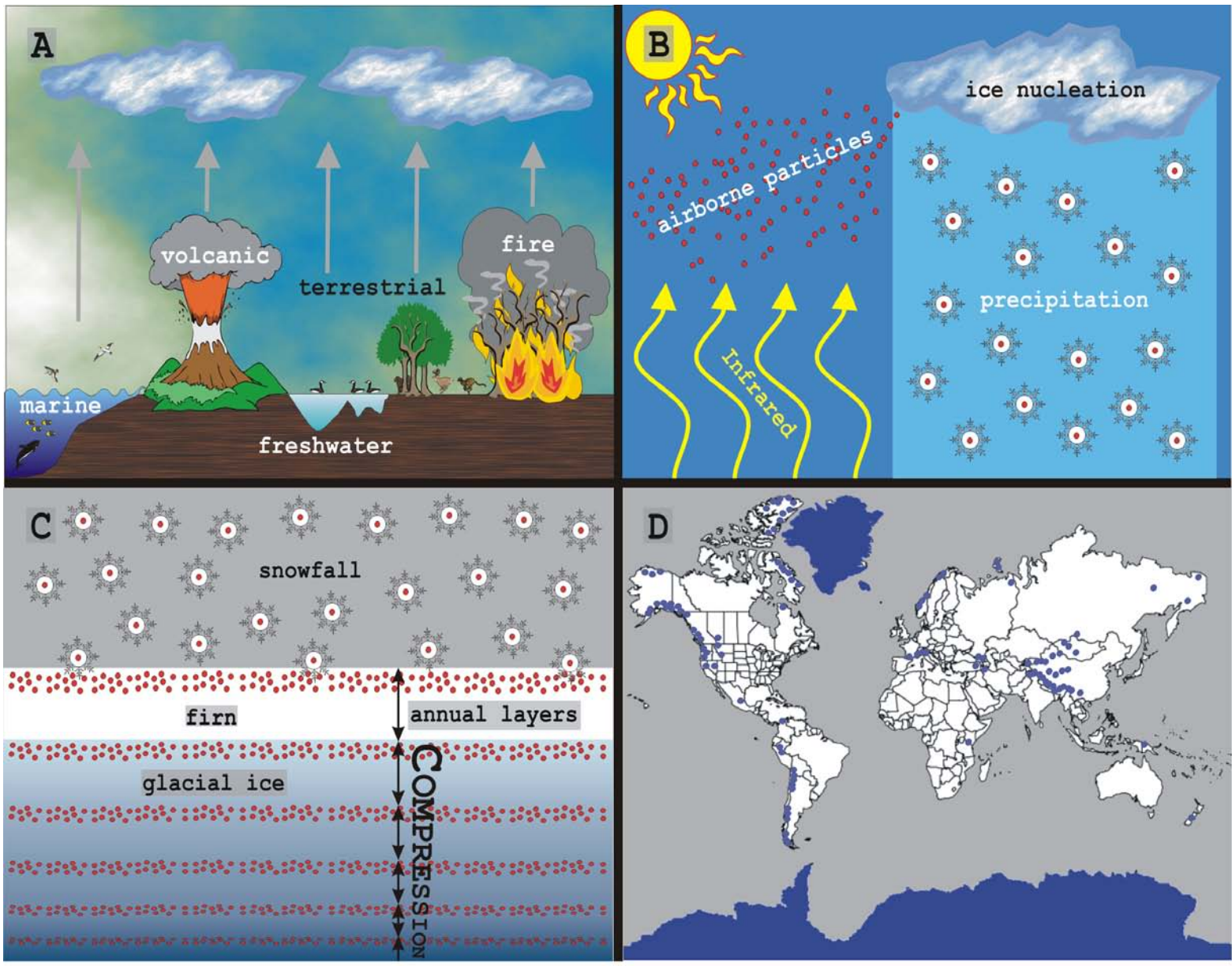

A
volcanic
terrestrial
fire
marine
freshwater
B
ice nucleation
airborne particles
precipitation
Infrared
C
snowfall
firn
annual layers
glacial ice
COMPRESSION
D

Figure 1

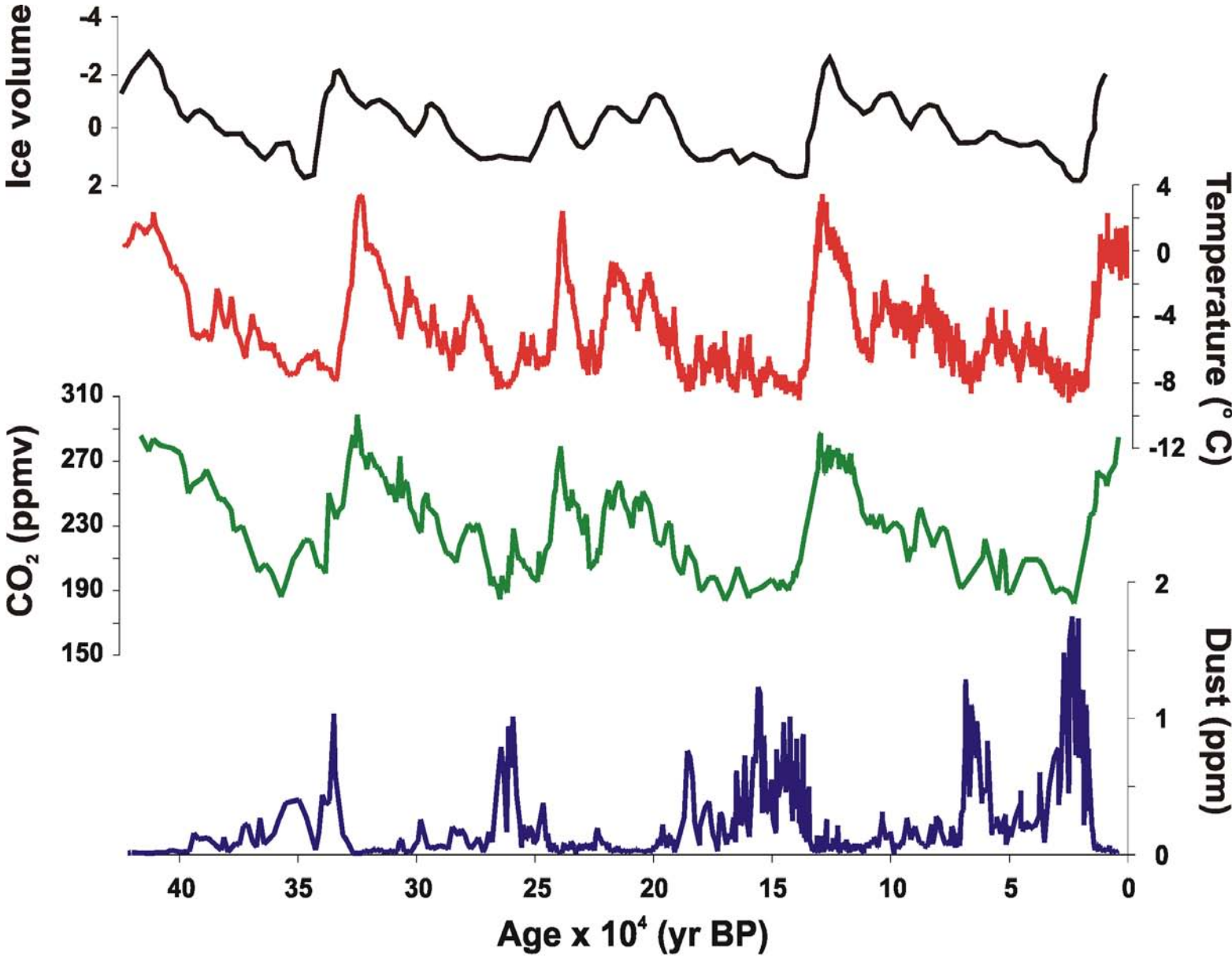

Ice volume
-4
-2
0
2
Temperature (° C)
4
0
-4
-8
-12
CO2 (ppmv)
310
270
230
190
150
Dust (ppm)
2
1
0
40
35
30
25
20
15
10
5
0
Age x 10^4 (yr BP)

Figure 2

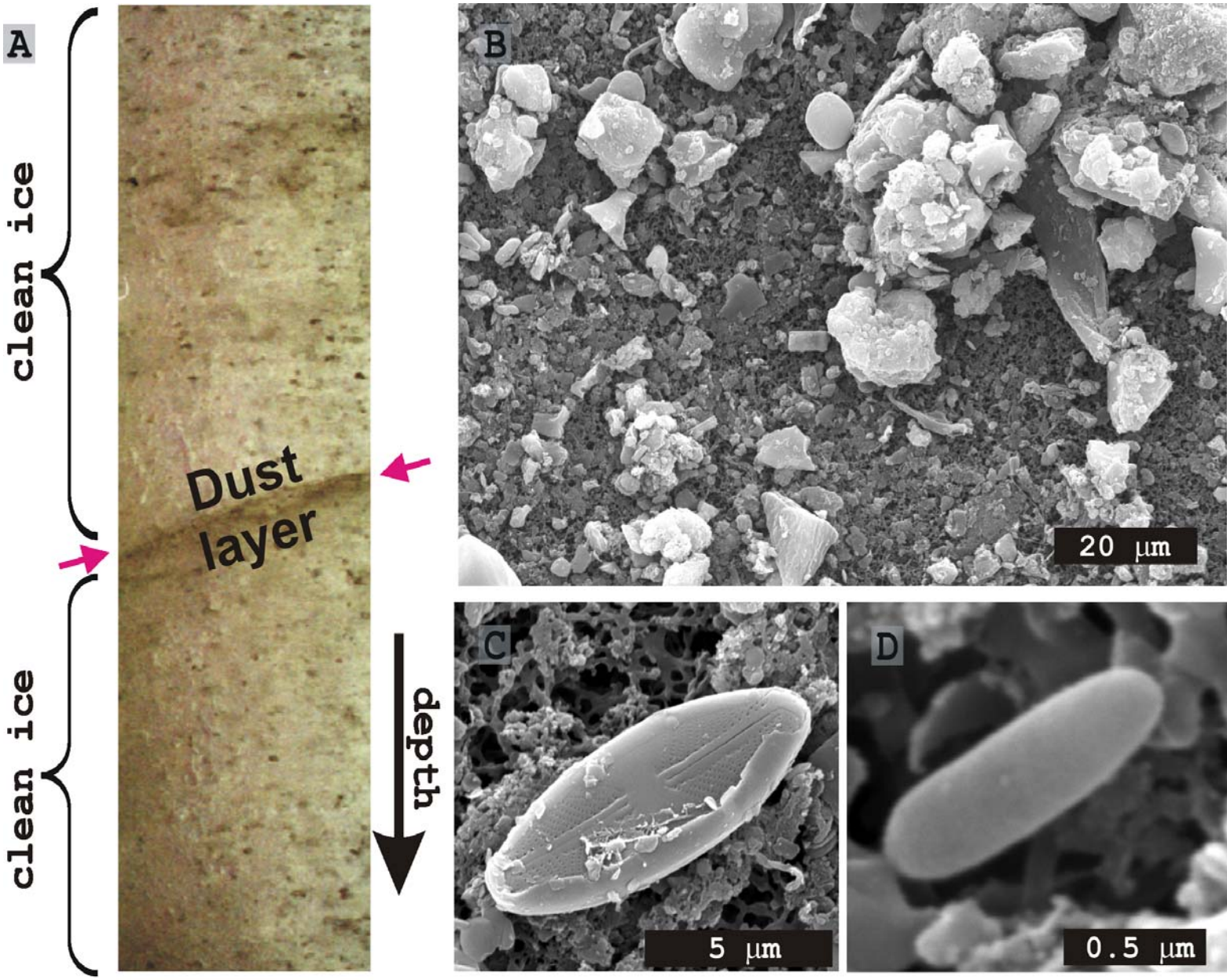
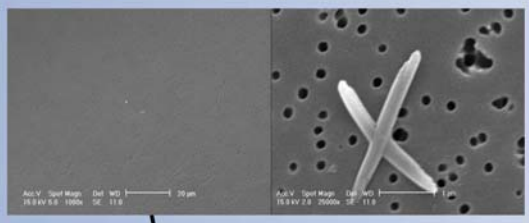


Figure 3

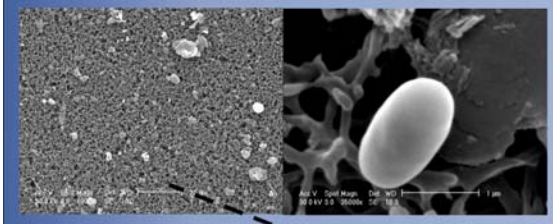
Biological Material in Ice

Polar Regions

Lake Vostok, Antarctica

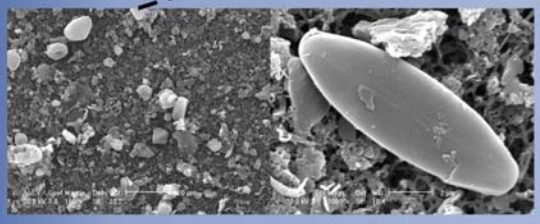


Taylor Dome, Antarctica

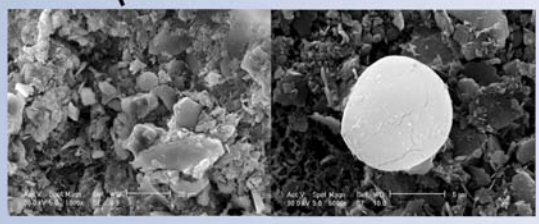


NGRIP Summit, Greenland

Non-Polar Regions



Sajama Ice Cap, Bolivia



Guliya Ice Cap, China

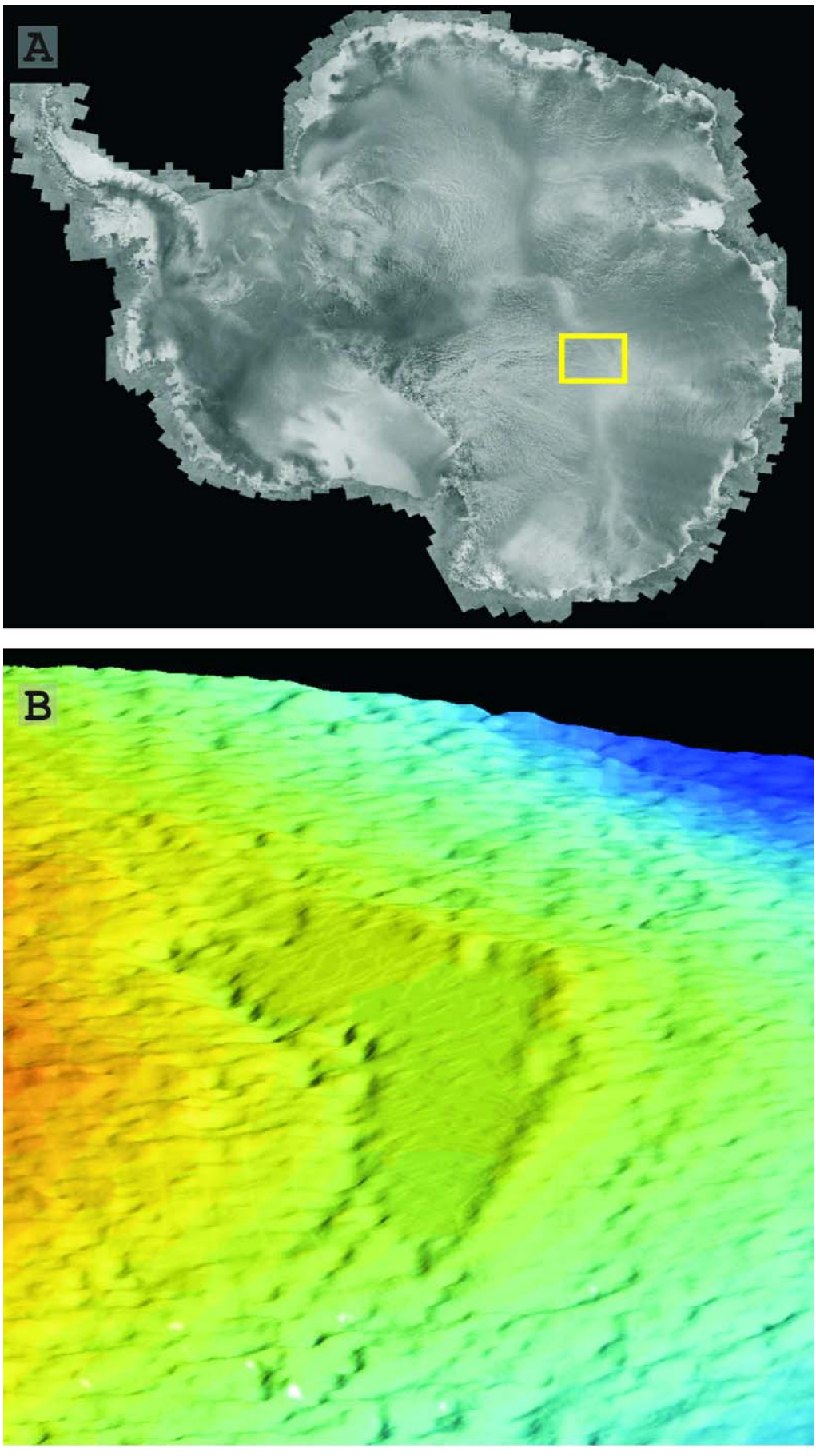


Figure 5

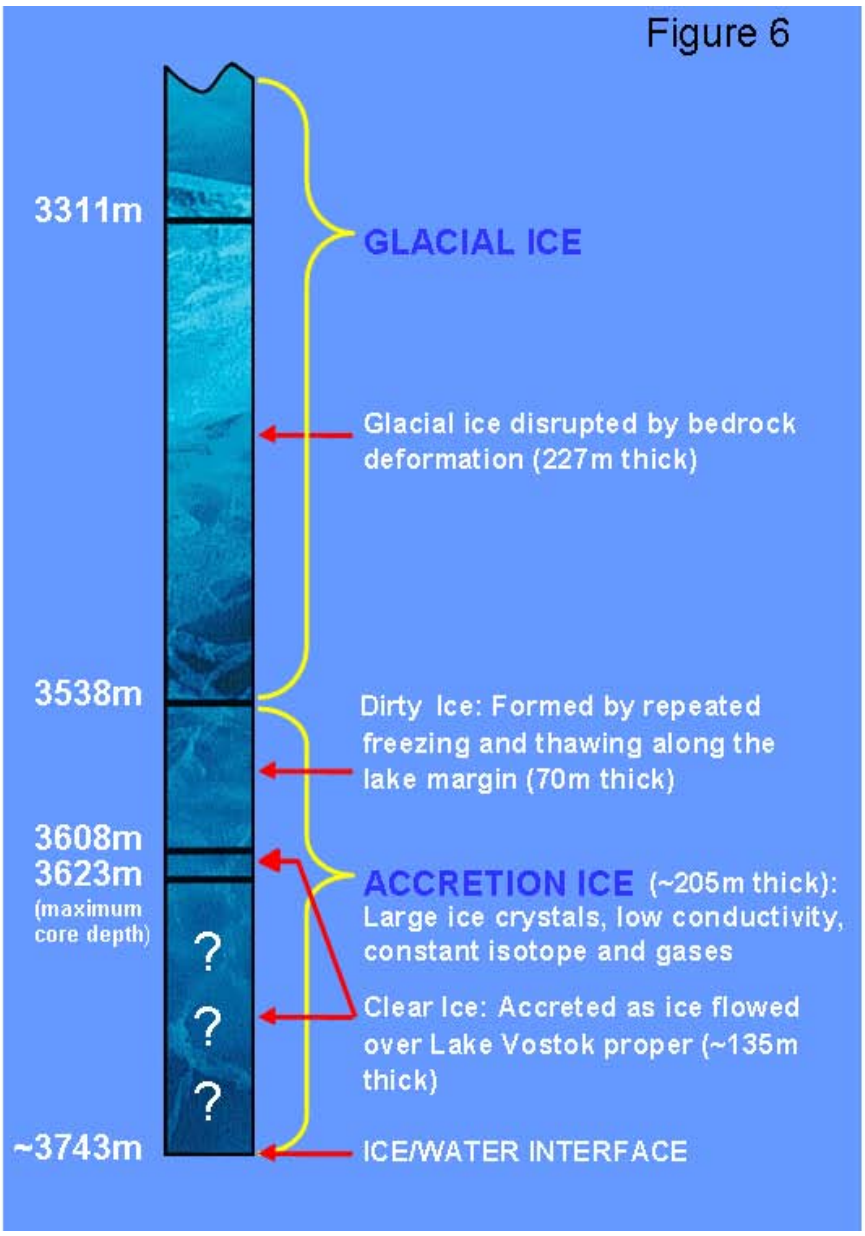


Figure 6
3311m
GLACIAL ICE
Glacial ice disrupted by bedrock deformation (227m thick)
3538m
Dirty Ice: Formed by repeated freezing and thawing along the lake margin (70m thick)
3608m
3623m
(maximum core depth)
ACCRETION ICE (~205m thick): Large ice crystals, low conductivity, constant isotope and gases
?
?
?
Clear Ice: Accreted as ice flowed over Lake Vostok proper (~135m thick)
~3743m
ICE/WATER INTERFACE

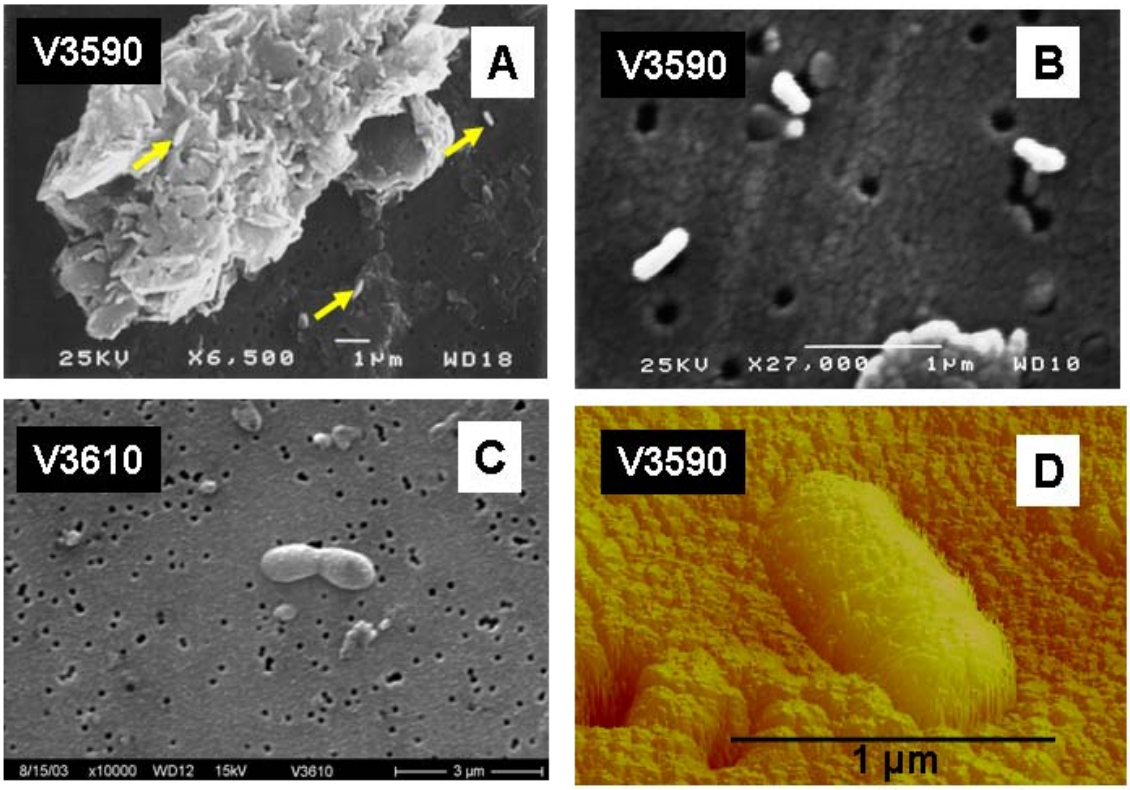
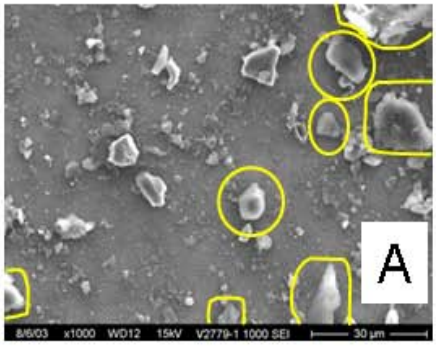
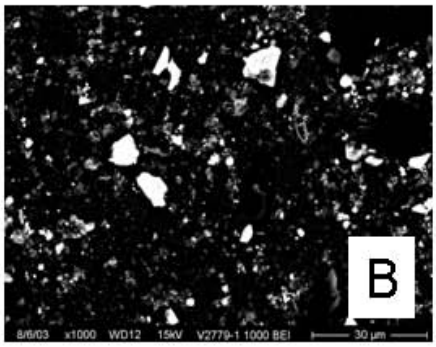
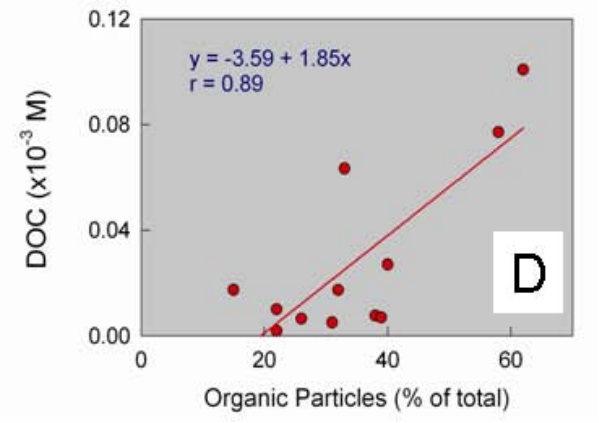
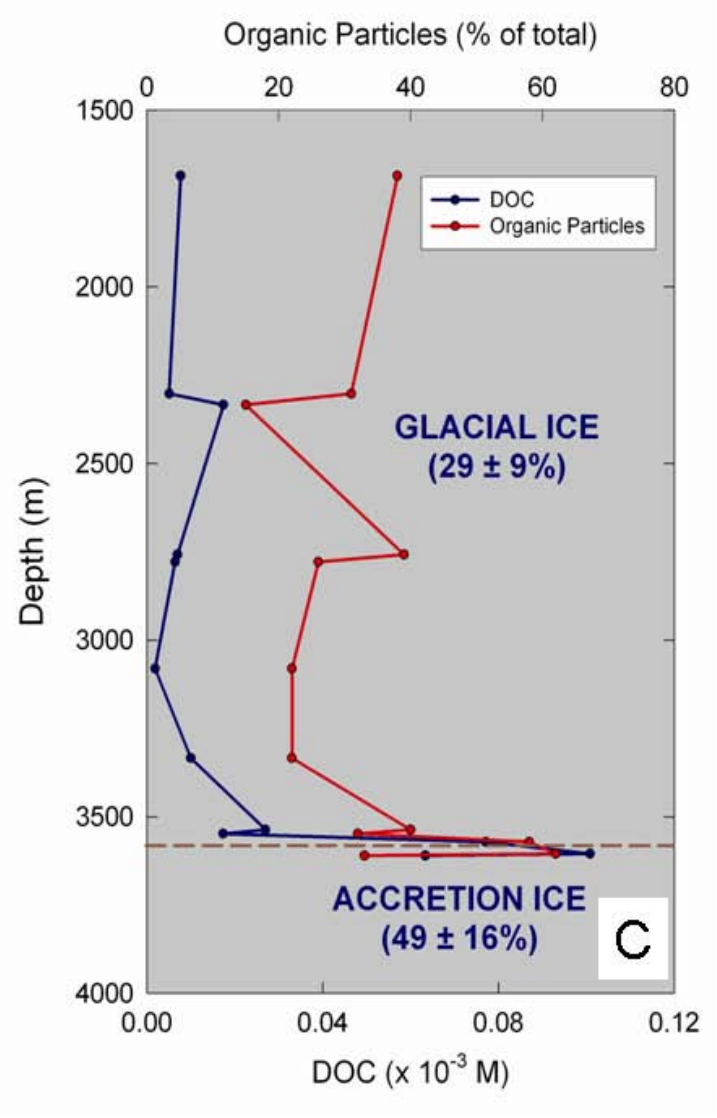

V3590
A
25KV X6,500 1µm WD18
V3590
B
25KV X27,000 1µm WD10
V3610
C
8/15/03 x10000 WD12 15kV V3610 3 µm
V3590
D
1 µm

Fig 8


A


B


y = -3.59 + 1.85x
r = 0.89
DOC (x10^{-3} M)
Organic Particles (% of total)
D


Organic Particles (% of total)
DOC
Organic Particles
GLACIAL ICE
(29 ± 9%)
ACCRETION ICE
(49 ± 16%)
Depth (m)
DOC (x 10^{-3} M)
C

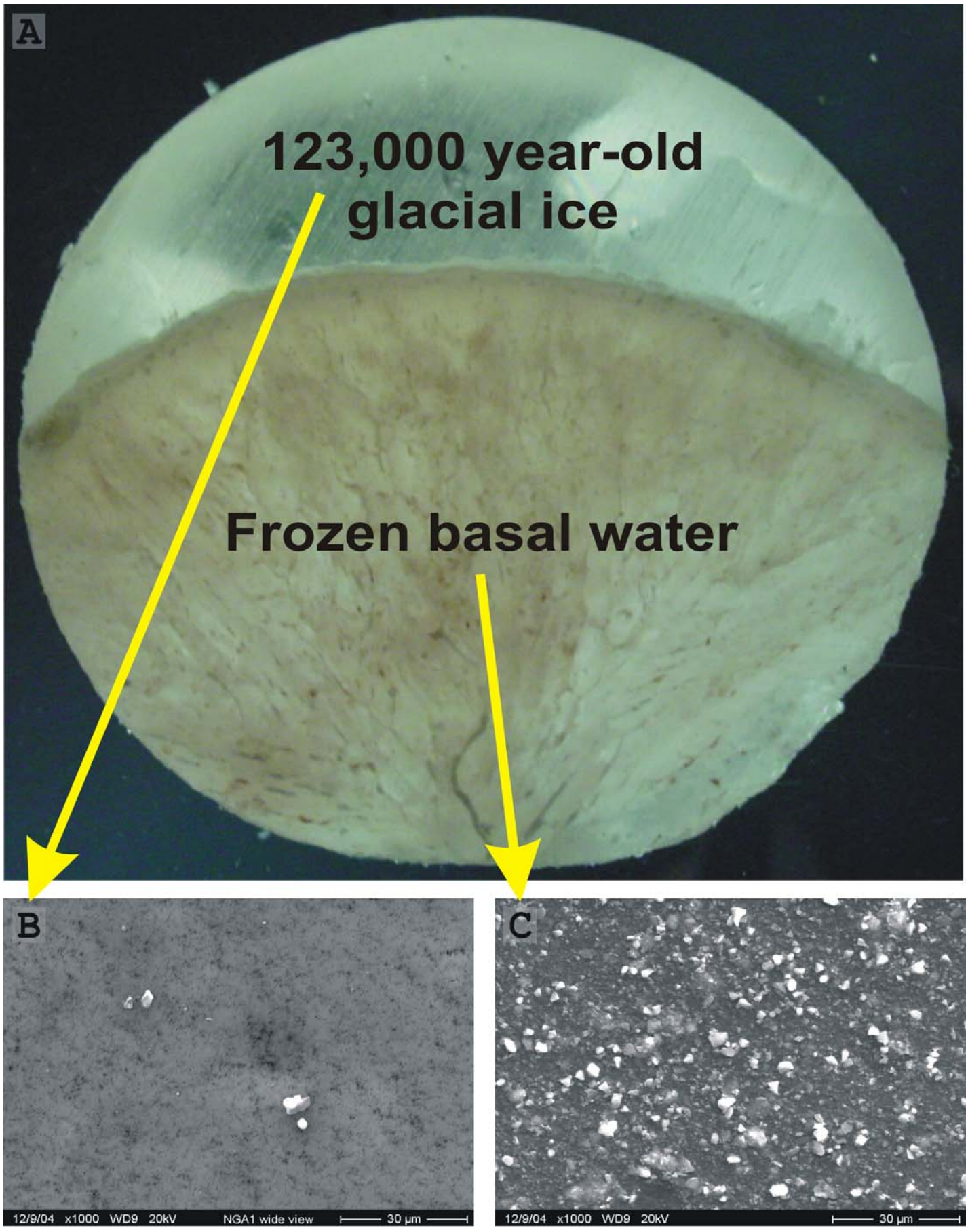

A
123,000 year-old
glacial ice
Frozen basal water
B
12/9/04 x1000 WD9 20kV NGA1 wide view 30 µm
C
12/9/04 x1000 WD9 20kV 30 µm

Figure 9

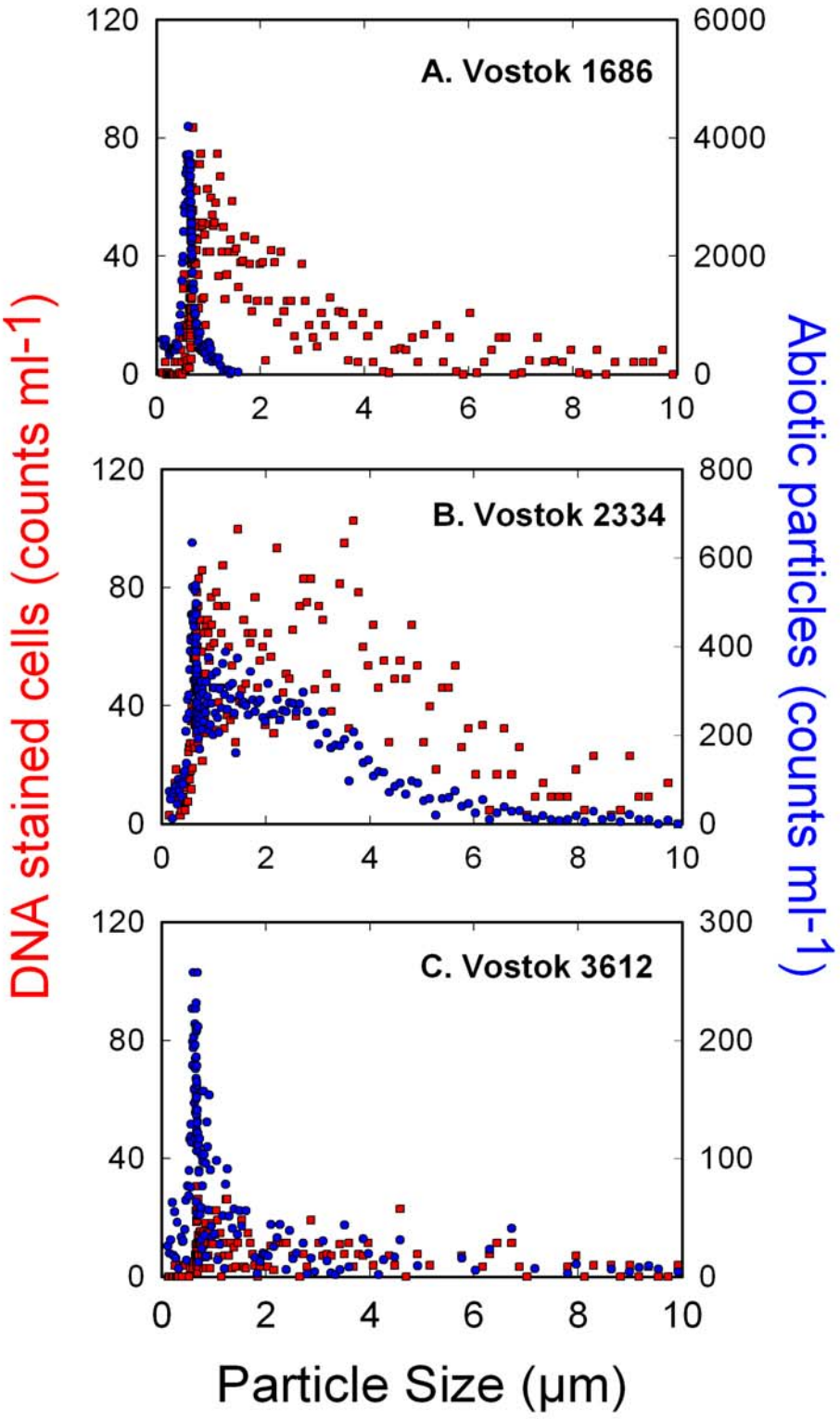
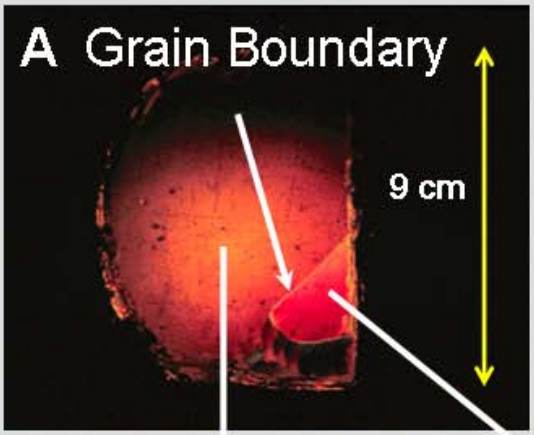
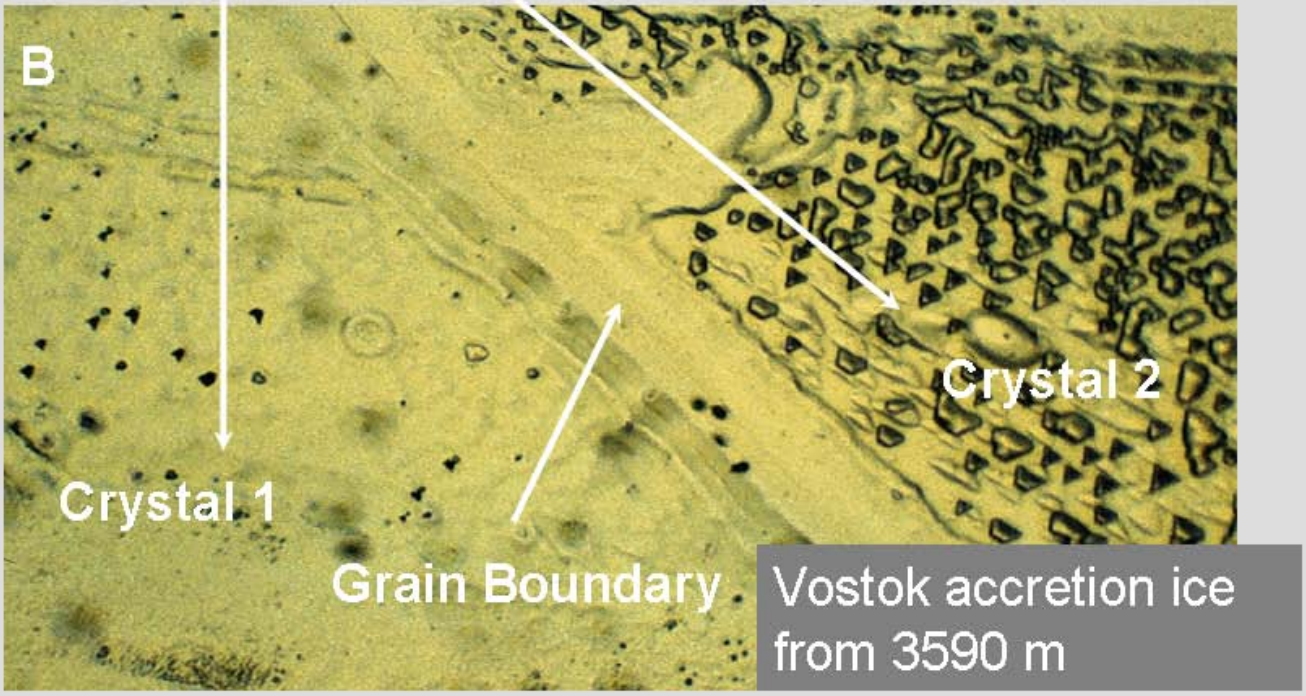

A. Vostok 1686
B. Vostok 2334
C. Vostok 3612
DNA stained cells (counts ml-1)
Abiotic particles (counts ml-1)
Particle Size (µm)
120
80
40
0
6000
4000
2000
800
600
400
200
300
100
0
2
4
6
8
10

Figure 10

Fig. 11



The grain boundaries between crystal grains in environmental ice may provide a viable liquid habitat for microbes.



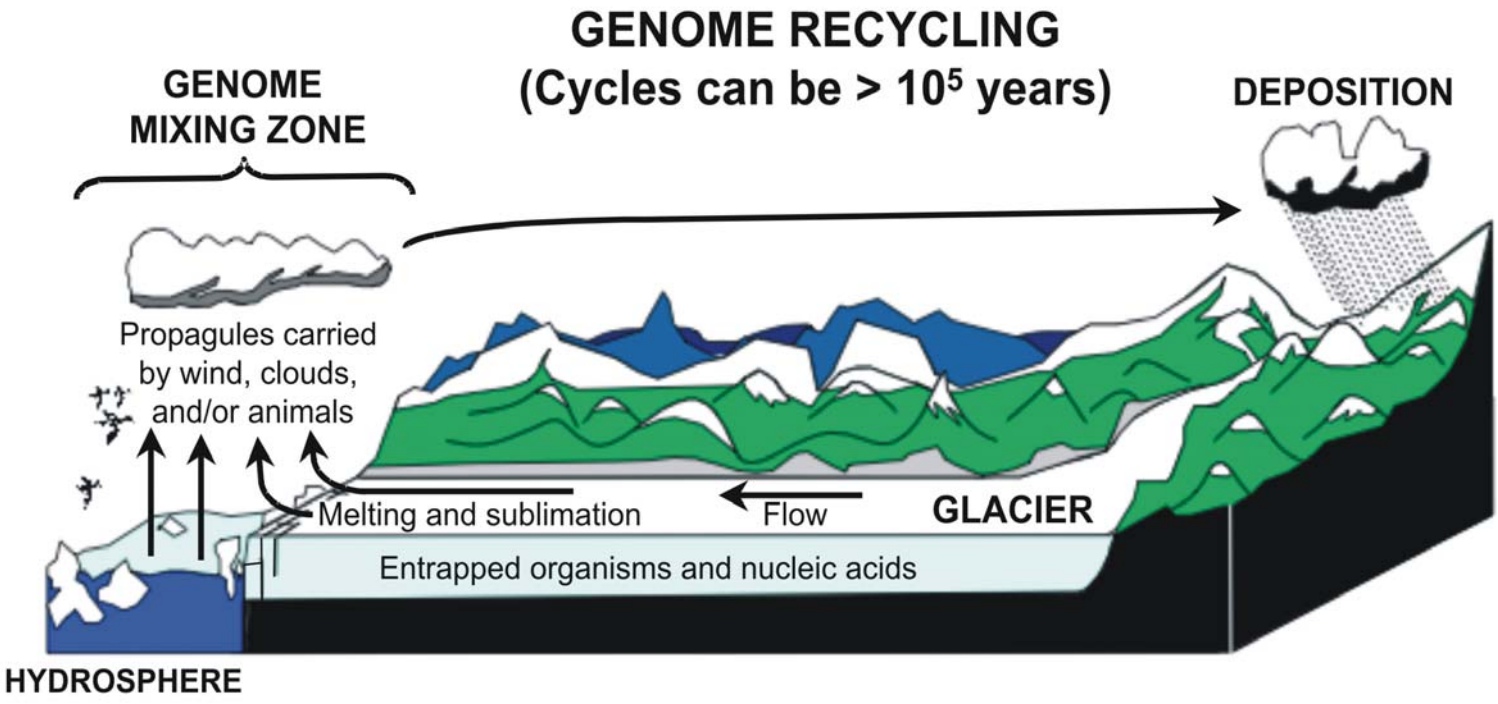
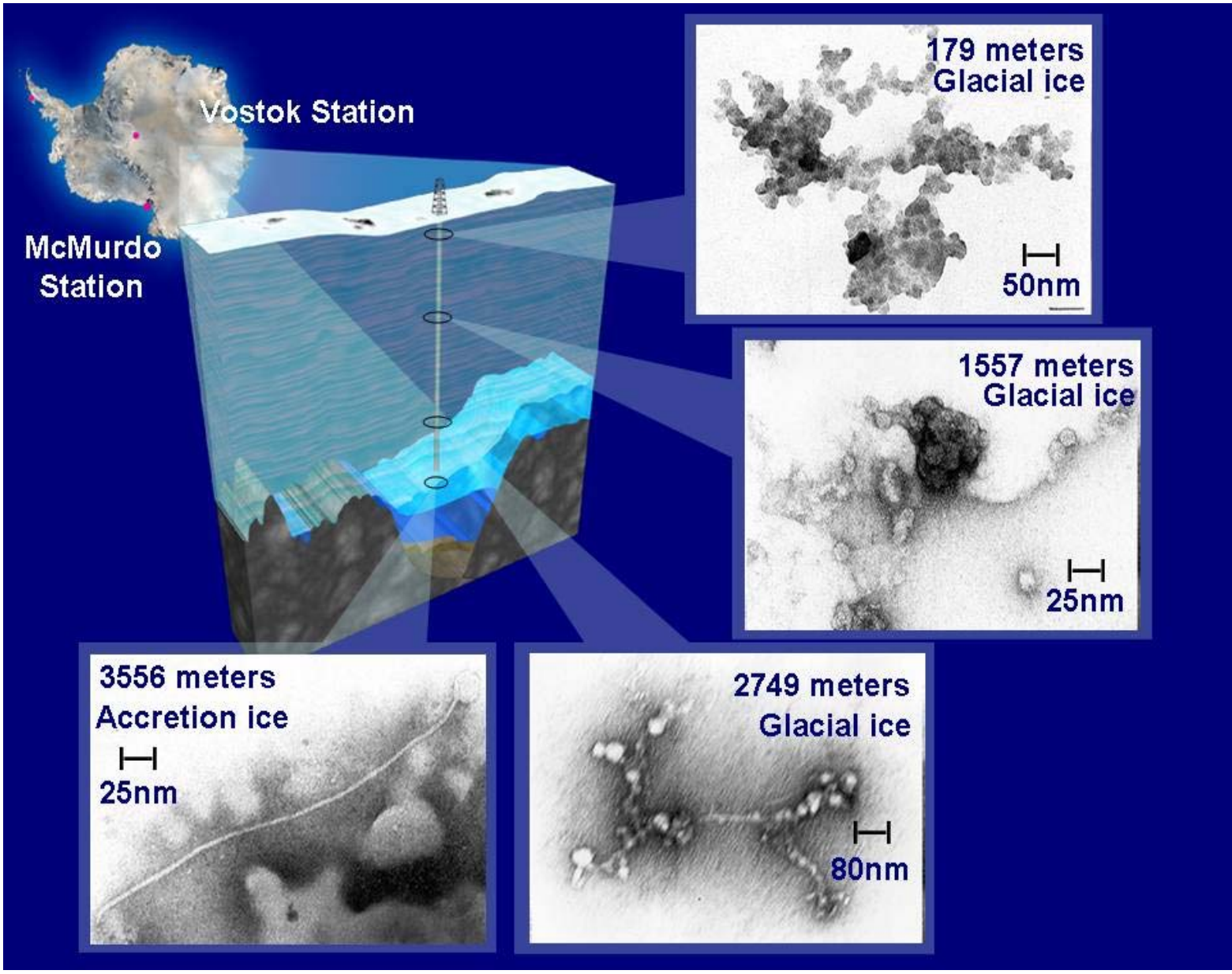

GENOME RECYCLING
(Cycles can be > 10^5 years)
GENOME
MIXING ZONE
DEPOSITION
Propagules carried
by wind, clouds,
and/or animals
Melting and sublimation
Flow
GLACIER
Entrapped organisms and nucleic acids
HYDROSPHERE

Figure 12


Vostok Station
McMurdo Station
179 meters
Glacial ice
50nm
1557 meters
Glacial ice
25nm
3556 meters
Accretion ice
25nm
2749 meters
Glacial ice
80nm