

Новости с **озера Восток** в Антарктиде

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indescribable

Elusive **microbial life** at the
uppermost water layer in
subglacial **Lake Vostok**,
East Antarctica

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***Russian-French GDRI consortium**

RUB from RFBR, RosHydroMeth

GDRI consortium

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- AARI FSBI, St Petersburg, **RU**
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 - Valery Lukin – logistics
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 - German Leitchenkov
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 - Philippe Normand

INMI, Moscow

LTHE, FR
Jean Martins

Culturing
Microscopy

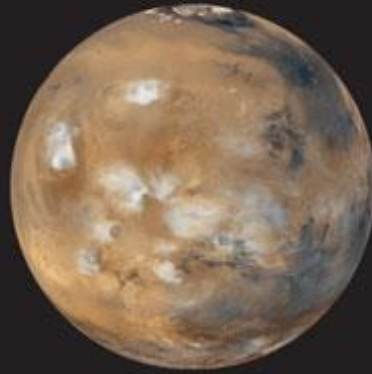
Cell counts

Repetition
Culturing

Life around us?



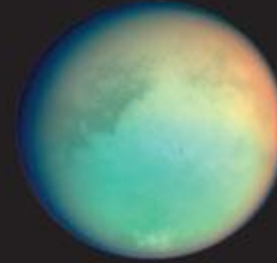
Earth



Mars



Ganymede



Titan



Europa



Enceladus

Searching for extraterrestrial life is one of the aims of astrobiology, and there are several potentially habitable worlds in the Solar System, in addition to Earth. Two of Jupiter's moons ([Ganymede](#) and [Europa](#)) and two of Saturn's moons ([Titan](#) and [Enceladus](#)) harbour **liquid-water oceans**. And [Mars](#) probably had vast liquid-water environments during much of its early history.

Lazcano 2012 Nature 488: 160-1

Pinheiro et al 2012 Science 336_341-344 **Six new xeno-nucleic acids (XNAs) capable for Darwinian evolution and folding into defined structures**

Anhydrohexitol nucleic acid, or HNA amongst others: CeNA, LNA, ANA, FANA and TNA

Working on **Earth-known** microbial **DNA/RNA**

4 primary nucleotides based on:
cytosine, guanine, adenine and
thymine - C, G, A, T

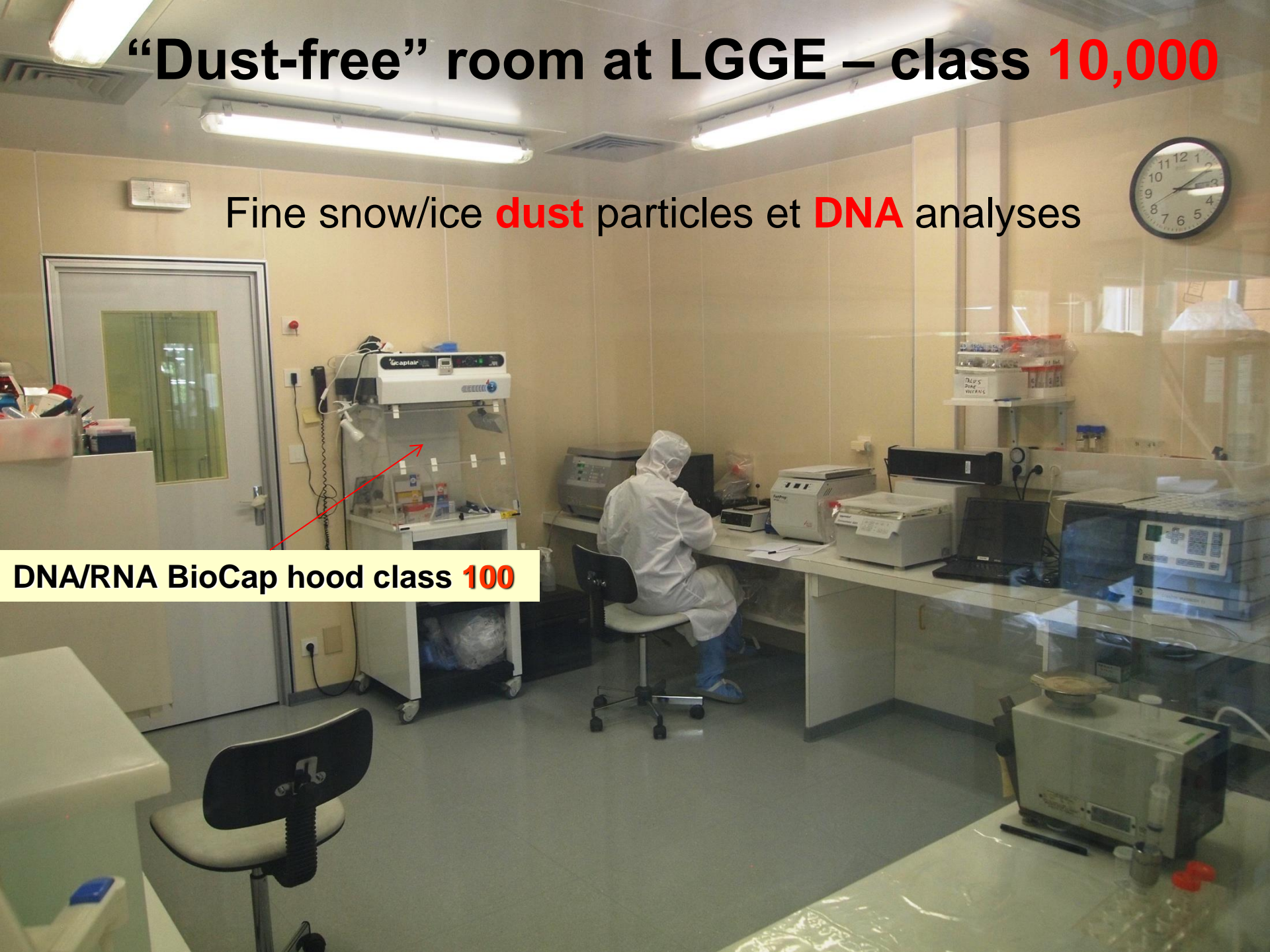
GenBank sequence database

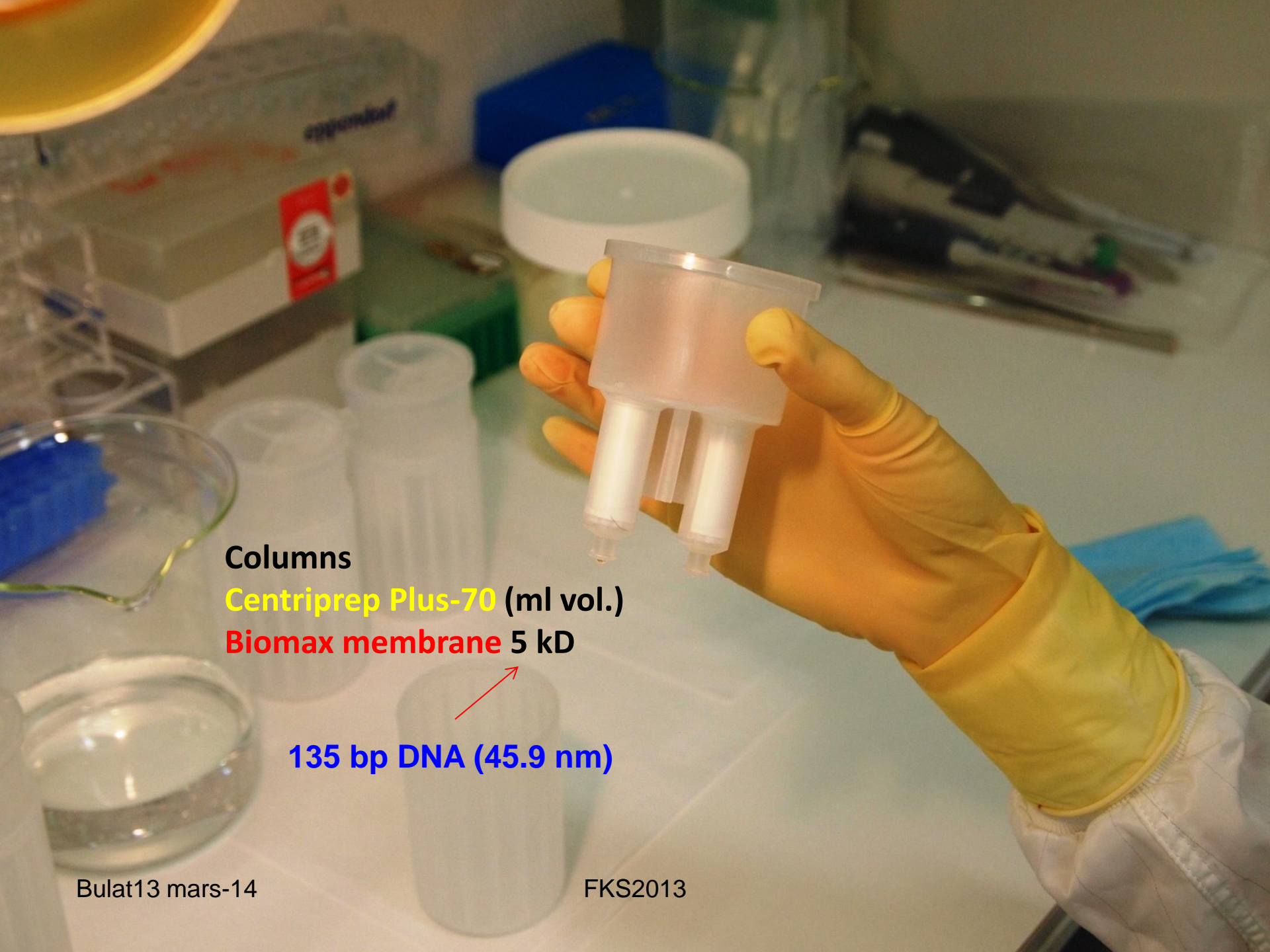
“Dust-free” room at LGGE – class 10,000

Fine snow/ice **dust** particles et **DNA** analyses



DNA/RNA BioCap hood class 100



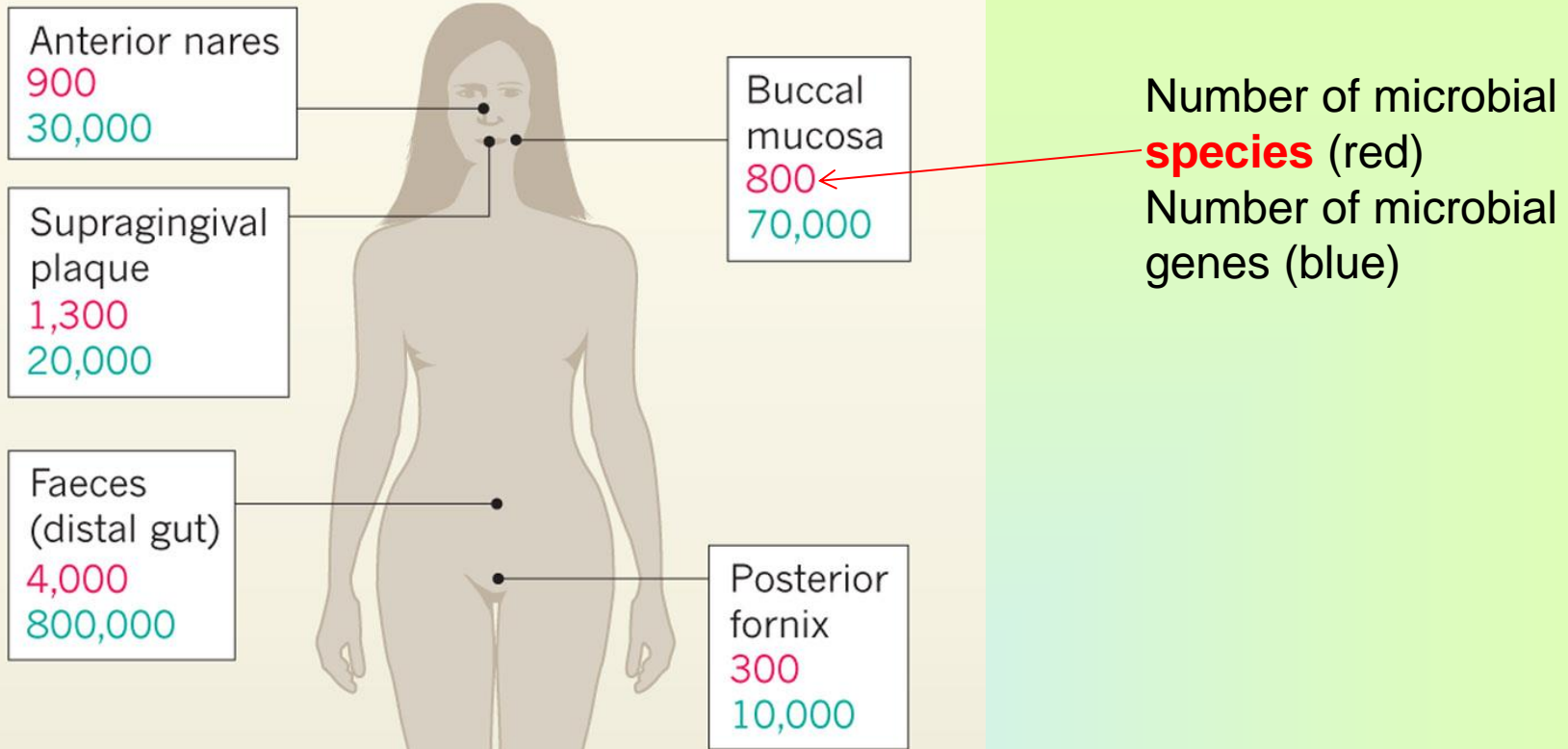


Columns

Centriprep Plus-70 (ml vol.)

Biomax membrane 5 kD

135 bp DNA (45.9 nm)



**Microbial inhabitants
outnumber our body's own
cells by about ten to one**

Vostok **ice** for Biology

REQUIREMENTS:

- Comprehensive **Biological controls**

- Sham/mock DNA extraction
- Negative PCR
- Ice core wash water
- Lab Environment (dust)
- Vostok drill fluid
- Outer-core (optionally)

235 seqs Feb 2013

**Contaminant
databases**

Vostok **ice** for Biology

REQUIREMENTS:

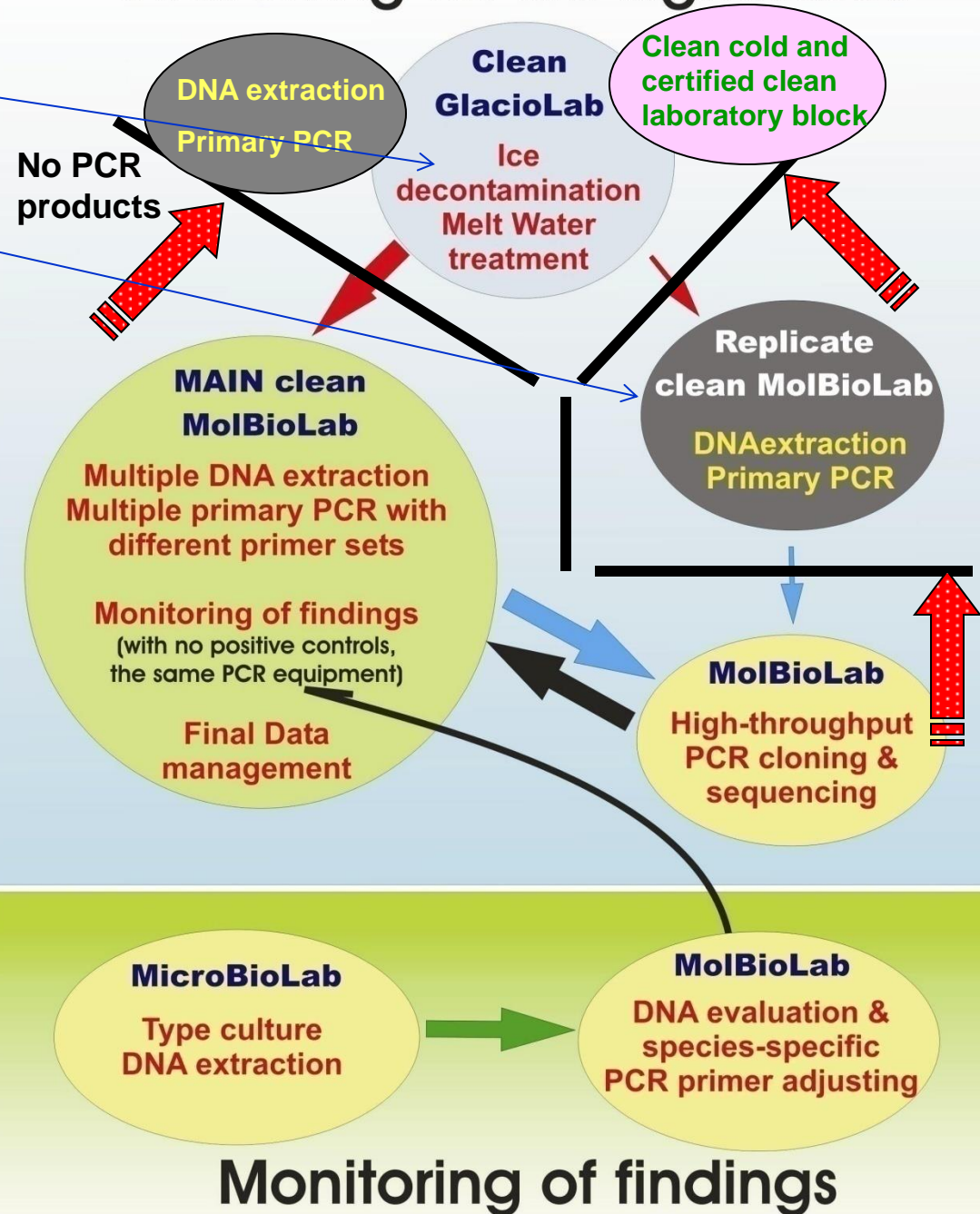
- **Authentication** of findings **by ecology**
(environmental conditions)

*“Everything is everywhere,
but, the **environment selects**”
– Baas Becking, 1934*

Searching for life signatures

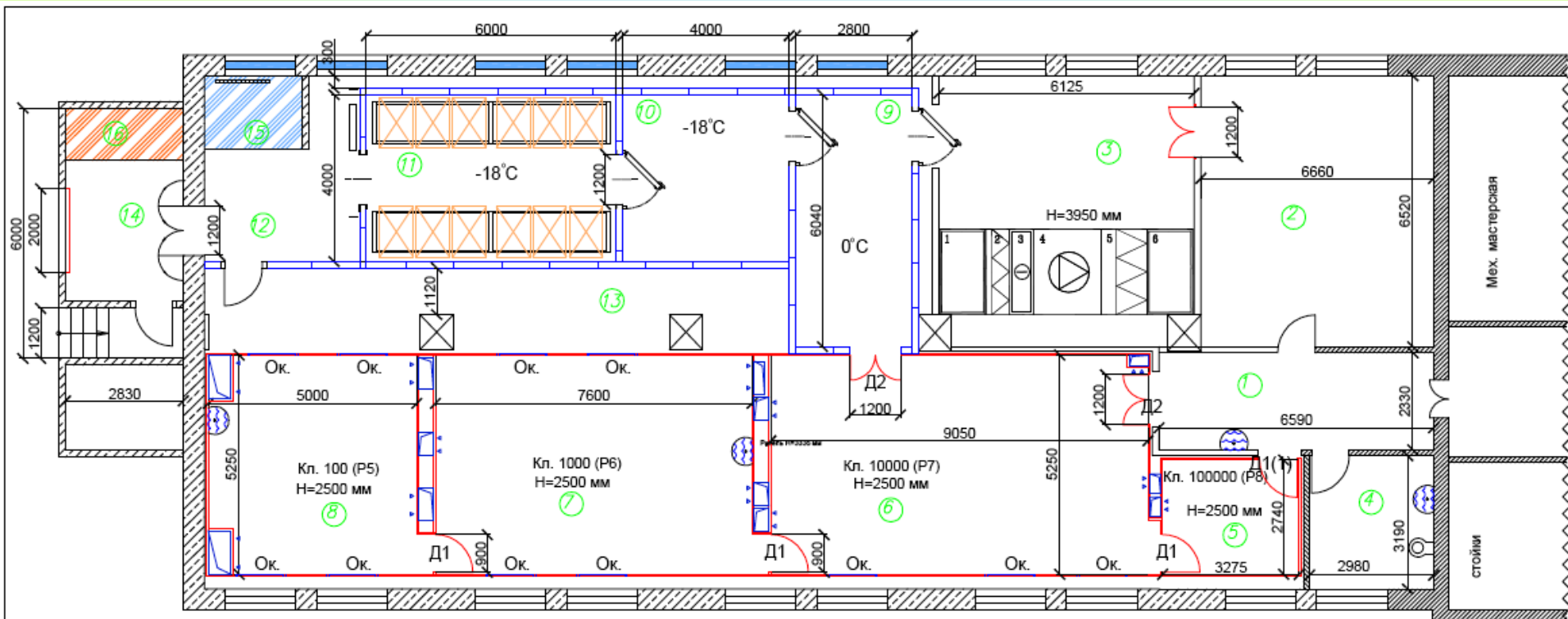
Gatchina – PNPI?

Dubna – JINR?



A **framework** for biological studies adopted for Lake Vostok

PNPI NRC Kurchatov Inst Gatchina clean et cold rooms astrobiology laboratory





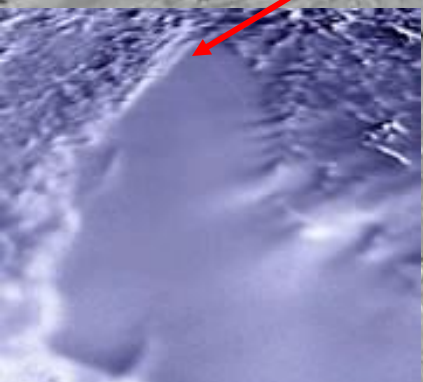
Vostok station (+3488m)

(78°S, 106°E)

(since 1957)

67 kPa – 67% oxygen

1260 km from
the coast



Absolute minimum -89.2°C
Average ann. Temp. -55.1°C

Lake Vostok (-3663m)

RADARSAT, CSA

Vostok SURFACE

SNOW

Vostok Surface snow

(0 – 130m up to 4 Kyr)

Below 3m the snow is pristine

Very clean 'life-hostile' environ

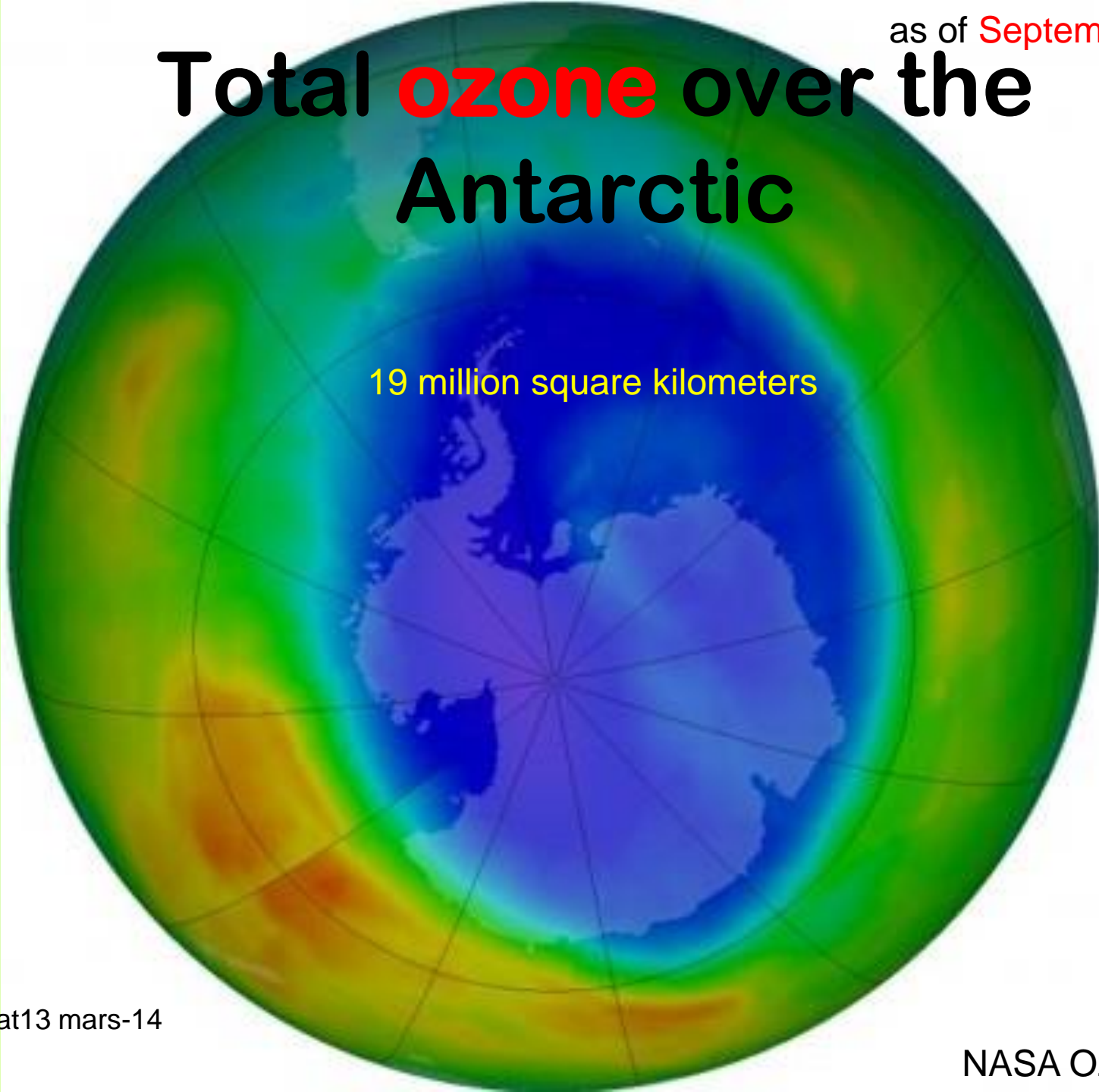
NO FREE (only BOUND) WATER!

- Low accumulation – **2.1-2.4g /cm²/a**
- Low Aeolian dust input
– **2 μm** mode; **10-15** ppb (summer time) → (6-6.5 cm/a – snow / needles!)
- Harsh **irradiation (UV-B, C etc.)** ('ozone hole' – Aug-Nov)
 - + Water-Oxygen → **free radicals**
 - **Oxidized organics** (photochemical reactions at ice-air interface)
- Low **freezing temperatures** – below **-36°C** (**-55.1°C** ann.)

as of September 11, 2012

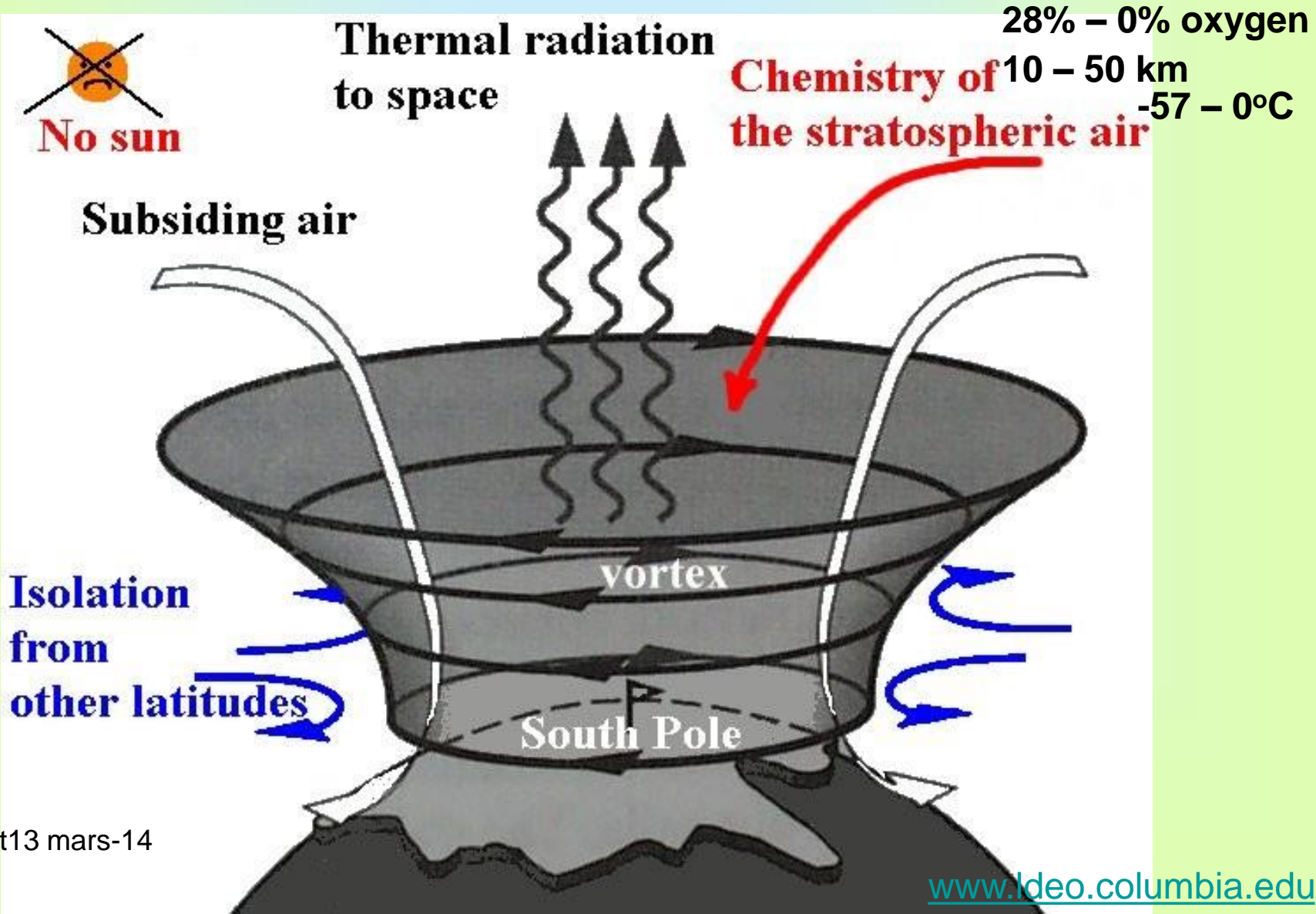
Total ozone over the Antarctic

19 million square kilometers



May – August
at Vostok

Antarctic **polar vortex** effect



Vostok MEGA- et Colossal- snow 2011

Snow **drifts** by wind for max. **10 km** distance

Отбор проб снега для геохимических и биологических исследований

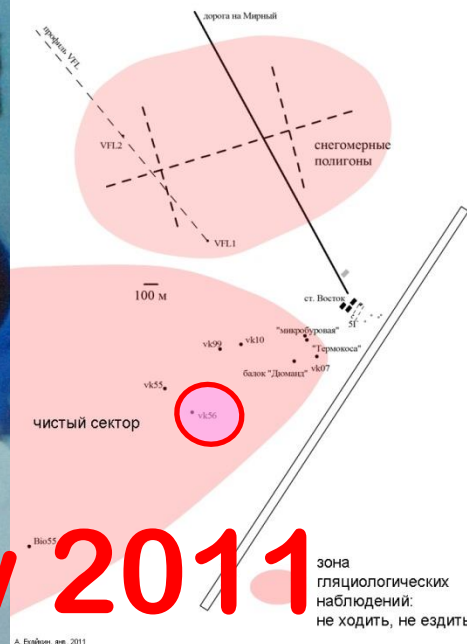
CS11 (78,4722S, 106,7796E)
(VostokColossalSnow2011 (RAE 56))

Программа исследований 3-м шурфа:

1. Стратиграфия
2. Космическая пыль (20-см)
3. Биология (20-см)
4. ^{10}Be (3-см)
5. Основные ионы (3-см)
6. Дейтерий и кислород-18 (3-см)
7. Бета-радиоактивность (5-см)

OK!

Trouble!



Vostok Colossal-snow 2011

Courtesy of A. Ekaykin et al (RAE56)

• **216km** from coastal Progress – **PMS11-1**

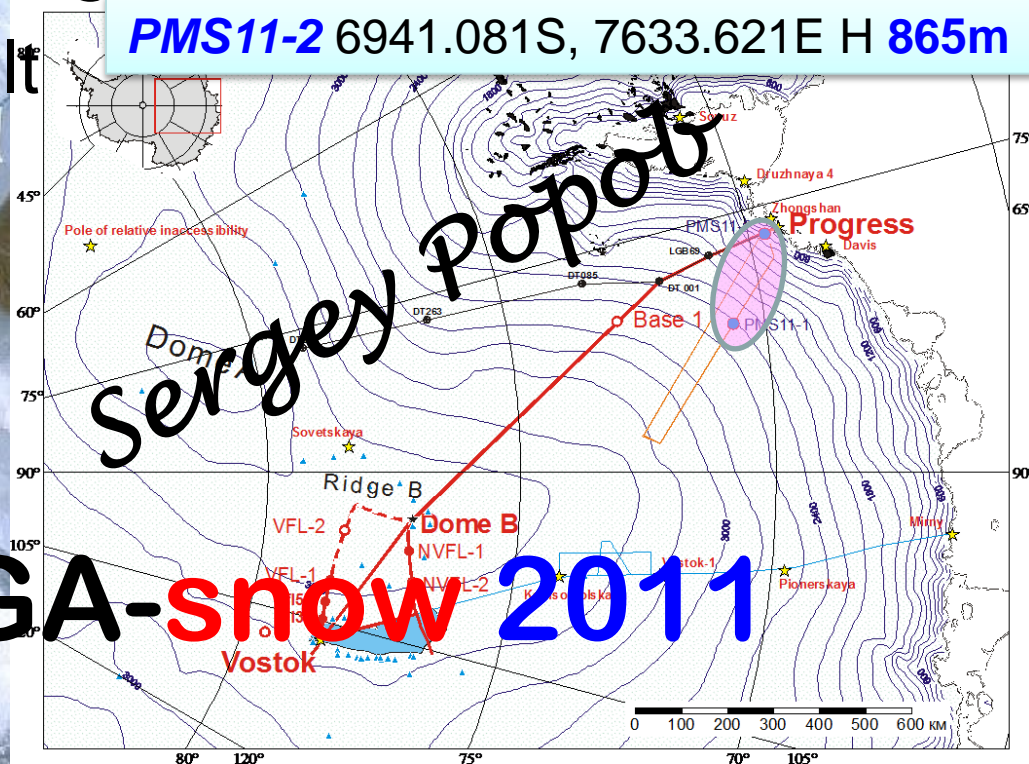
– Crate 23L → **8.6L** melt

PMS11-1 7034.925S, 8124.103E H **2376m**

• **29km** from coastal Progress – **PMS11-2**

– Crate 23L → **7.6L** melt

PMS11-2 6941.081S, 7633.621E H **865m**



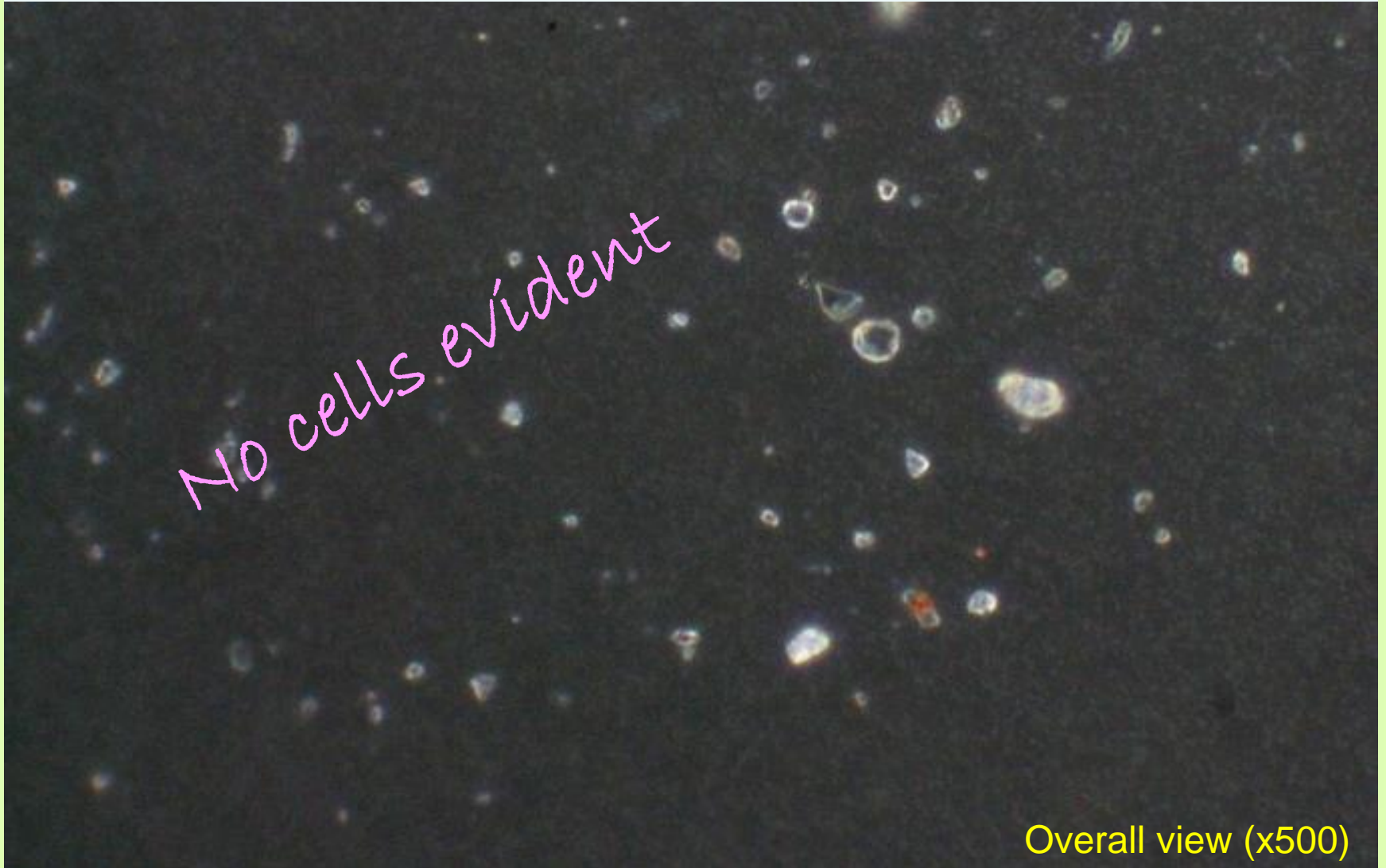
Vostok MEGA-snow 2011

Vostok MEGA- et Colossal- snow 2011 - RESULTS

Dust particle counts

Sample	N/g	M/g (ppb)	Masse(0.8-2)	Masse (2.2-5)	Mass >5
Vivendi ELGA-26	1347	7.63	2.79	4.30	0.00
Ultra-pure water	577	1.45	1.45	0.00	0.00
	3549	9.2	7.28	1.17	0.00
PMS11-1	4821	12.14	9.09	2.27	0.00
216 km Progress	5375	16.19	10.45	4.11	0.00
PMS11-2	4933	14.6	10.22	3.29	0.00
29 km Progress	4241	11.8	8.80	2.00	0.00
	3811	10.7	7.90	1.86	0.00
CS11-15 Vostok	3643	11.94	7.52	3.41	0.00
2.8-3.0 m deep	3754	13.13	7.93	4.07	0.00

CS11-15 – light microscopy
OLYMPUS BH-2



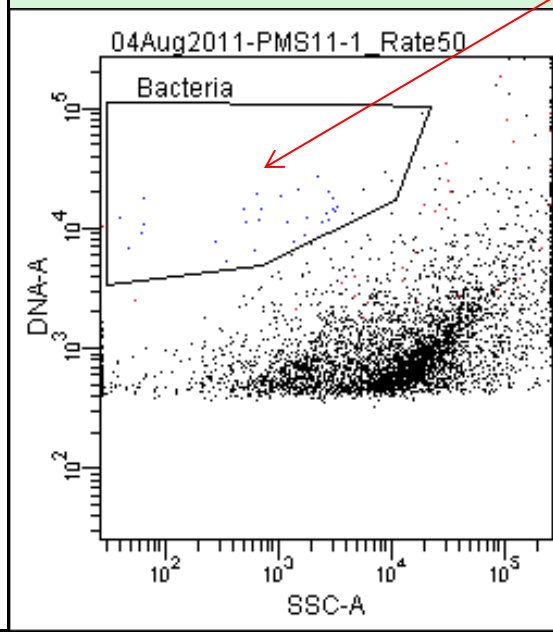
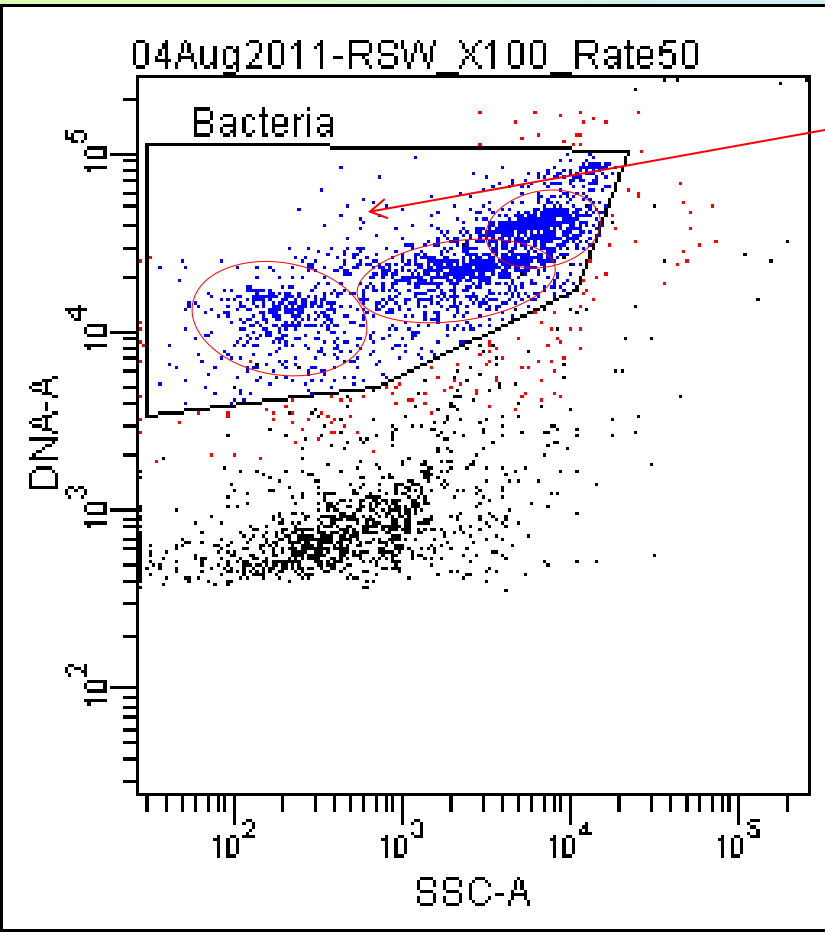
No cells evident

Overall view (x500)

Vostok MEGA- et Colossal- snow 2011 - RESULTS

Cell counts

Sample	Bacteria / ml
Roscoff sea water	30961
PMS11-1 - 216 km Progress	45
PMS11-2 - 29 km Progress	0
CS11-15 Vostok - 2.8-3.0 m deep	0



Vostok MEGA- et Colossal- snow 2011 - RESULTS

PCR - phylotypes

Sample	Bacteria / ml
PMS11-1 - 216 km Progress	45

V4-v8 16S rDNA amplicon sequencing →

HA-Pseudomonas sp (*alpha-proteo*) - 80%

99% *Ewingella americana* (*gamma-proteo*, Enterobact.)

Bradyrhizobium sp(*elkani*) (*alpha-proteo*)

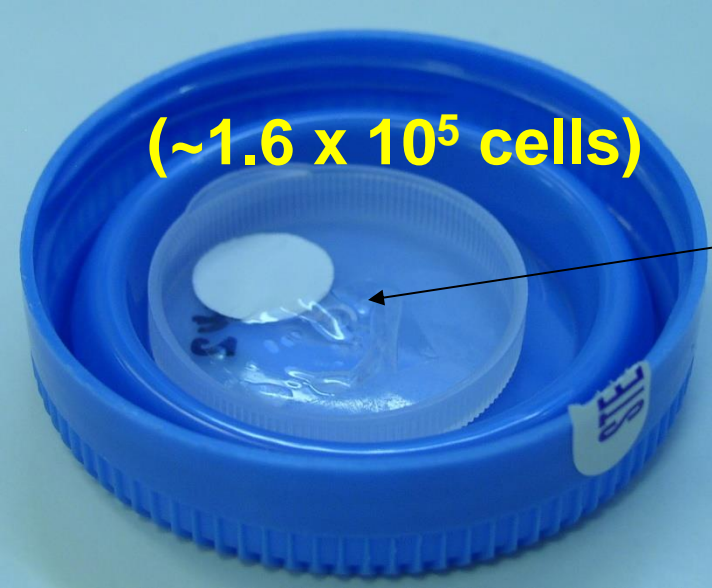
Clinical specimens including wound,
sputum, urine, stool, blood etc.
Sources of infection – domestic air
conditioning units, ice baths etc.

Pre CONCLUSION

- Vostok [even 29km from coast!] **SURFACE snow** contains a **few(?) cells / ml** - **NO CELL populations** [lifeless]

Background contamination?

Vostok SURFACE solar RADIATION



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Summer (January) 2008:
exposure during one month

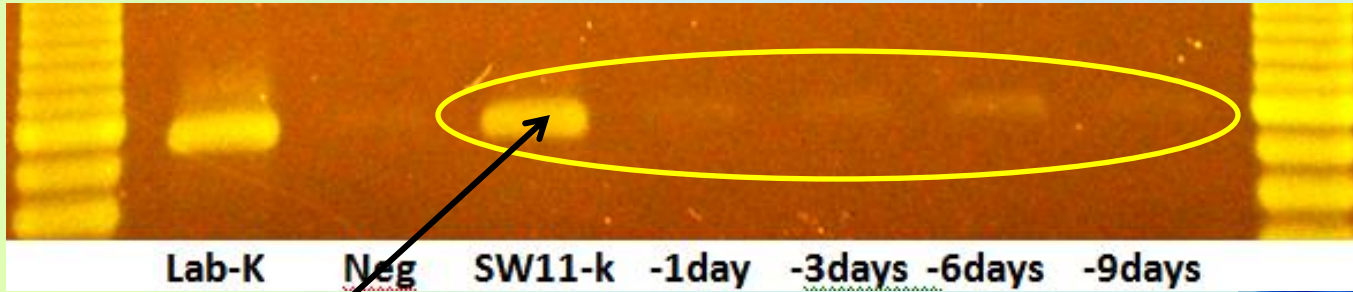
BIO-EXPOSURE 2011



Bulat13 mars-14

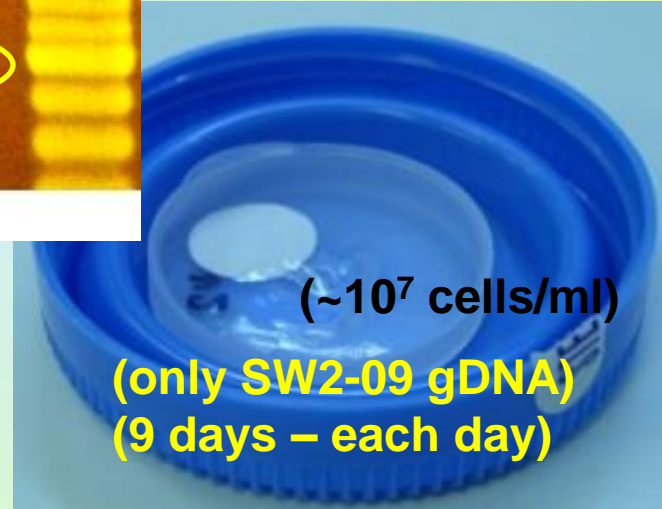
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BIO-EXPOSURE 2011



No exposure at Vostok

RESULTS



No signals
(v4-v8^{890bp}, v3-v5^{590bp}, v6^{170bp} reg.
16S rDNA) even **in one[1st] day!**

CONCLUSION

**Intensive solar (UV-B?)
radiation**

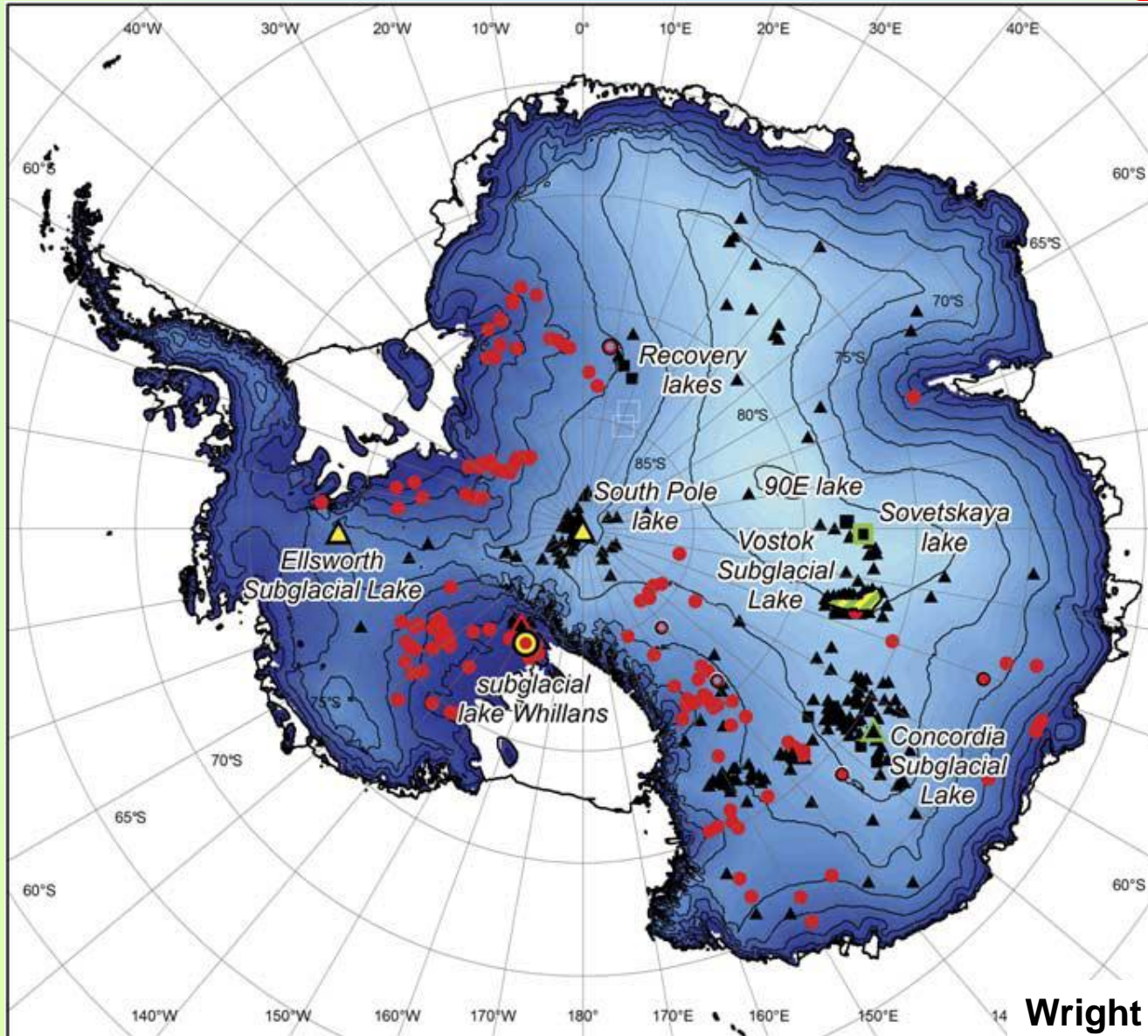
as effective cell/DNA 'killing' factor?

Arctic et Antarctic **PERMAFROST**
bioExposure trial in a progress

Vostok **ICE CORE**

Towards the subglacial Lake
Vostok

SALE Subglacial Antarctic Lake Environments - 379





Lake Vostok

known since **1994**

Published in *Nature* (Kapitsa et al., **1996**)

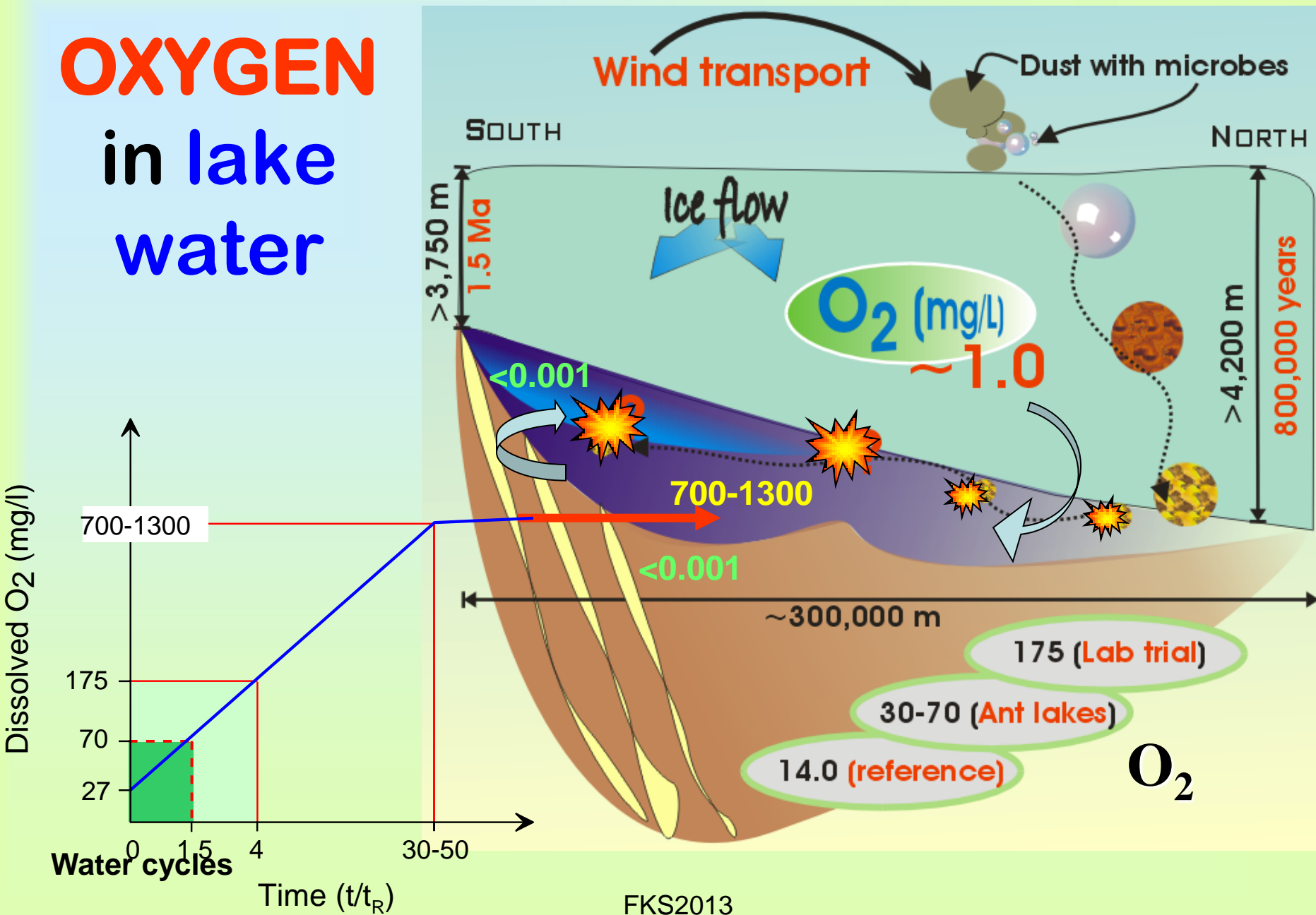
RADARSAT, CSA

Friendly environment?

FREE (LIQUID) WATER!

- Deeply ice buried (in dark) – **4 km**
- High pressure – **337-377 bar**
- Permanently BUT not very cold – **-2.65°C**
- Likely **oxygen supersaturated** - **800 mg/L**
(upper bound **700-1300 mg/L**)

OXYGEN in lake water





Vostok station (+3488m)
(since 1957)

1260 km from the coast

PRISTINE ENVIRON

Absolute minimum -89.2°C
Average ann. Temp. -55.1°C

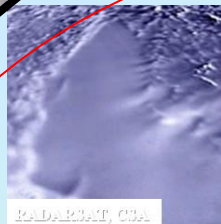
Lake Vostok (-3663m)

RADARSAT, CSA

No life

ISOLATED

May host life



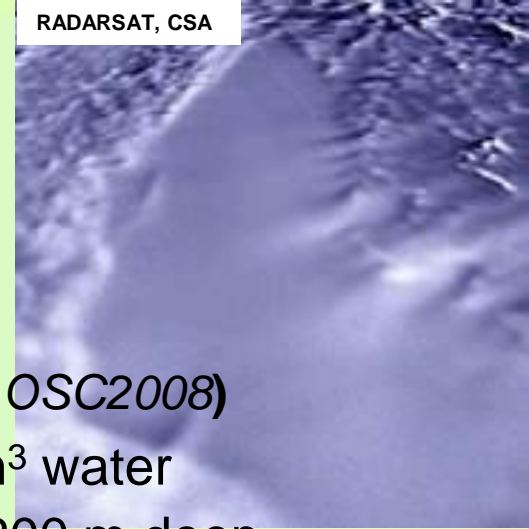
Lake Vostok known since 1971

Much less hostile environ?

FREE LIQUID WATER

- Deeply ice buried
- High pressure
- Permanent BUT not very cold – -2 / -3°C
- Like oxygen supersaturated

FRIENDLY ENVIRON?



Lake Vostok **settings**

- **Huge** subglacial lake (*Masolov et al., SCAR OSC2008*)
 - **275 x 65** km in size; **15 500** km² area; **6 100** km³ water
 - **Two main basins** with the ridge between 150-200 m deep
 - Average depth - **300** m; max depth – ~**1650** m (south basin)
 - Water renewal – **80-100** Kyr
- **Buried** beneath 2.0-0.8 Myr old **3750-4200** m thick ice sheet
- **Isolated** from surface biota for >**14** Ma
 - Age of water (melted ice) - ~**1 Ma**
- **No hydrological links** to other lakes

2.0-0.8 Ma years old

Vostok ice core

19.02.1990 (78°S, 106°E)

North

South

Meteoric ice

Ледниковый пол

0.8 Ma

2.0 Ma – 3538m

3650m

~4km
from coast

4200 м

Accretion

Уровень моря Melting zone

Lake water body

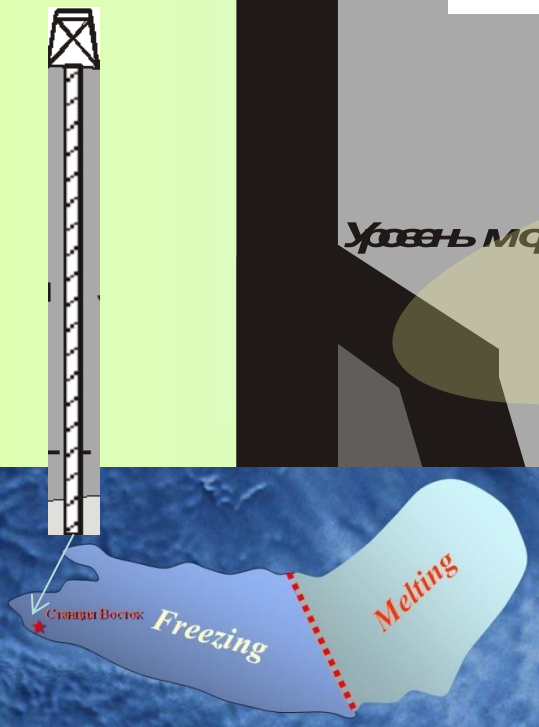
680m

Gases

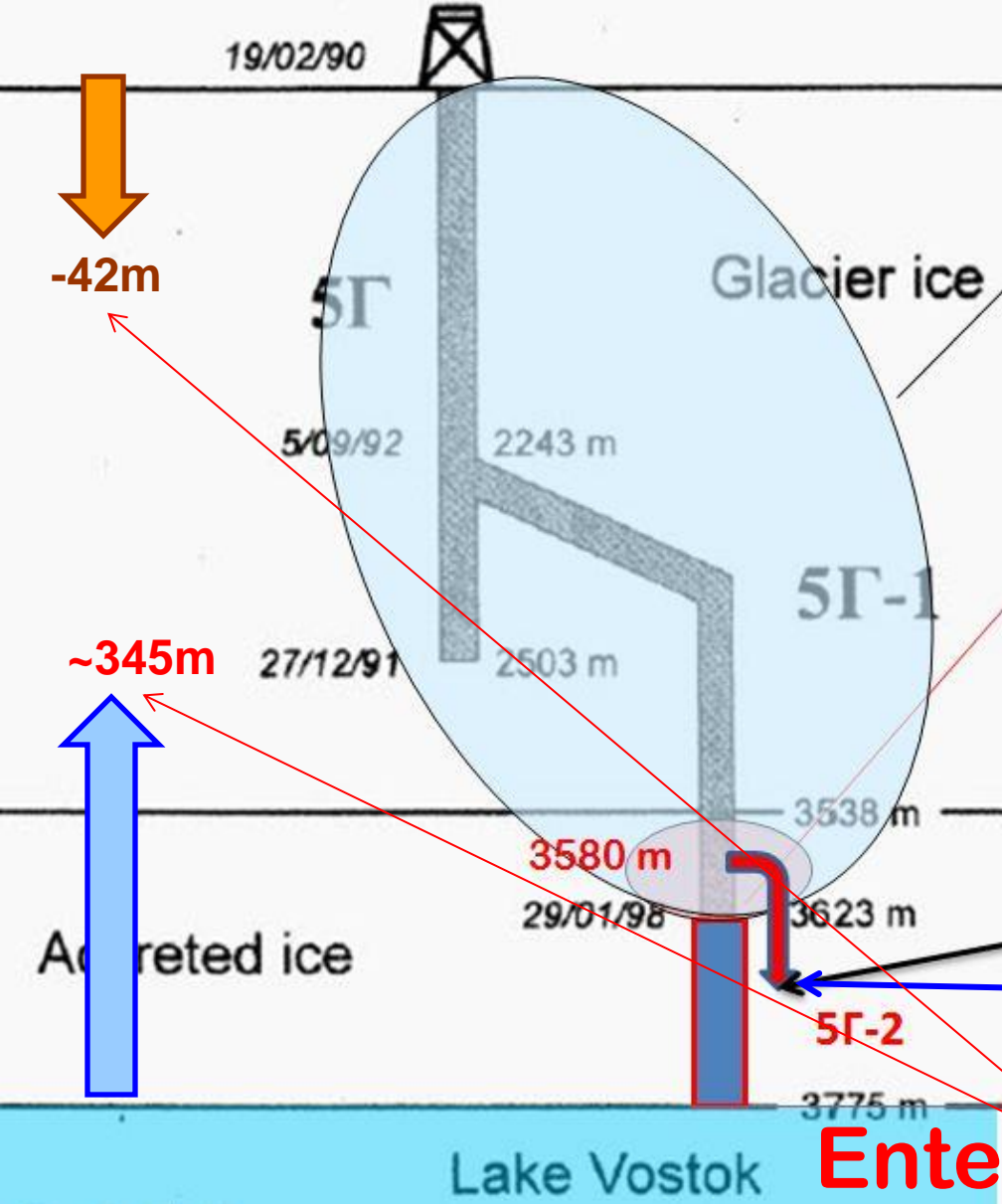
Accretion (lake) ice

Accr. rate – <10 mm/a

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5G-1[2] Vostok borehole



Russian-French-US
 collaborative program of Deep
 Drilling and Ice Core Study at
 Vostok station

Each party obtained 1/3 part of core

- ... **stopped for almost 8 years**
- 3650 m – 2005/06
 - 3659 m – 2006/07
 - 3667 m – 2007/08
 - 3599 m – 2008/09
 - 3650 m – 2009/10**
 - ~80m
 - 3720 m - Jan 21, 2011**
 - 3750 m – ice-water boundary
 - 3769.3 m – Feb 05, 2012**

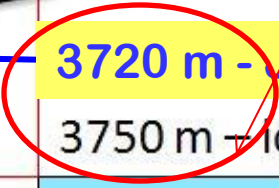
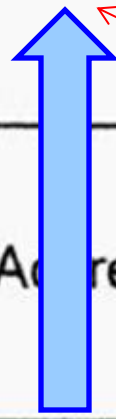
30m left!

Enter!

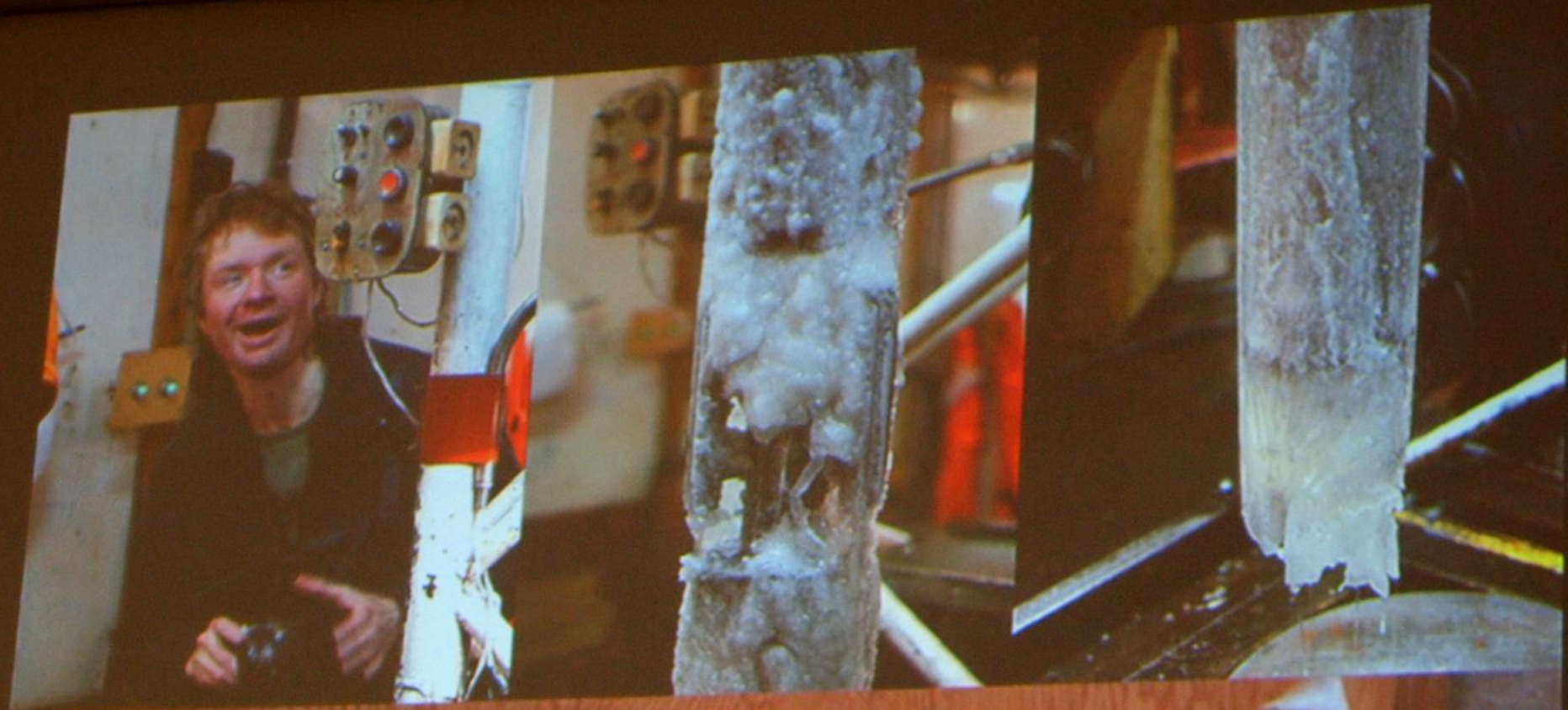


-42m

~345m



~80m

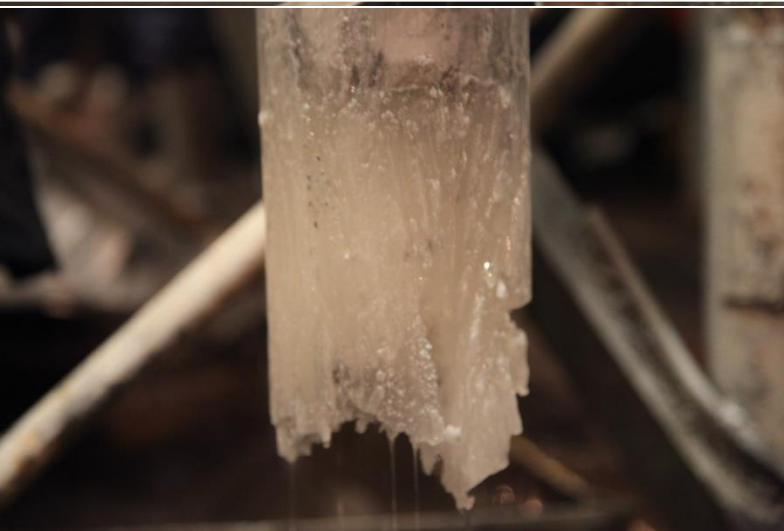


3769,30 m

3768,m



Lake water frozen



FKS2013

We believe
Whatever we find

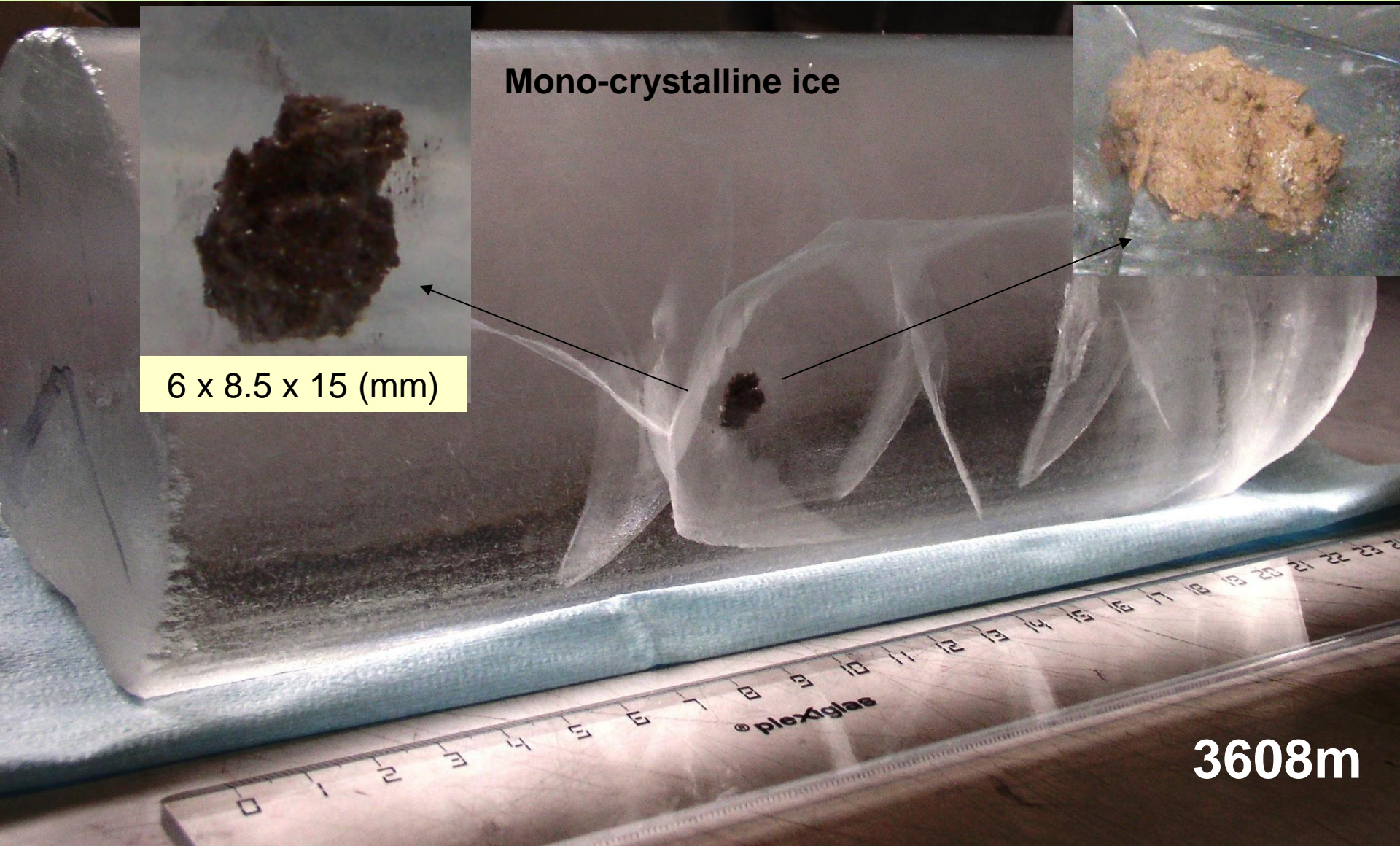
It's going to be **special stuff,**
unique stuff, life forms we've
never encountered before

Accretion ice **BIO** features

Accretion ICE	NO GAS <small>GAS contents</small>	NO DOC <small>DOC (ppbC)</small>	Major ions*
TYPE 1 Sediments	Low (10^{-2}) CO ₂ , CH ₄ , O ₂	10 ± 9	Mg ²⁺ Ca ²⁺ SO ₄ ²⁻ (S ²⁻) (no NH ₄ ⁺ , nitrate)
TYPE 2 Very clean	Very low (10^{-3}) (no O ₂ , CH ₄)	5.6 ± 4.6	- (no NH ₄ ⁺ , nitrate)
Conditions	Low aerobic Anaerobic	Ultra- Oligotrophic No heterotrophs	No e-donors? (Hydrogen?)

Many oligotrophic lakes have DOC's in the range of 100 to 500 ppb

3608BK - Big 'Kamina'



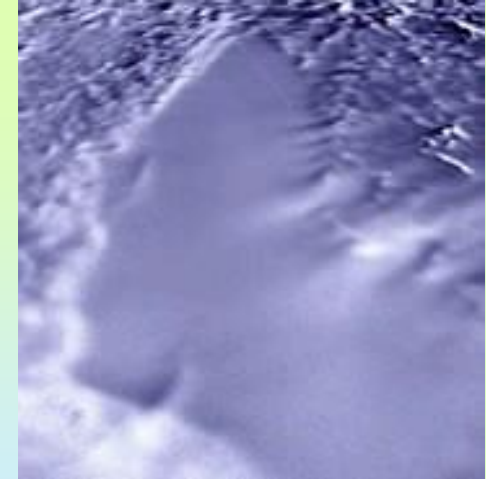
Mono-crystalline ice

6 x 8.5 x 15 (mm)

3608m



RADARSAT, CSA



Microbes in lake water

'Oxygenophilic' (?)

Earth-unknown !

chemolithoautotrophic

piezophilic psychrophiles

Superoxide, hydrogen peroxide, singlet O, hydroxyl radicals etc. **vs.** carotinoids, catalase, superoxide dismutase etc.

Bacterial metabolic pathways

Accretion ice type 1 et water

E-donor	E-acceptor	Carbon	Process	Microbes
H ₂	SO ₄ ²⁻	CO ₂	Sulfate reduction (H ₂ S)	<i>Sulfate-reducers</i>
H ₂	O ₂ (in mineral inclusions)	CO ₂	Hydrogen-oxidation	<i>Hydrogen-oxidizers</i>
H ₂	CO ₂	CO ₂	Methanogenesis (CH ₄)	<i>Methanogenic archaea</i>
S ²⁻ S ⁰ In mineral inclusions	O ₂ (NO ₃ ⁻)	CO ₂	Sulfur oxidation (SO ₄ ²⁻ - acidic)	<i>Sulfur-oxidizers</i>

RECALL: Gas content is very low - 0.001 vs. 0.1 g/cm³

RESULTS

Bacteria in Vostok ice core et
drill-frozen water

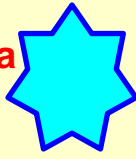
Vostok **cells/phylotypes** (16S rRNA - v2-v5 reg.) - **SUMMARY**

Ice type	Sample (m)	Cells/ml	(% Similarity with closest sequence in GenBank)
Snow (50 yr)	4.0-4.3	0-0.02	Contaminants
Glacier	122	1.9	
4.5 - 760 kyr	2005	2.4	
	2054	3-24	
	3471	1-4	
	3489	0	
	3504	1-5	
	3519	0-1	
Accretion I	3547	0	Contaminants
30 - 40 kyr	3548	1	Contaminants
	3561	4-9	Contaminants + thermophile
	3607	ND	Thermophile (92%) Uncultured bacterium (410 bp, AF532061) ???
	3607-re	1	
	3608BK-re	ND	(95%) <i>Ilumatobacter fluminis</i> (526 bp, NR_041633) Actino
	5G2-3608	0	Contaminants
Accretion II	3613	3	
0 - 30 kyr	3621	2	
	3622	0.6	
	3635	4.7	
	5G2-3646	0	Contaminants
	3650	3.1	
	3650	4777	→ untreated surface
No more water pockets	3659	12	
	5G2-3714	0	
	5G2-3764	0	
Lake water	5G2-water* w2ori	167	Refer to below

99-100% - thermophile
Hydrogenophilus
thermoluteolus β-Proteob



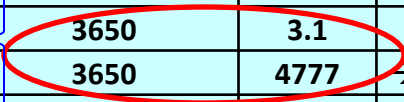
Sulfate-reducing bacteria
Hydrogen-oxidizing bacteria
Methanogenic archaea
Sulfur-oxidizing bacteria



•Lake water frozen on a drill bit

99% - glacier sediment

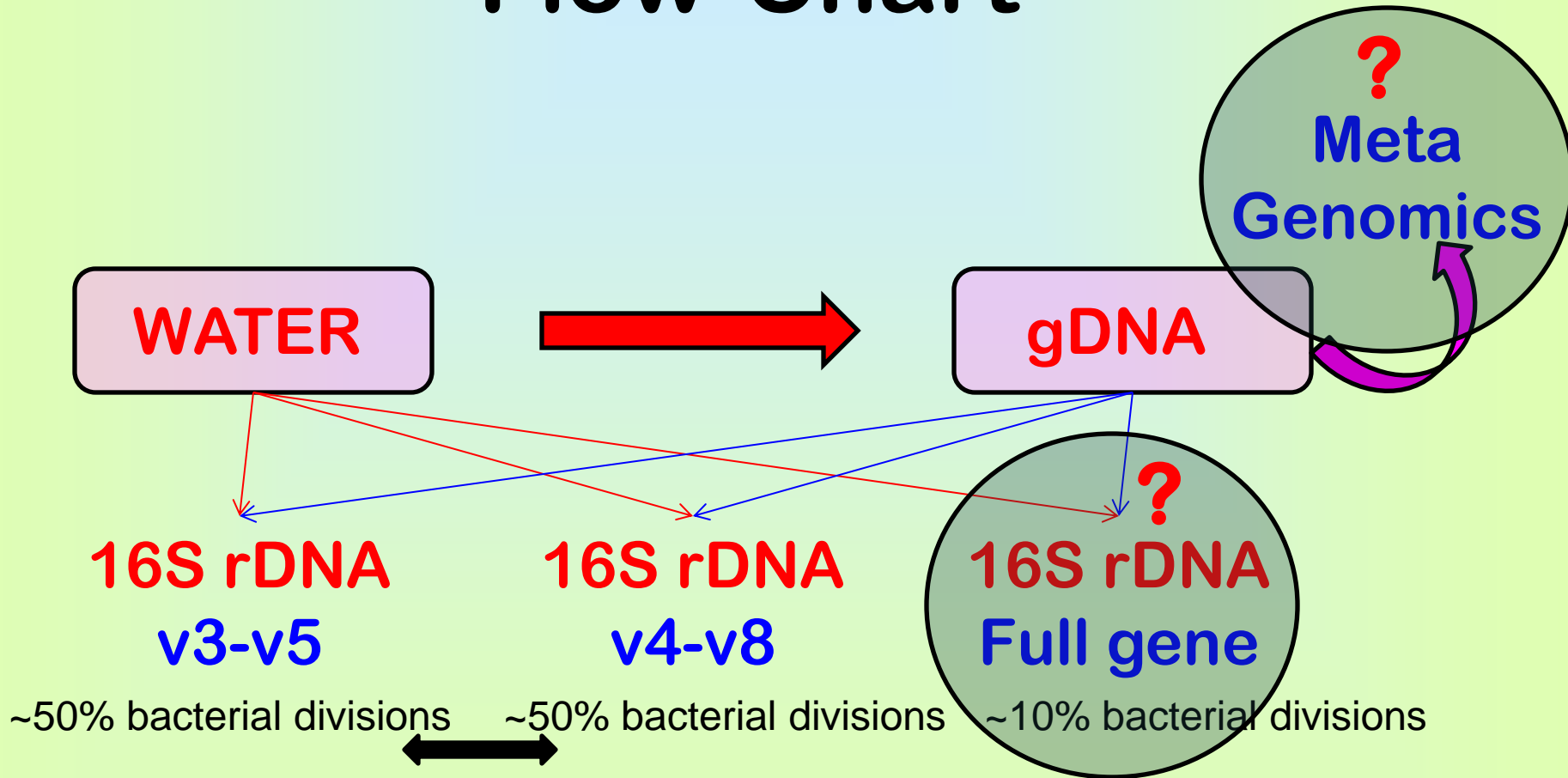
No more water pockets





Bulat13 mars-14

Water-DNA/PCR Flow-Chart



Discovered in 'technological' lake water – mars 2013

2 identical clones – **100%** *Microbacterium sp (ginsengisoli)* (soil, plant roots) or *Microbacterium sp (aliphatic hydrocarbon-contaminated soil)* – *Actinobacteria*

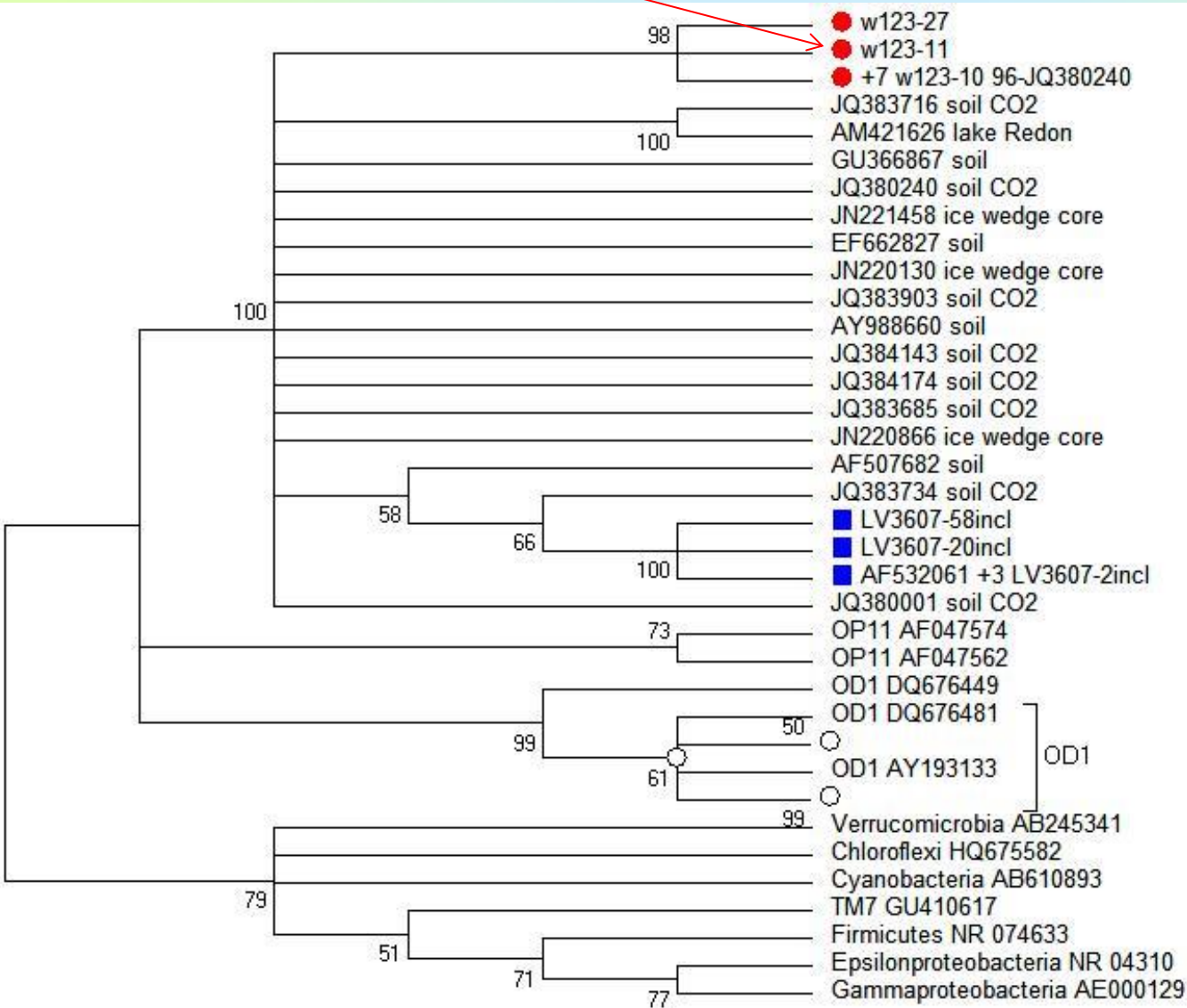
1 clone – **99%** *Sporosarcina sp. SS16.7* (coastal sea sediments of King George Island, **Antarctic**) while can be met elsewhere – surface **lake water** (*Sporosarcina saromensis* - An et al., 2007), **clinical** samples and even as contaminants in **clean room** facilities (*Sporosarcina contaminans* - Kampfer et al., 2010) – *Firmicutes* (Bacillales; Planococcaceae)

22% (7 clones) – w123-10 et al <**86%** OD1 – **???**

3 allelic vars

Lake ice II 3607m (92%) Uncultured bacterium (410 bp, AF532061)

~~22%~~ – w123-10 et al <86% OD1 – ???



Maximum Likelihood
- 369 sites

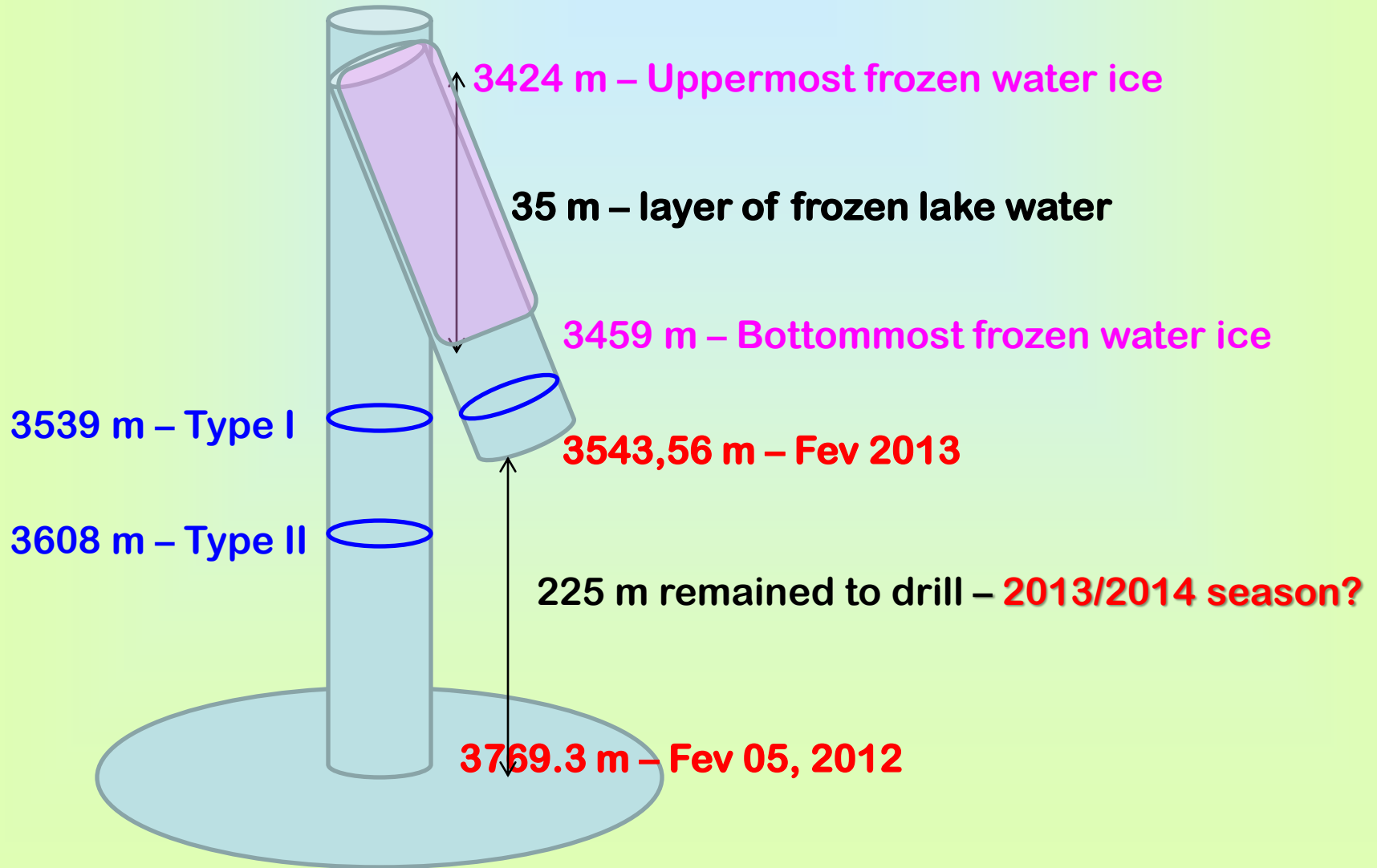
Preliminary CONCLUSION

Seems there is **UNKNOWN**
LIFE (population) in **upper layer**
of Lake Vostok water
(frozen on a drill)

Support may come with full-gene
sequencing

Proof may come late May 2013

5G-2[3] **Vostok** borehole fev 28, 13



Clean Vostok frozen water ice



SEVEN DAYS

The news in brief

RESEARCH

Life in Lake Vostok

Russian scientists say that they have found a hitherto-unknown type of bacterium in Antarctica's largest subglacial lake. Samples retrieved last year from Lake Vostok contain an "unclassified" bacterium whose DNA is less than 86% similar to known bacteria, Sergey Bulat of the Petersburg Nuclear Physics Institute in Gatchina announced at a meeting last week in Moscow. More tests and cleaner samples are needed to establish the physiology and biochemistry of the microbe, says Bulat. See go.nature.com/ydcmw4 for more.

Carbon spike

Atmospheric carbon dioxide concentrations rose by 2.59 parts per million (p.p.m.) in 2012, marking the sharpest increase since 1998, according to data from the US National Oceanic and Atmospheric Administration. Atmospheric CO₂ concentrations reflect rising global emissions, driven by developing countries, as well as variations in carbon uptake by plants and the oceans. The global average, calculated from measurements at the Mauna Loa Observatory in Hawaii and other locations, exceeded 395 p.p.m. in January, representing an increase of more than 70% from pre-industrial levels.

Anti-HIV trial

A clinical trial of the preventive powers of the anti-HIV drug tenofovir may have failed because women were not actually taking the drug. In 2011, the VOICE study, in 5,029 HIV-negative women in South Africa, Zimbabwe and Uganda, suggested that neither a vaginal gel nor tablets containing the drug could prevent HIV infection (see



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Rebuilding Naples' City of Science

The Italian government has pledged €20 million (US\$26 million) to help rebuild the City of Science (*Città della Scienza*), an iconic 12,000-square-metre exhibition centre and science museum in Naples that was destroyed by fire on 4 March (pictured). Italy's research minister, Francesco Profumo, said that the

government would work with local authorities on a plan to rebuild the museum in just 18 months. The museum's management is also seeking voluntary donations. Investigators had not announced an official cause for the fire as *Nature* went to press, but they were reported by the Italian media to suspect arson.

Nature 480, 10–11; 2011). Even though little unused product was returned, tenofovir was present in fewer than one-third of biological samples from participants assigned to receive it, study leaders revealed last week. Too few women took the drug to assess whether it did prevent HIV infection, although earlier studies have suggested it can work.

Elephant poaching

A surge in ivory poaching has driven the population of African forest elephants down to less than 10% of what could be supported by the available range. Researchers calculate that this species (*Loxodonta africana cyclotis*) lost 30% of its range and 62% of its population between 2002 and 2011 (F. Maisels *et al.* *PLoS ONE* 8, e59469; 2013).

The study was released as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) met in Bangkok; many scientists have urged CITES to clamp down on poaching and habitat destruction and to tackle demand for ivory (see *Nature* 494, 411–412; 2013).

Standard Higgs

The new particle discovered last year at CERN's Large Hadron Collider outside Geneva continues to behave just like the Higgs boson predicted by the standard model of particle physics, according to results presented last week at a conference in La Thuile, Italy. The latest data indicate that the boson decays into τ leptons as predicted, and also dampen earlier hints that

the boson decays into pairs of photons more often than the standard model allows. No evidence yet points to theories beyond the standard model, such as supersymmetry (see *Nature* 491, 505–506; 2012).

POLICY

Trading species

Polar bears (*Ursus maritimus*) will not be given increased protection under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), but it looks increasingly likely that several species of shark will. Delegates meeting in Bangkok for the sixteenth CITES conference, which governs the trade in many animals and plants, voted against banning the trading of polar-bear parts.

Life in Lake Vostok

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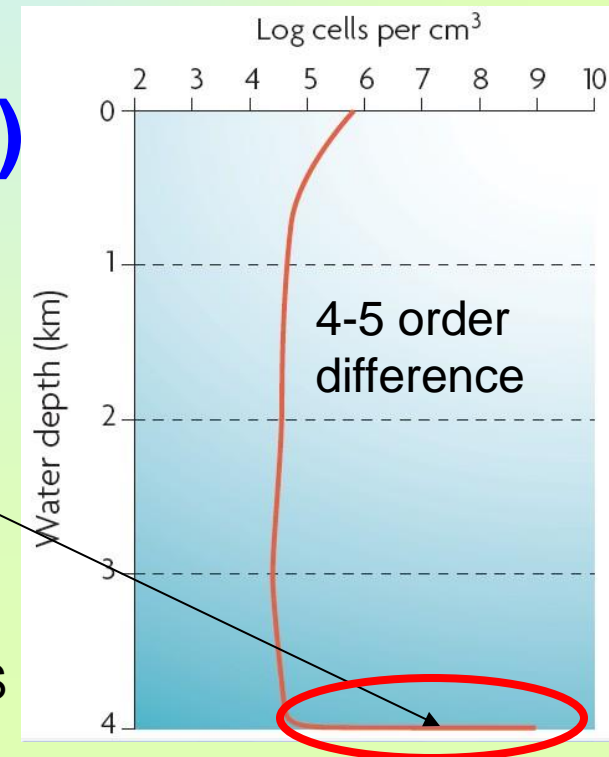
10 years FUTURE DIRECTIONS

– Rapidly frozen lake water (within borehole) – 2012/14



– Lake water column (680m)

– Lake sediments (>300m)



Subglacial **Lake Entry** on the Horizon in Antarctica

- Subglacial **Lake Vostok**, the largest known subglacial lake on earth (**3769.3m-1.5km**)
- Subglacial environments beneath two **West Antarctic Whillans** ice stream (**800m- 2m**)
- Subglacial **Lake Ellsworth**, West Antarctica (**3200m-160m**)

• **Feb 05, 2012 – Russia**

• **Jan 27, 2013 – US**

• **2013/2014? - UK**

