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Background information

The use of culture independent techniques to aquatic microbial ecology has showed that archaea are ubiquitous and abundant in prokaryotic planktonic assemblages from both freshwater and marine environments. In some freshwater lakes, mesophilic Crenarchaeota have been found to constitute between 1 and 10% of the total planktonic community. Recently, the discovery that some mesophilic Crenarchaeota can act as chemolithoautotrophs has broadened our view of archaeal metabolism and has posed questions on their impact on biogeochemical cycles.

Study Site

Lake Kivu (2°S, 29°E) is a deep meromictic and oligotrophic high altitude (1,463m a.s.l.) tropical lake. Kivu has a permanent thermal- and haline stratification that produces a wide oxic mixolimnion and a deep anoxic monimolimnion (from 60 to 489 m depth) which is very rich in both CO₂ and CH₄. Although most of this CH₄ comes from the reduction of magmatic CO₂, degradation of organic matter in anoxic conditions by active methanogenic Archaea counts for one third of CH₄ production.

Experimental desing

Water samples

- K-1 to K-4 sampling stations (see figure for details)
- 1L to 500 mL water filtrated through 5.0 and 0.22 µm pore size filters

DNA extraction

- CTAB+β-mercaptoethanol+PVP
- Centricon purification
- Only 0.22 µm filters analysed

16S rDNA extraction

- Universal PCR: UA21f/U958r
- Archaea nested-PCR: ARC344f-GC/ARC915r
- Crenarchaeota nested-PCR: CREN337f-GC/ARC915r



DGGE analysis

- 60°C, 160 V, 16h
- 20% to 80% denaturing gradient
- SybrGold® staining

Phylogenetic analysis

- Sequencing
- ARB-software analysis (*in progress*)
- Phylogenetic trees (*in progress*)

Results

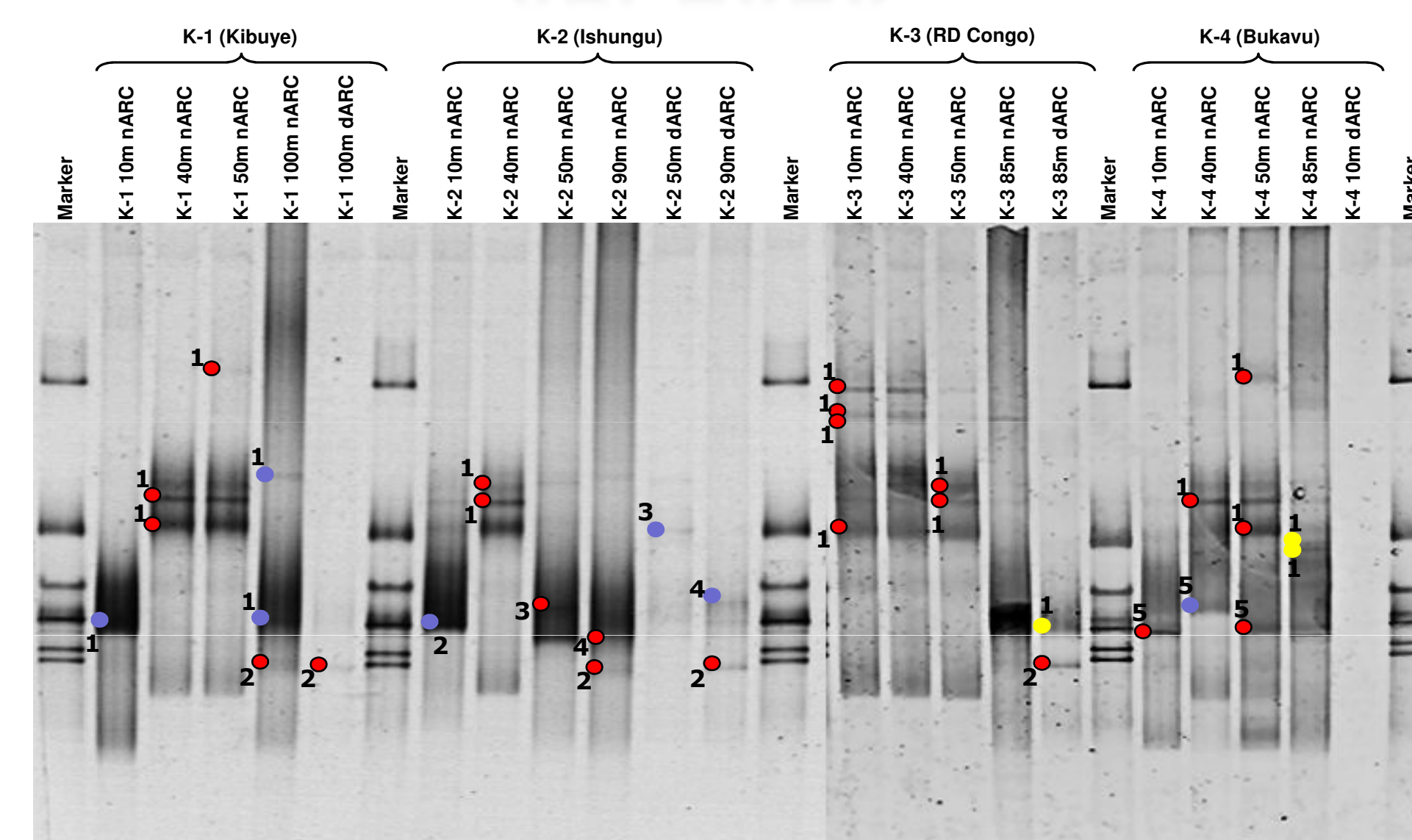


Bitacora of Lake Kivu (March-April 2007)

A sampling campaign was carried out during March-April 2007 in Lake Kivu. Four stations (labelled K1 to K4, see map) were sampled during the campaign. Thermal and chemical stratification of the water column with oxic and anoxic water layers were clearly defined in all stations. Water samples were collected every 10 meters along the vertical profile of each station and they were used for molecular fingerprinting analysis.



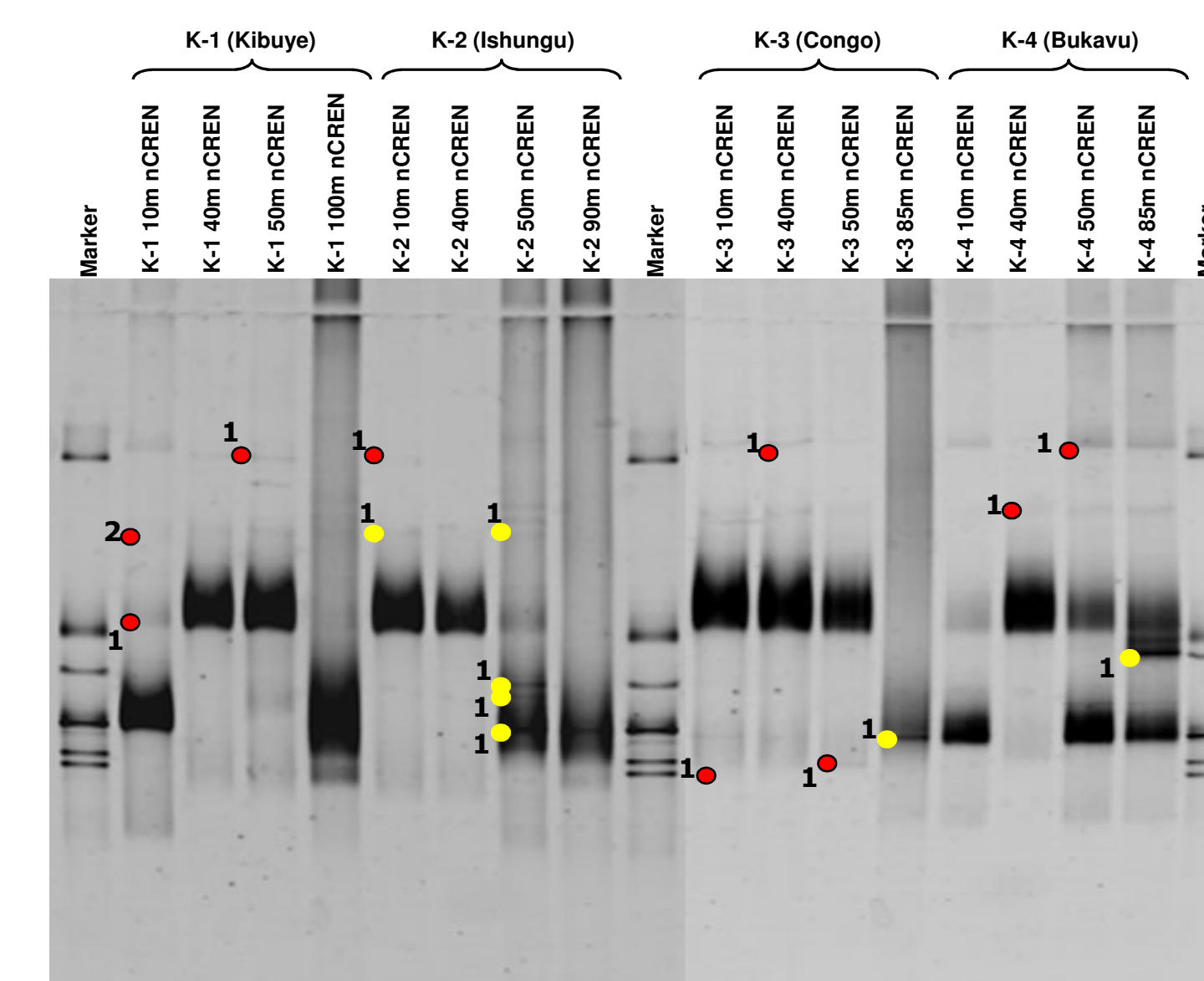
ARCHAEA



Code	Type	Clone	Environment
1	Crenarchaeota	MP104-SW-a5 (DQ088780)	Deep fracture water (crustal biome)
2	Crenarchaeota	VIARC-2 (UCR240005)	Freshwater lake
3	Crenarchaeota	NRP-N (AB243805)	Rice paddy soil (anaerobic)
4	Crenarchaeota	GA55 (AB046184)	leachate from landfill site
5	Crenarchaeota	PET1-19 (AY278101)	Soil, grassland, Crenarchaeota group 1.1b
1	Euryarchaeota	ASC34 (AB161335)	Petroleum cont soil
2	Euryarchaeota	Methanosaeta sp. Clone A1 (MSP133791)	Methane formation from crude oil
3	Euryarchaeota	ESYB47/EJ_CO2 (AB119603/AY454667)	Estuarine sediment
4	Euryarchaeota	MB-01 (AY175367)	Methanogen peatland
5	Euryarchaeota	pTN-19 (AB182768)	Rice paddy soil
1	Unknown		

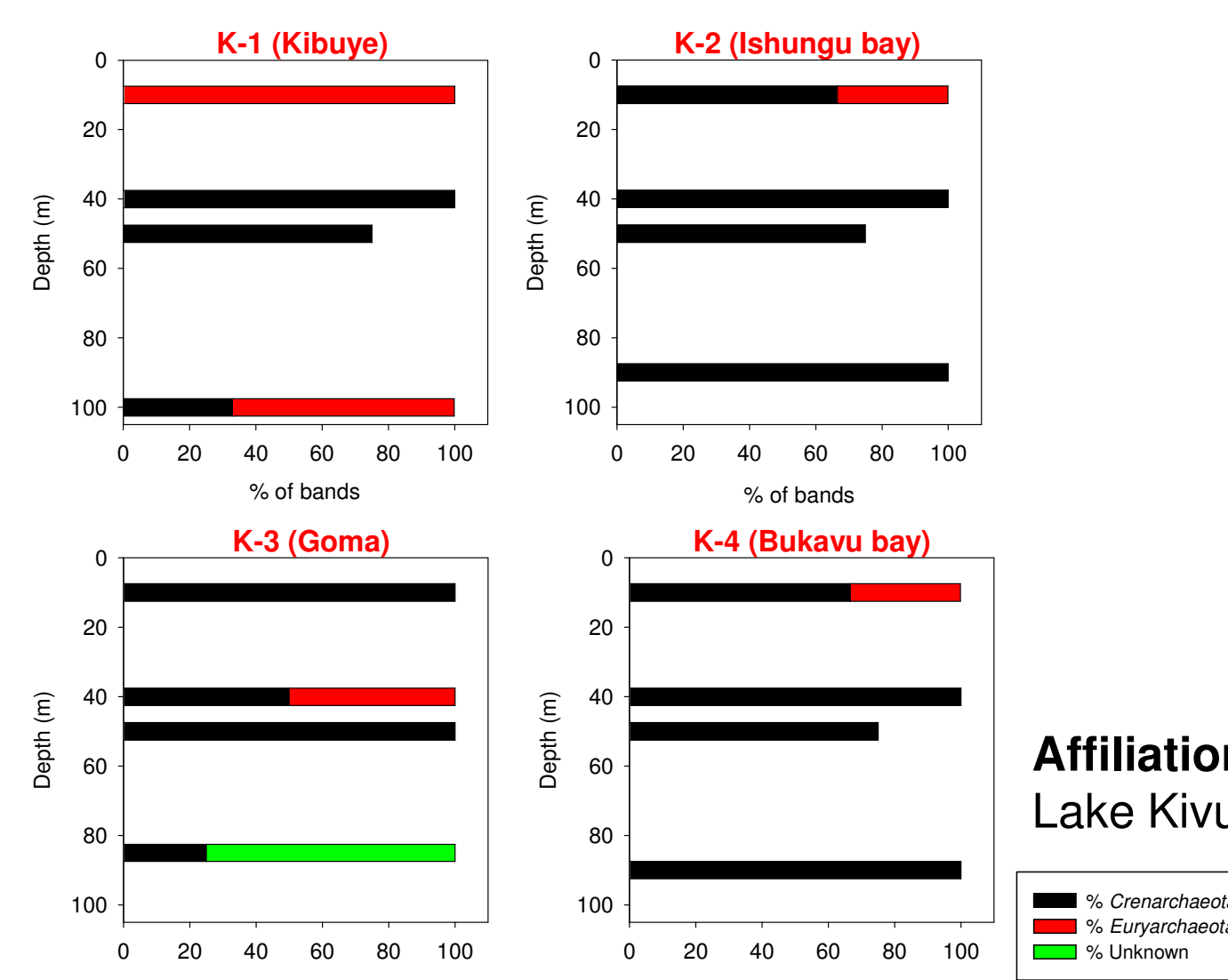
Negative images of SybrGold®-stained DGGE fingerprints of 16S rRNA gene amplicons from Archaea (above) and Crenarchaeota (below) planktonic community thriving in Lake Kivu. Archaea and Crenarchaeota nested-PCR products loaded onto 20%-80% DGGE gels gave clear and distinct band profiles. Some of the obtained bands were common in the different samples analysed while some others were depth specific. We are currently trying to optimize PCR-DGGE performance to obtain more clear fingerprints.

CRENARCHAEOTA



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1	Unknown		

Affiliation (in %) of 16S rRNA gene sequences retrieved from DGGE fingerprints of Lake Kivu samples.



Preliminary conclusions

- Planktonic archaea seems to be widely distributed in Lake Kivu.
- The main archaeal phylotypes recovered were mainly assigned to:
 - *Euryarchaeota*: mainly methanogenic uncultured clones (as expected according to lake chemistry).
 - *Crenarchaeota*: mainly related to different non-thermophilic clones from anoxic environments.