

## Opening the archaeal box in an oligotrophic freshwater environment

M Llrós<sup>1, 2</sup>, T Garcia-Armisen<sup>3</sup>, SA Crowe<sup>4</sup>, F Darchambeau<sup>5</sup>, C Morana<sup>6</sup>, C Michiels<sup>3</sup>, M Schmitz<sup>2</sup>, K de Saedeleer<sup>2</sup>, L Montante<sup>2</sup>, F Roland<sup>5</sup>, W Thiery<sup>6</sup>, S Bouillon<sup>6</sup>, AV Borges<sup>5</sup>, P Servais<sup>3</sup> & JP Descy<sup>2</sup>

<sup>1</sup> Department of Genetics and Microbiology, Autonomous University of Barcelona, Spain.

<sup>2</sup> Laboratory of Freshwater Ecology, Facultés Universitaires Notre-Dame de la Paix, Belgium.

<sup>3</sup> Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Belgium.

<sup>4</sup> Nordic Center for Earth Evolution, University of Southern Denmark, Denmark.

<sup>5</sup> Chemical Oceanography, Université de Liège, Belgium.

<sup>6</sup> Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven, Belgium.

Owing to the wide use of both culture-dependent and -independent techniques, knowledge on the biology of Archaea is rapidly increasing widening capabilities to detect and culture them. An important role for some archaeal groups (e.g., methane (CH<sub>4</sub>)-related and ammonia-oxidizing archaea) has been envisaged in global biogeochemical cycles. However, many challenges still remain. Importantly, archaeal taxonomic assignments are limited due to both the lack of cultured representatives for all archaeal groups and a dearth of full-length 16S rRNA gene sequences. The objective of this research is to expand our knowledge on archaeal diversity and ecology in freshwater oligotrophic environments using Lake Kivu as a natural laboratory.

Lake Kivu (East Africa) is a deep (maximum depth 489 m), oligotrophic, and high altitude (1,463 m) lake of tectonic origin. The water column is split into two layers— an oxic mixolimnion (the oxycline situated around 20 to 60-m depth depending on the season) and an anoxic monimolimnion rich in dissolved salts, carbon dioxide and CH<sub>4</sub>, due to permanent stratification. Consequently, these water layers exhibit contrasting physico-chemical properties and they thus harbour different microbial populations.

In a previous snapshot study (2007), Archaea accounted for less than 5.0% of total prokaryotes (DAPI counts) and DGGE fingerprints revealed the presence of ammonia-oxidizers in oxic waters, whereas methanogenic lineages were most prevalent in anoxic waters. In order to gain a deeper insight into this archaeal community, water samples from different depths were collected three times (October 2010, June 2011, and January 2012) at three different pelagic sites and analysed by 454 FLX pyrosequencing (~250 bp reads) and quantitative PCR targeting 16S rRNA and distinct functional genes. A wide array of environmental metadata and process rate measurements was collected in parallel. Preliminary results revealed on average ca. 160 different operational taxonomic units (OTUs, 0.03 cutoff) in samples obtained in the main lake, whereas 22 OTUs were obtained from a separated and isolated basin. In all samples analysed to date, nearly half of retrieved OTUs were singletons suggesting that the archaeal assemblage in Lake Kivu was dominated by a few abundant OTUs. The archaeal richness was higher in the oxic-anoxic transition and in the fully anoxic water layer than in the oxic water compartment. In general, the permanent stratification of the water column has driven a niche differentiation of archaeal communities with ammonia-oxidizing Thaumarchaeota dominating the oxic water layer, putative heterotrophic phylotypes related to the Miscellaneous Crenarchaeotal Group present in the oxic-anoxic transition and Euryarchaeota methanogenic lineages dominating the anoxic

layer. Quantitative analyses revealed similar vertical profiles between marine Thaumarchaeota 16S rRNA gene and archaeal amoA gene copy numbers, suggesting that the restriction of Thaumarchaeota to the oxic layer is related to their reliance on aerobic ammonium oxidation to support growth. Overall, our results on the distribution of archaeal phylotypes in Lake Kivu are consistent with observations from marine environments. Anoxic waters support greater archaeal diversity but favour euryarchaeotic species related to CH<sub>4</sub> cycling. In contrast, oxic waters exhibit restricted diversity, supporting largely aerobic Thaumarchaeota sustained by oxidizing ammonium.