



Marine Jurassic microbes in a continental karst setting and their implications for ecosystem conservation.

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Located within an evaporitic basin with
~150 mm annual precipitation, the
Cuatro Ciénegas basin supports more
than 70 endemic species and, uniquely,
abundant living stromatolites forming
the basis multilayered food webs that
include snails, crustaceans, and fish.

Amazingly this occurs in an extremely
salty and oligotrophic water.

Given the high levels of
endemism and biodiversity,
an 85,000-ha area is currently
designated as a federal “Area
de Protección de Flora y
Fauna,” providing for nature
conservation alongside
sustainable development
activities

**Aquatic ecosystems are
under increasing
anthropogenic pressures
worldwide and especially in
arid regions of the
developing world.**

CCB is no exception.



Given the considerable conservation interest of the CCB due to its high levels of endemism and its status as an aquatic oasis in severe desert environs, a better understanding of the sources of water sustaining the springs is critical.

Why CCB is so diverse?



Geology matters



México: Imagen desde el espacio

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad
Mosaico 2002 de imágenes Modis sin nubes del satélite Terra,
bandas 1, 4, 3 (RGB), resolución espacial 250 metros,
sobre un modelo digital de terreno.

Our questions:

- Where the water comes from?
- Why there are so many species in CCB?
- Which are the evolutionary forces that shape these communities

How we did it?

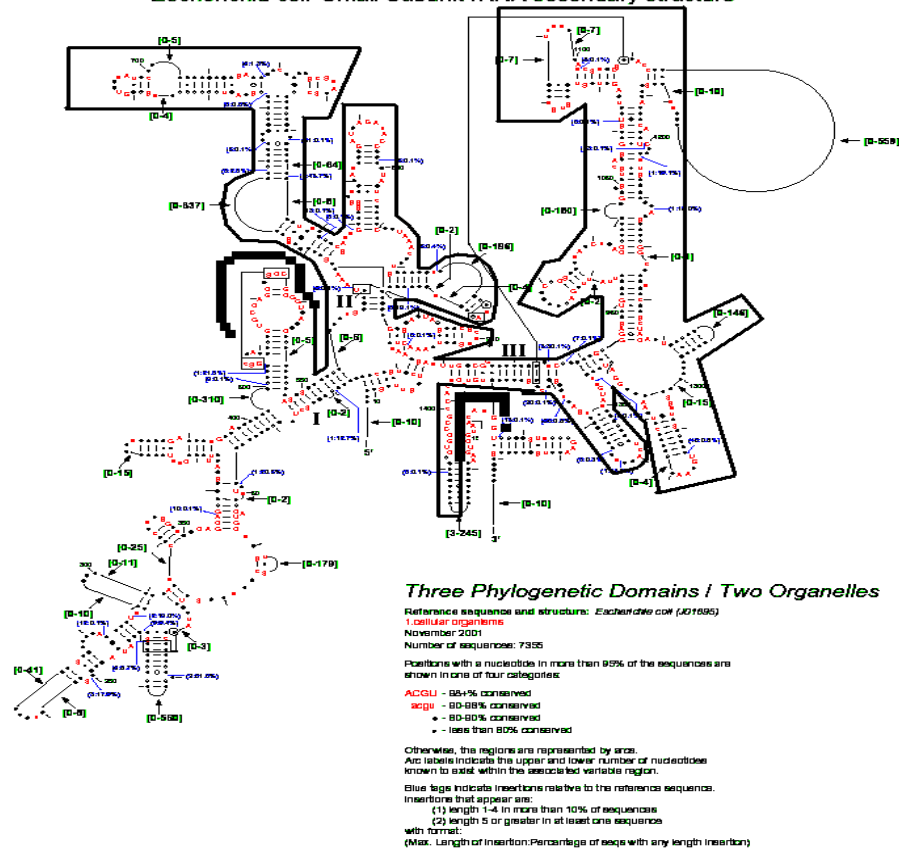
© Dean A. Hendrickson







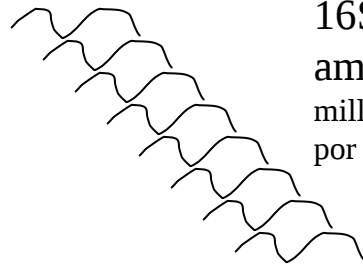
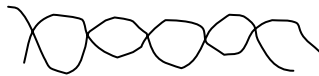
Phylogenetic conservation superimposed onto the
Escherichia coli Small Subunit rRNA secondary structure



Citation and related information available at <http://www.molcomb.utexas.edu>

16S amplified zone

MUESTRA DE AGUA



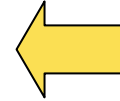
DNA Total de la muestra. CONTIENE MILLONES DE
HEBRAS DE MILLONES DE ORGANISMOS,
MUCHOS DE ELLOS DIFERENTES

Gen del ribosoma
16S
amplificado
millones de veces
por PCR.



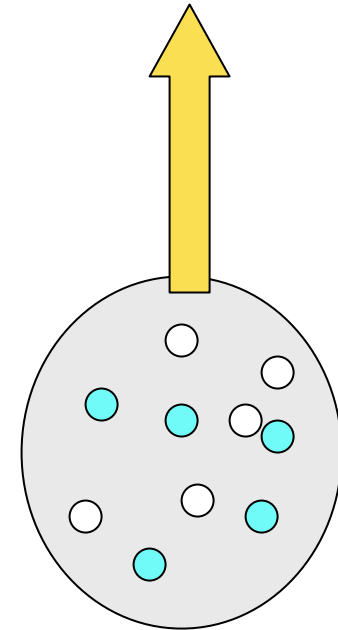
Librería de
clonas de
ADN
ambiental

Alinear, identificar la
diversidad y BLAST

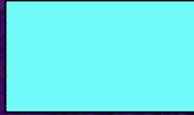


ATTTCGCTTAAATTCGTC
ATTTCGTTTAAATTCCTC

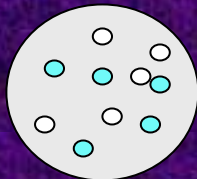
Secuenciar varias clonas por sitio



MUESTRA DE AGUA



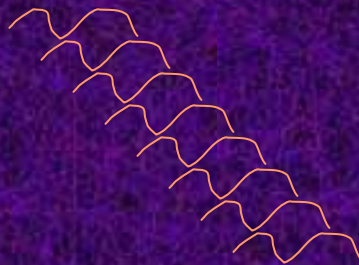
Crece las
bacterias en
medios de
cultivo
diferentes



Aislar DNA
de cada cepa



Secuencias
de cepas
cultivables



Alinear, identificar la
diversidad y BLAST



Identificación de la
especie por similitud



Construcción de
filogenia



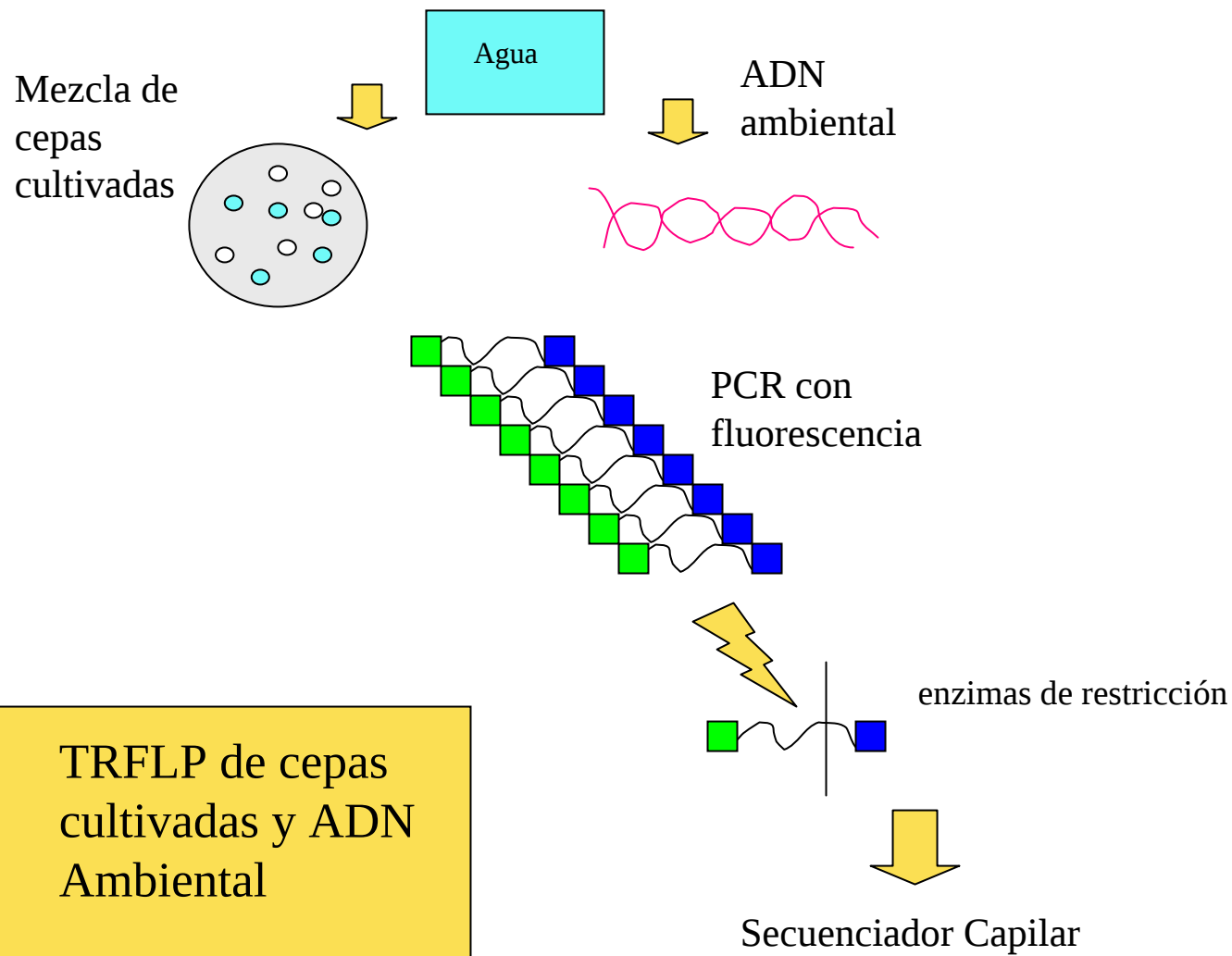
ATTTCGCTTAAATTCGTC
ATTTCGTTTAAATTCCTC



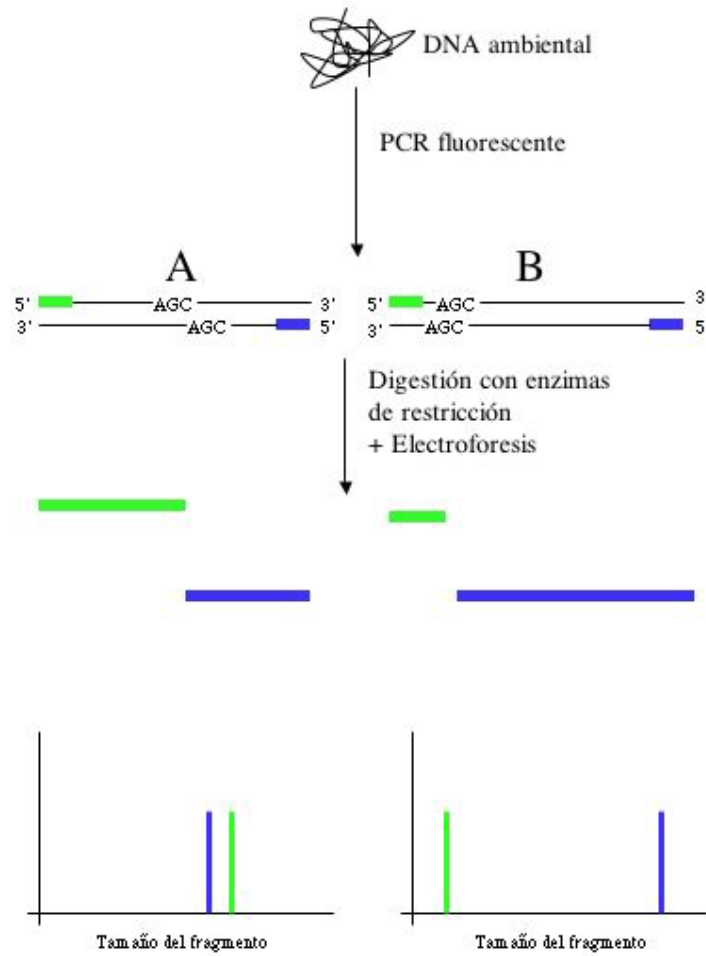
Secuenciar



Gen del ribosoma 16S amplificado millones
de veces por PCR.



¿Porqué se pueden hacer estudios de comunidades con esta técnica?



Porque separa los organismos con sitios diferentes

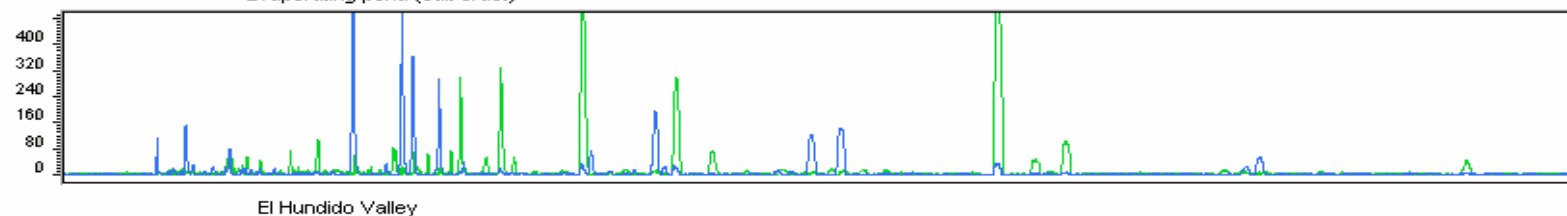
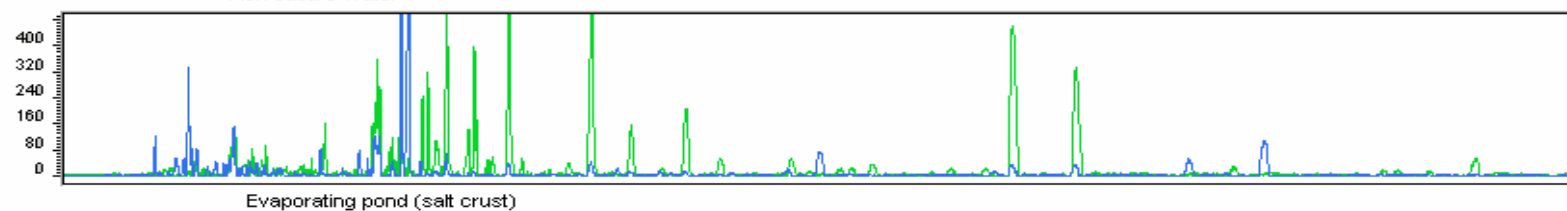
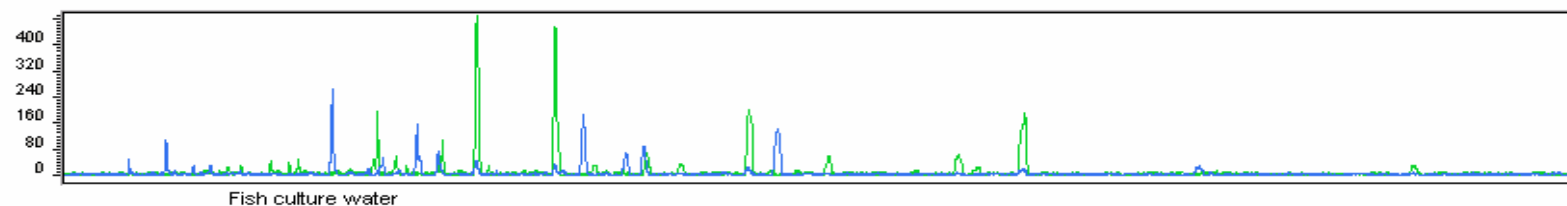
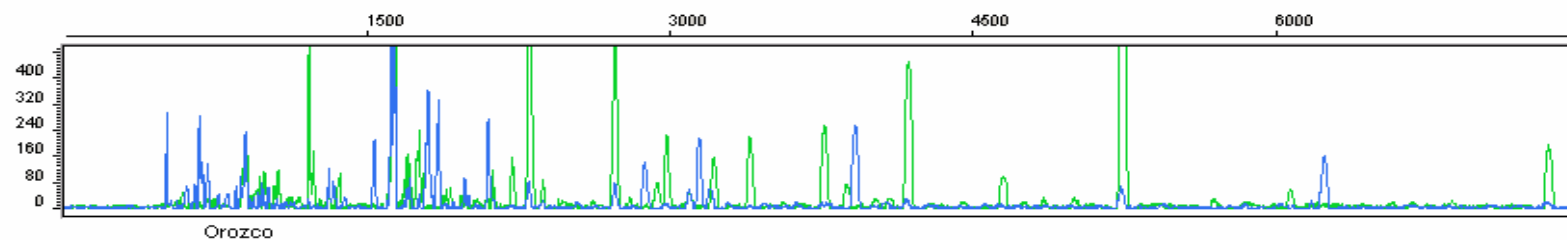
What we
found?

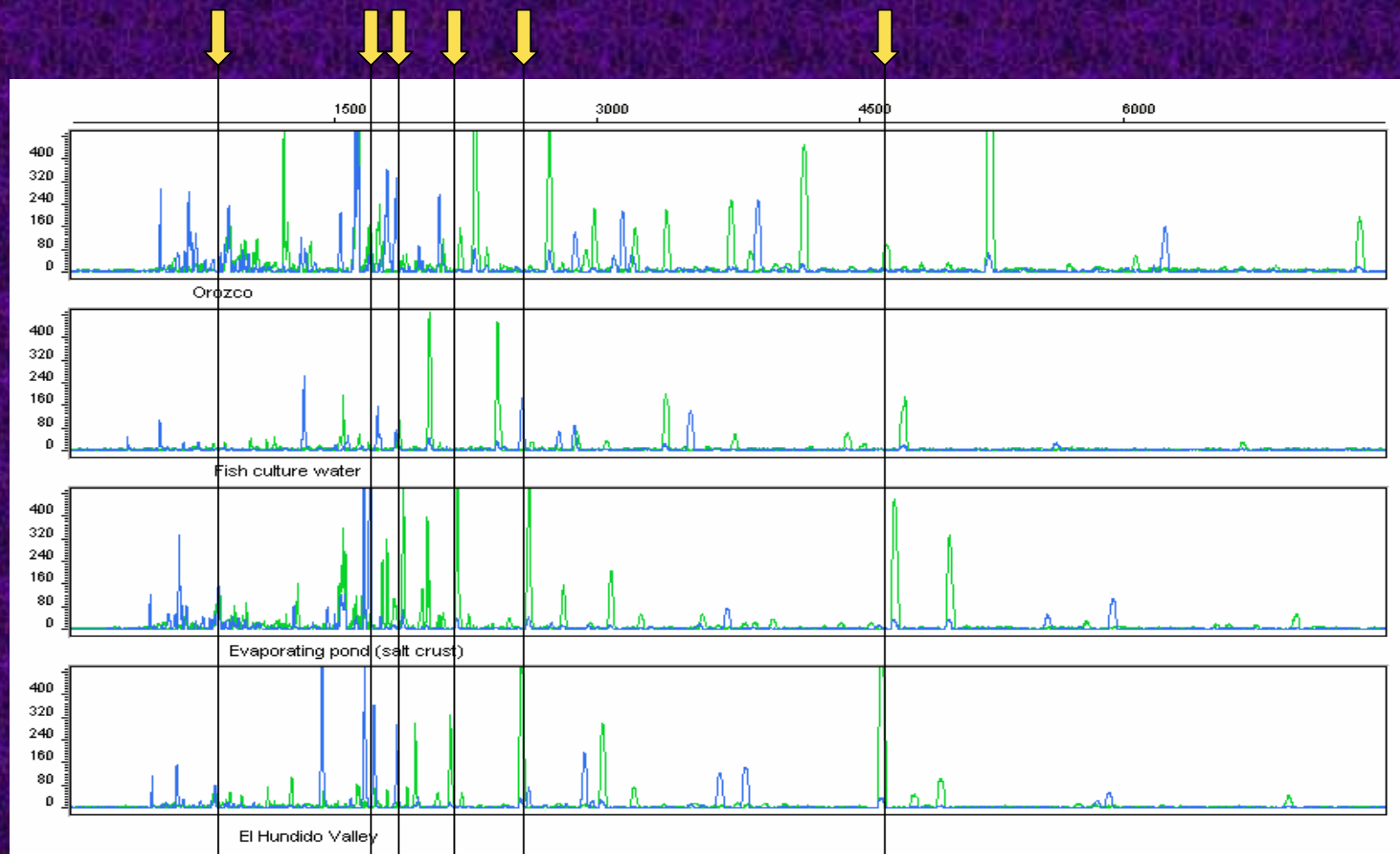
TRFLP

As a way to map the edge of the
aquifer

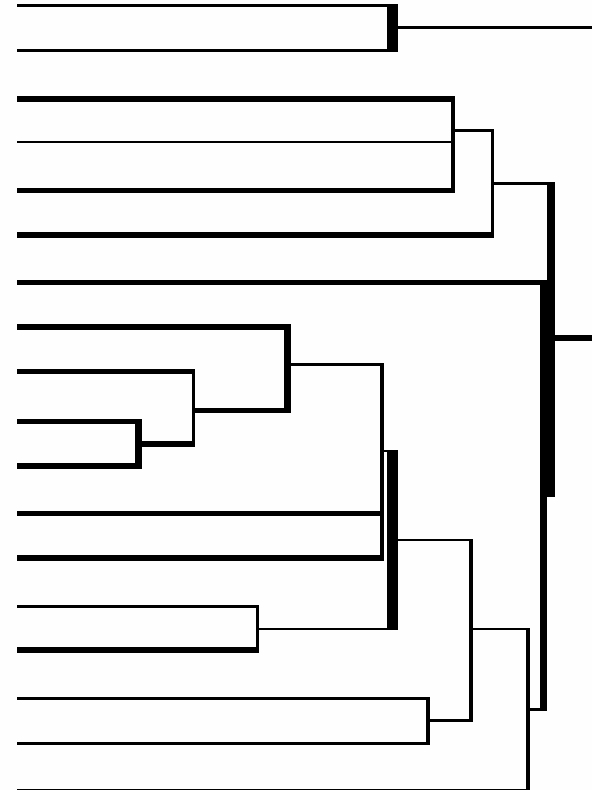
TRFLP

As a hint to microbial diversity

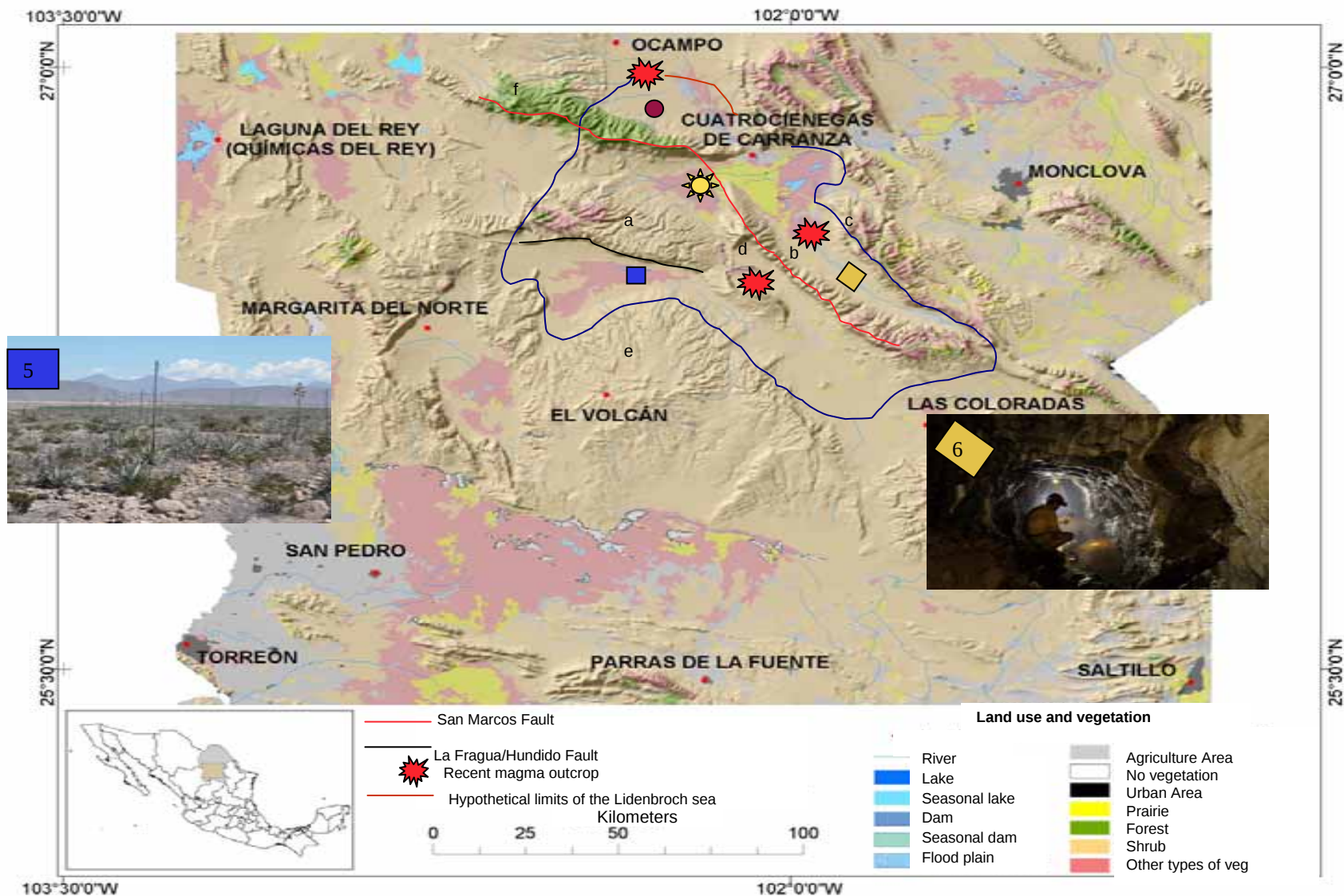




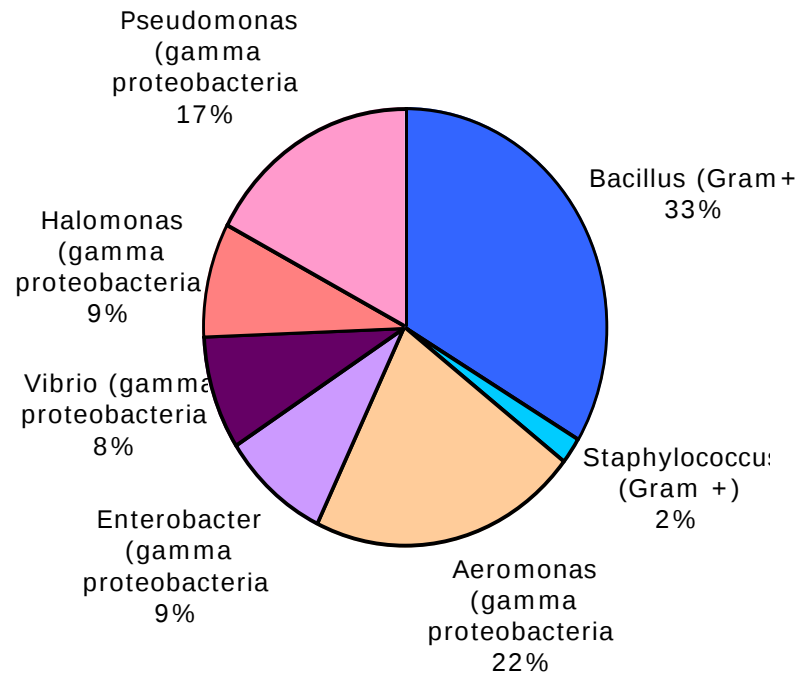
- 12 años Churince (manantial)
- 11 años Becerra (manantial)
- 2 años Churince
- 27 años Calaveras
- 26 años El Hundido
- 29 años Calaveras
- 9 años Mesquites
- 5 años Churince
- 16 años Escobedo
- 2 años El Hundido
- 28 años Calaveras
- 8 años Mesquites
- 7 años Mesquites
- 22 años Poza campana
- 14 años Orozco
- 25 años Los Hundidos
- 4 años Churince
- 24 años Mina Rosario



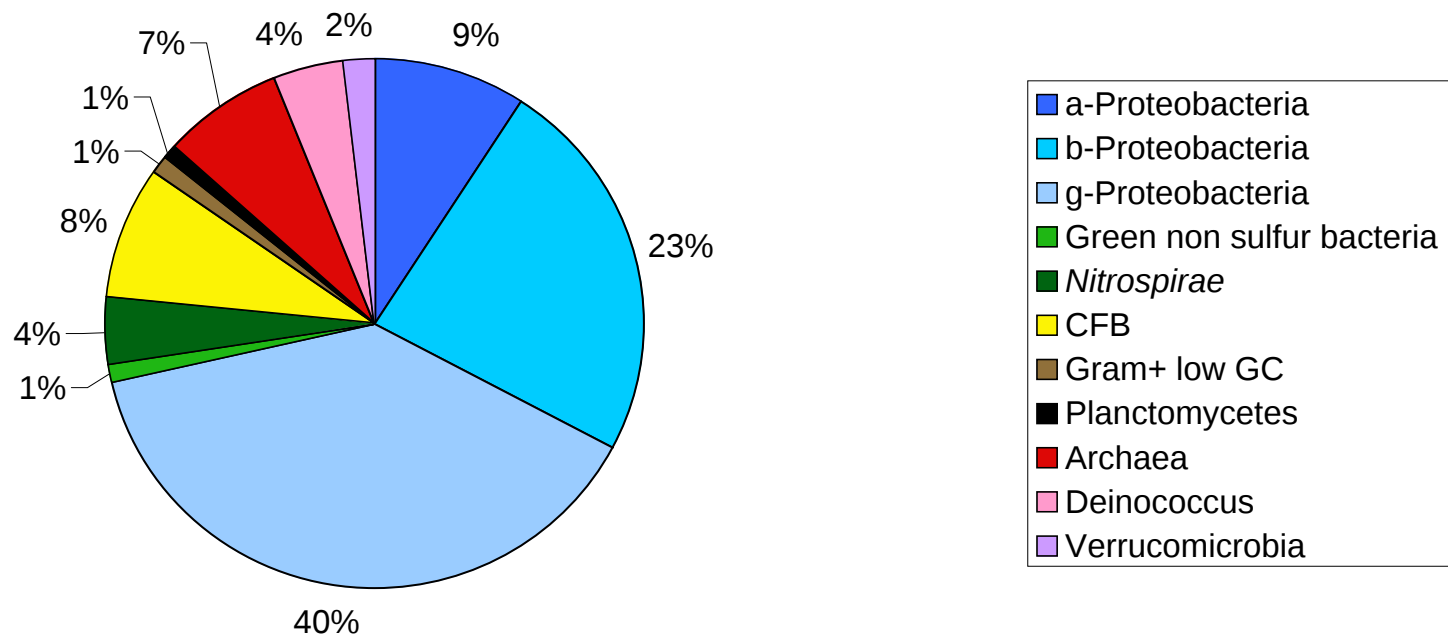
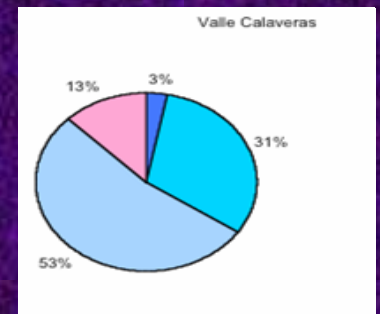
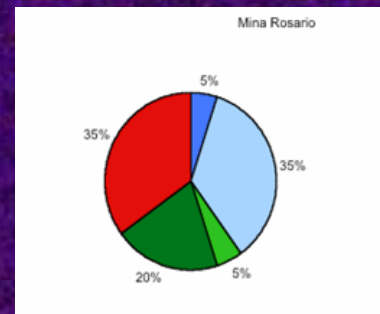
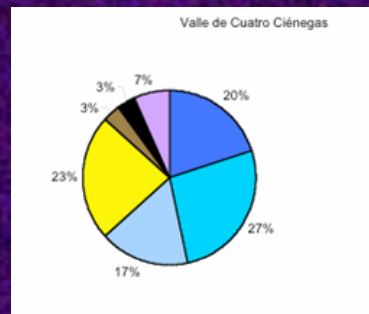
At least 4 valleys are connected



Sequences as a way to describe diversity



Phylogenetical affiliation of 16S rDNA 250 cultivated strains

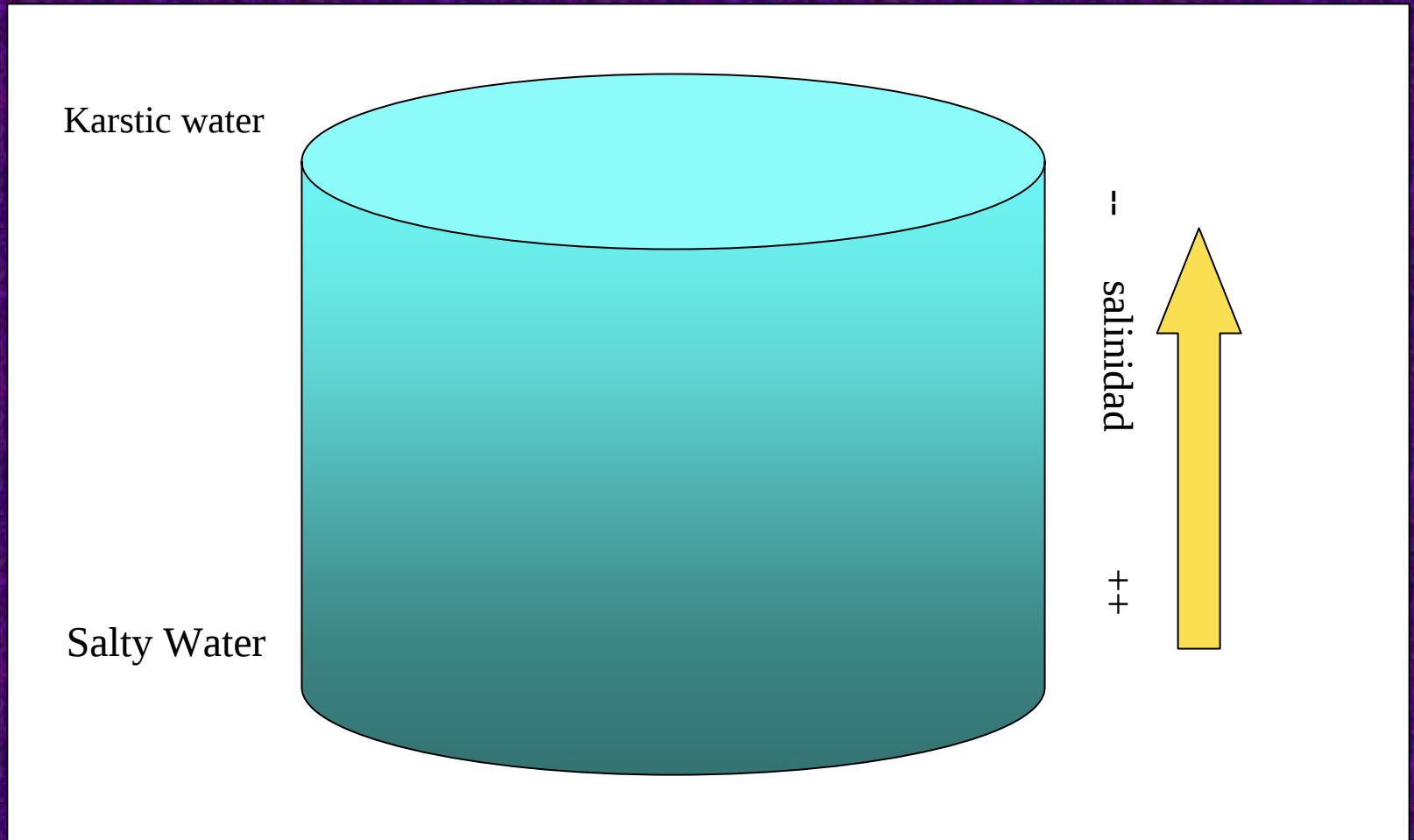


Phylogenetical affiliation of 16S rDNA clone library 96 clones

How can we explain
so much diversity
in an extreme
environment?

Even if we are sub sampling it

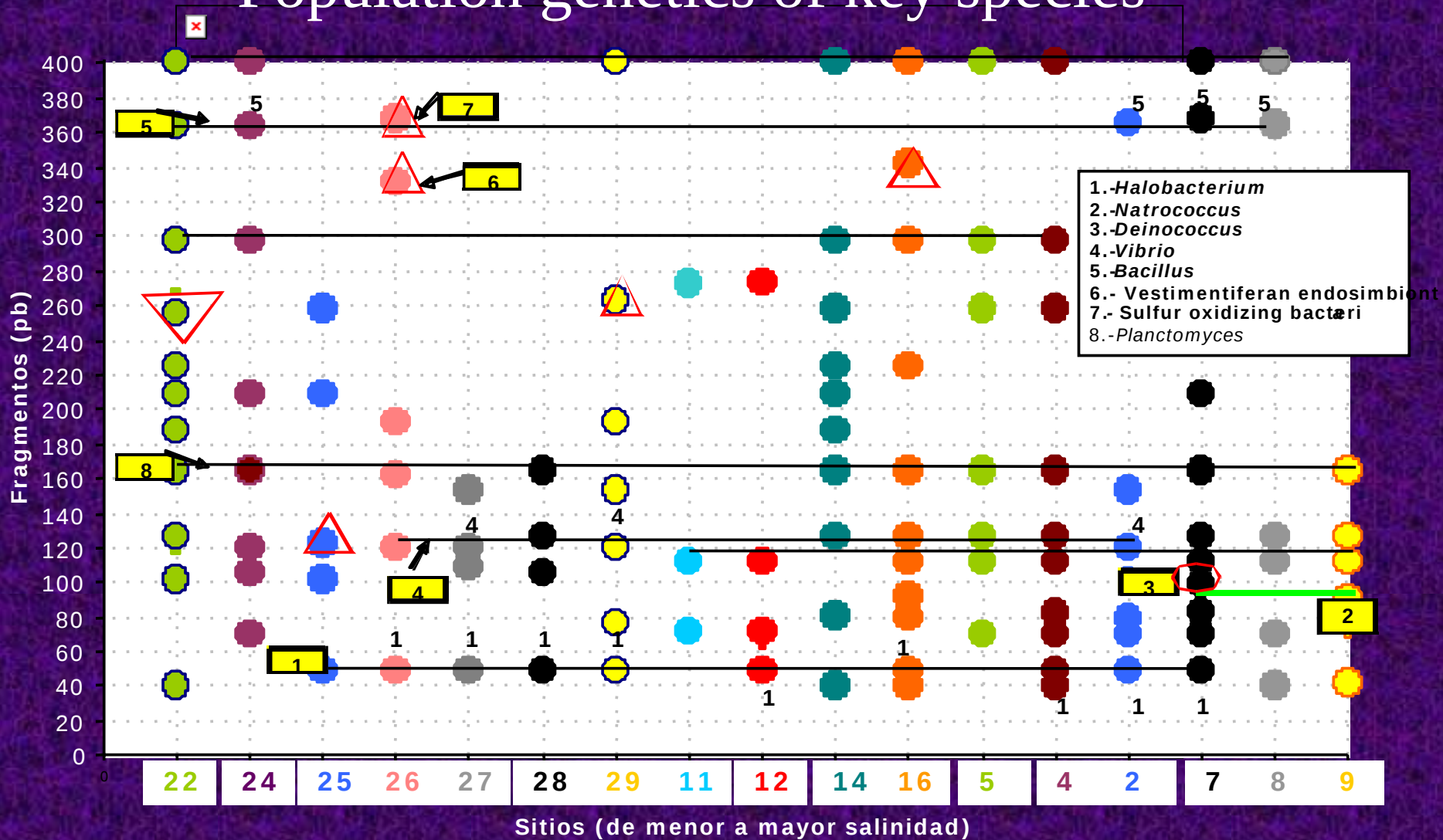
Interphases are the key?





Or random process as genetic
bottle necks and high
extinction/speciation process?

Population genetics of key species



TRFLP is used to identify the common peaks in an environmental gradient

Our favorites

- *Bacillus*: 18 species including several marines (*B. Aquamaris* (yellow) has an ample distribution). 1000 isolates

species concept

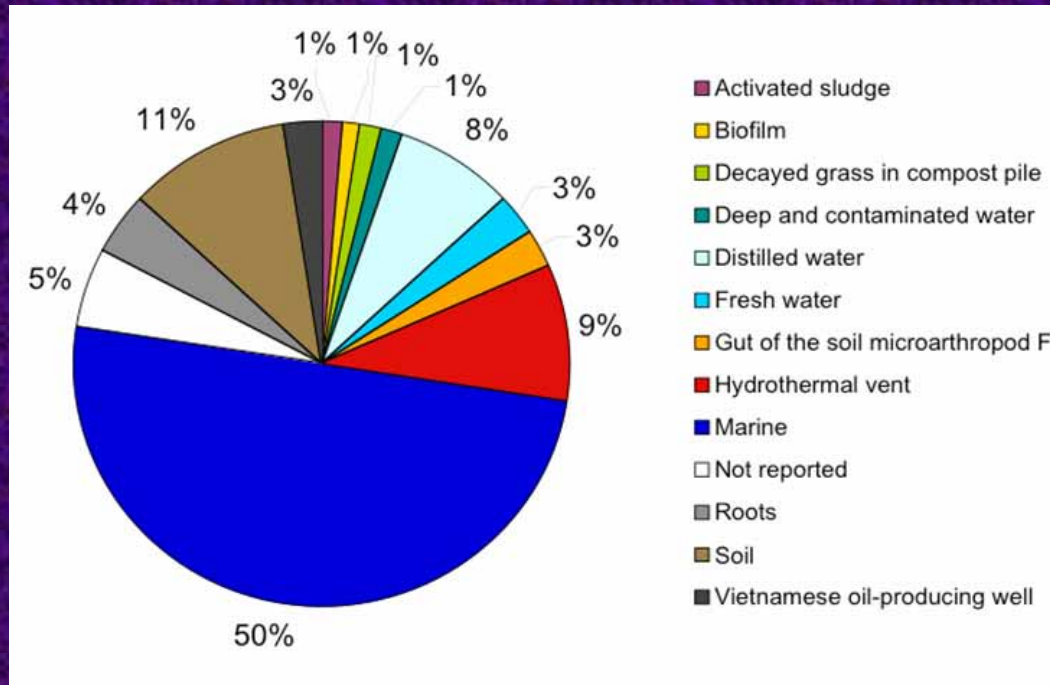
- *Aquatic rhizobiacea*
- *Pseudomonas*

Population structure and Gene flow

250 strains of each genera to
sequence at least 4 genes for
molecular population genetics.

But more
interesting

We found the sea at
CCB!!!



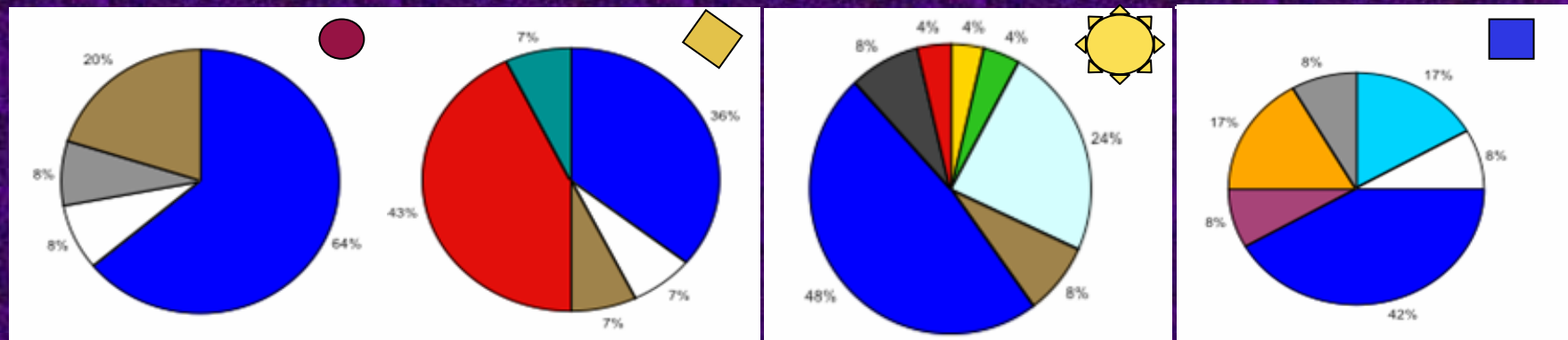
Total sample

Symbols of sampled valleys

Valle de Cuatro Ciénegas
 Valle del Mundo
 Valle Calaveras
 Mina Rosario



16S sequence affiliation total and by valley



Valleys samples

Symbols of sampled valleys

Valle de Cuatro Ciénegas

Valle de la Lluvia

Valle Calaveras

Mina Rosario



RDB data on Source of closest relatives

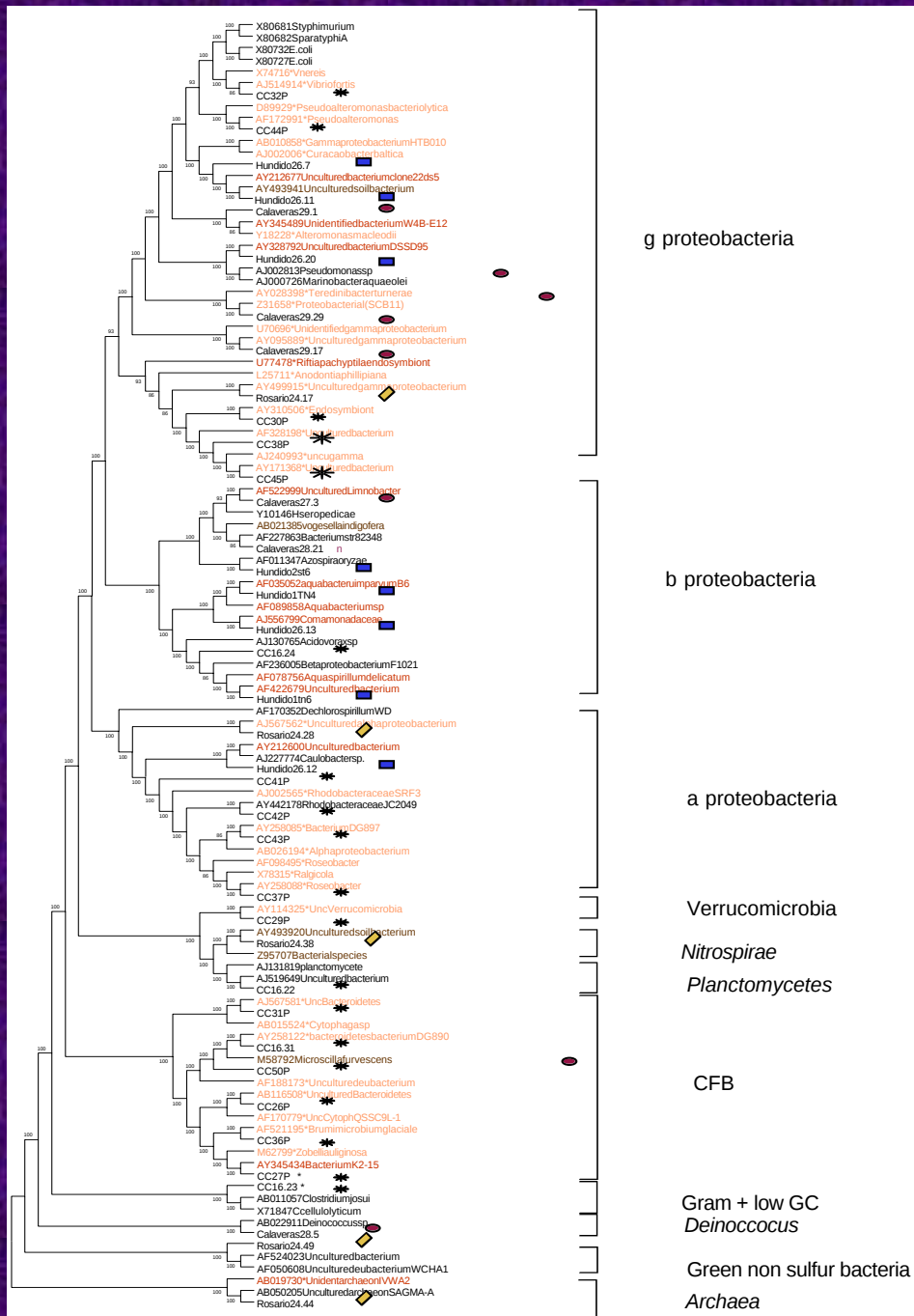
Marine

Soil

Fresh water or mineral water

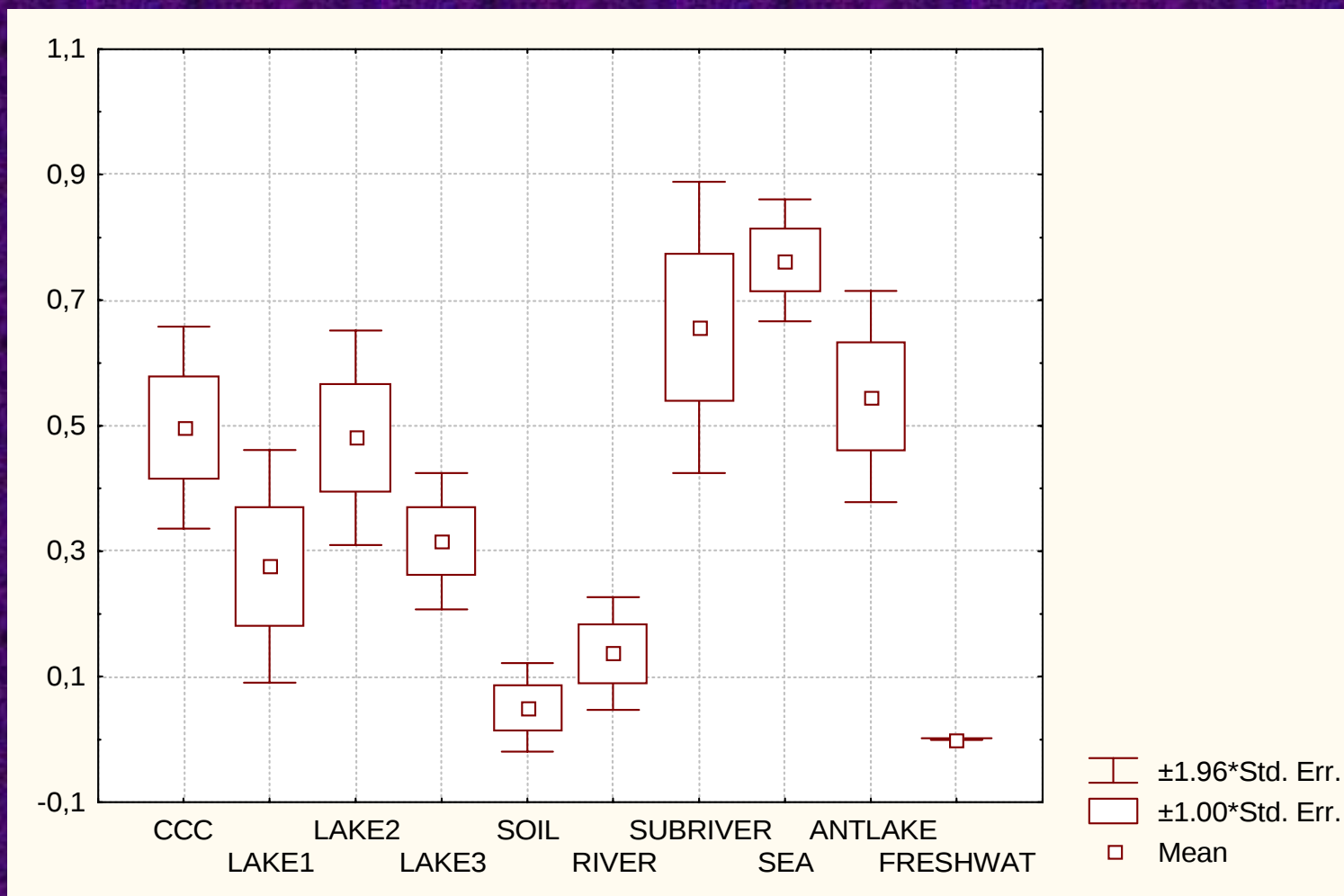
Volcanic

Not reported or others



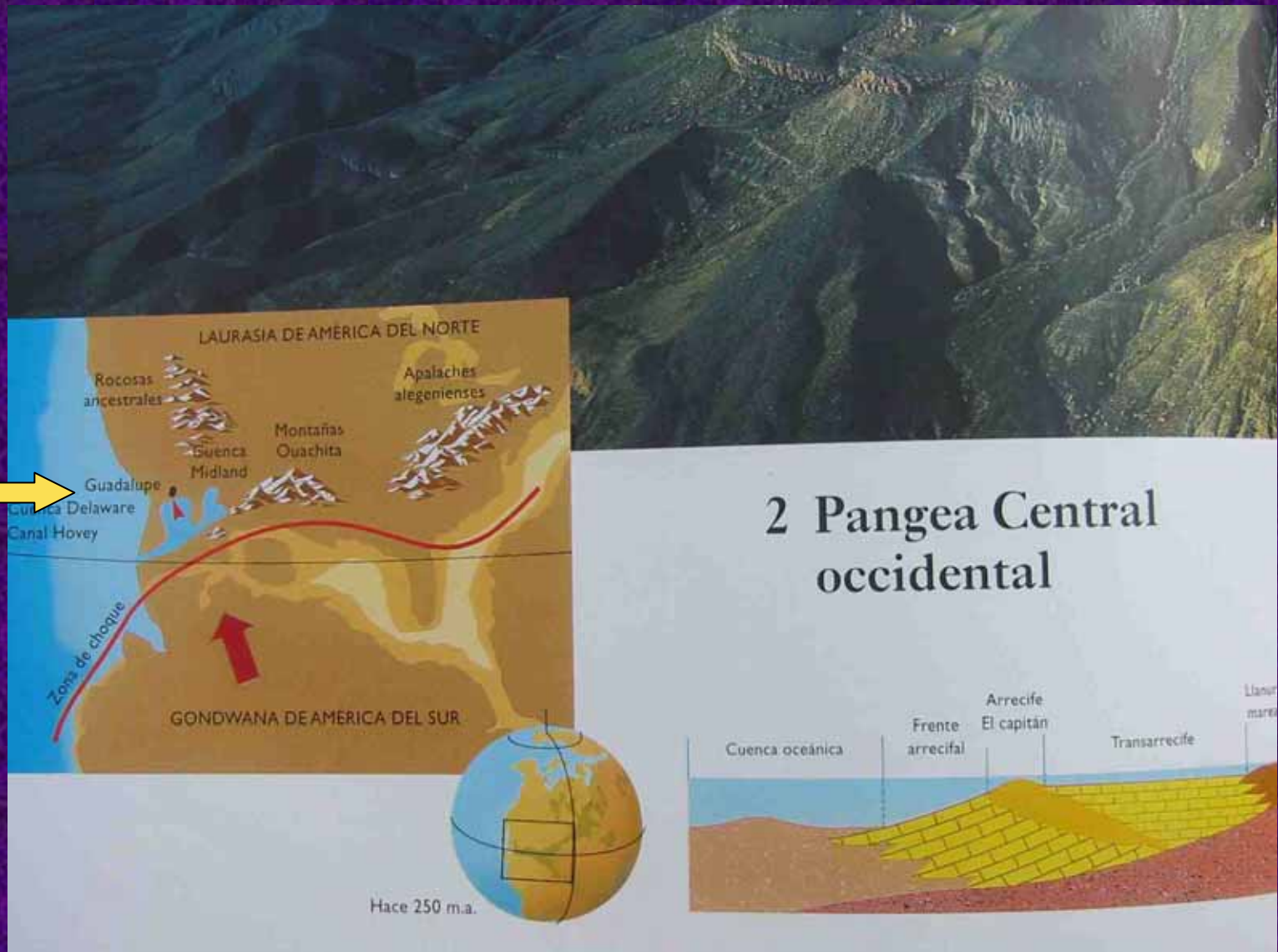
Author	N analyzed clones	Source of clones
Quiza2004 (CCQ)	35	Ponds, Cuatro Ciénegas Cahuila
Asanmayor (LAKE1)	21	Sulfurous lakes: Gs-lake and Miralake Spain
Emergasso (LAKE2)	31	Atacama desert, Northern Chile
Umayoun 2002 (LAKE3)	63	Mono Lake, California
Buske 1997 (SOIL)	39	Soils of the arid southwestern United States
Yokigishi 2002 (RIVER)	27	Changjiang River, China
Engert 1998 (SUBRIVER)	16	Sulphur river, Parker cave, Kentucky
Michard 2001 (SEA)	68	Cariaco Basin
Rowman 1999 (ANTLAKE)	30	Hypersaline antarctic lakes
Williams2004 (FRESHWATER)	17	Drinking water in a distribution system simulator

Indice de que tan marinas son en una matriz: 1 mar 0 continente

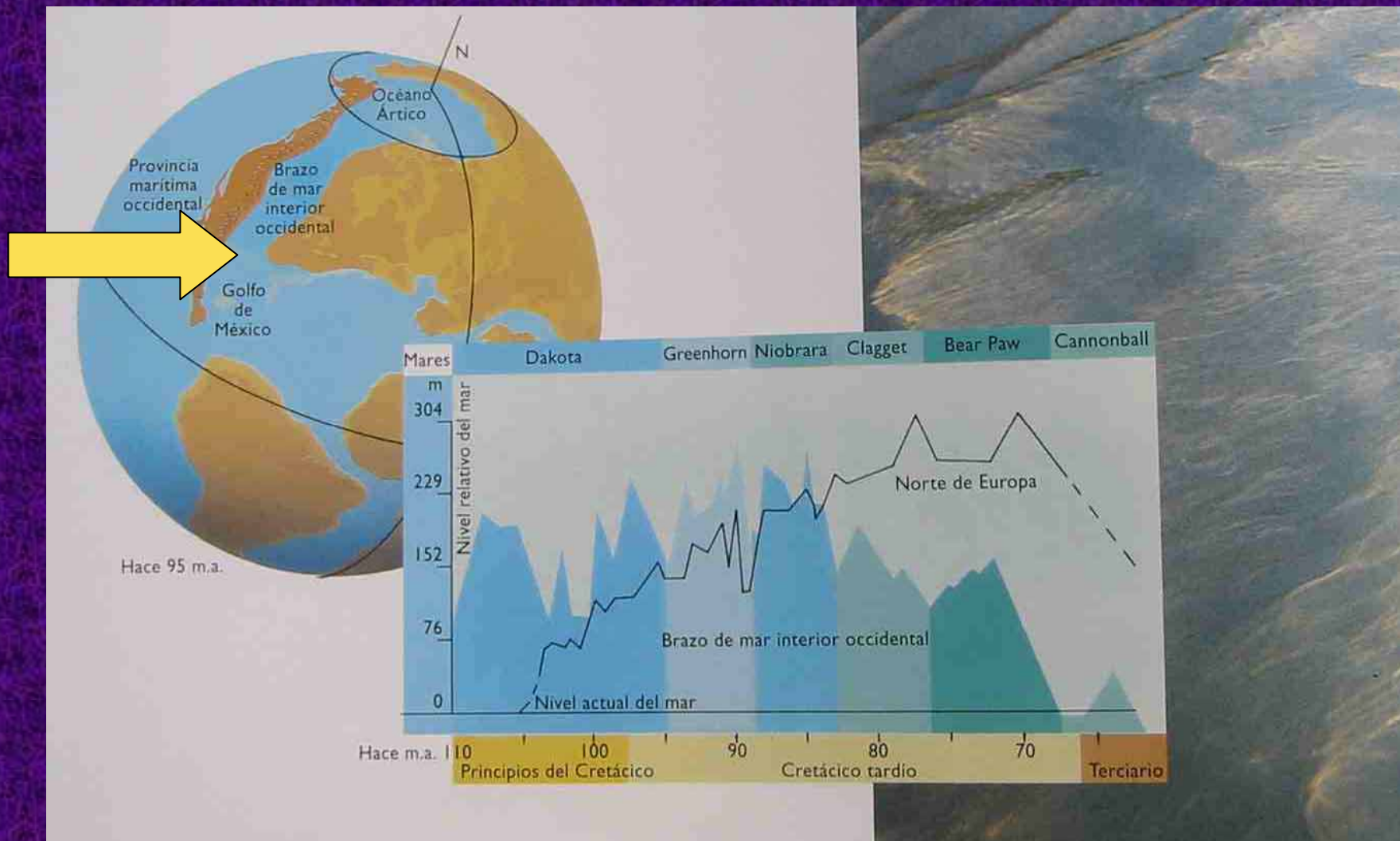


Comparación de clonas del mar vs de agua continental

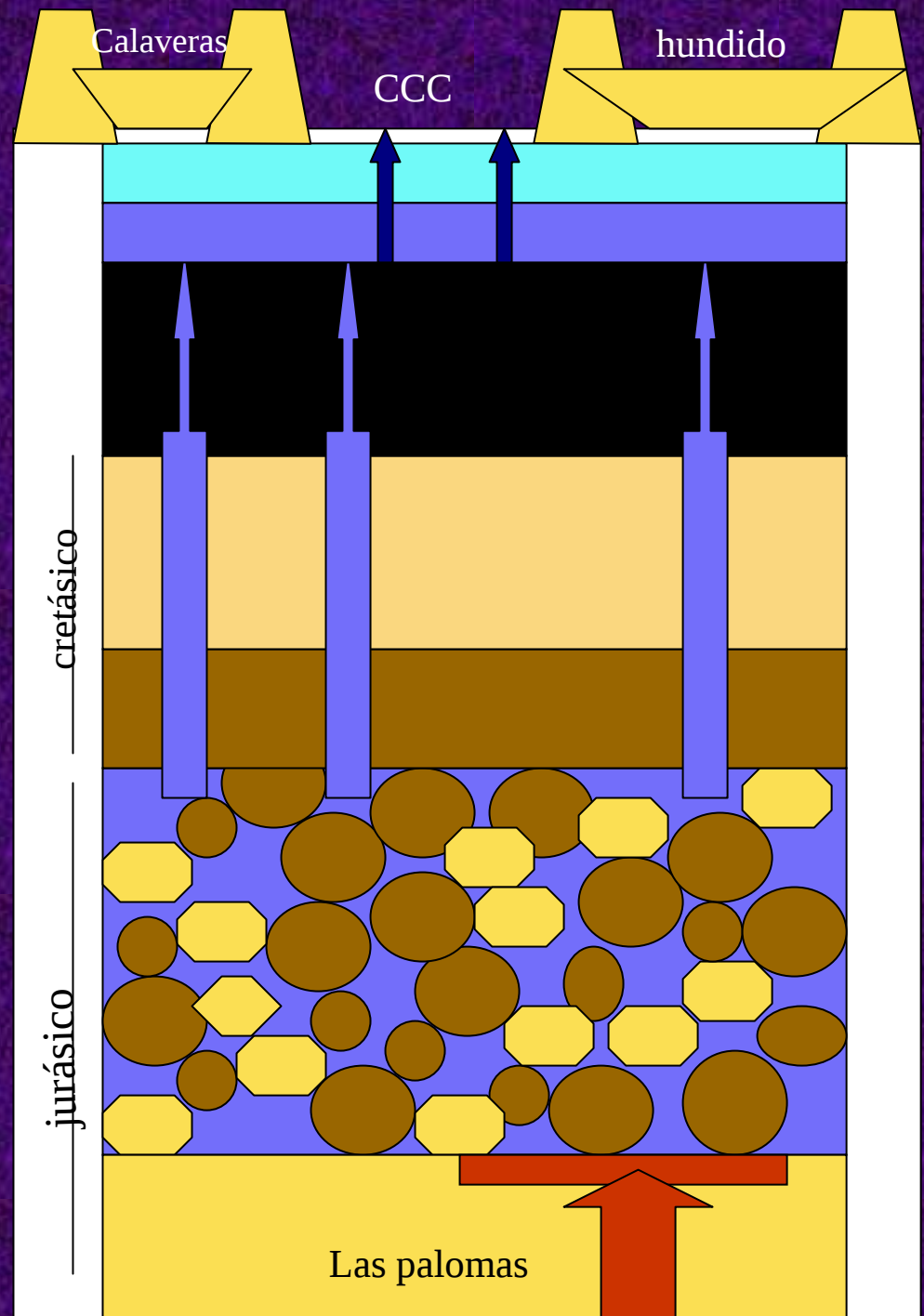
How this could
happen?



200 million years ago



How the sea
could be
stored and
preserved
alive?

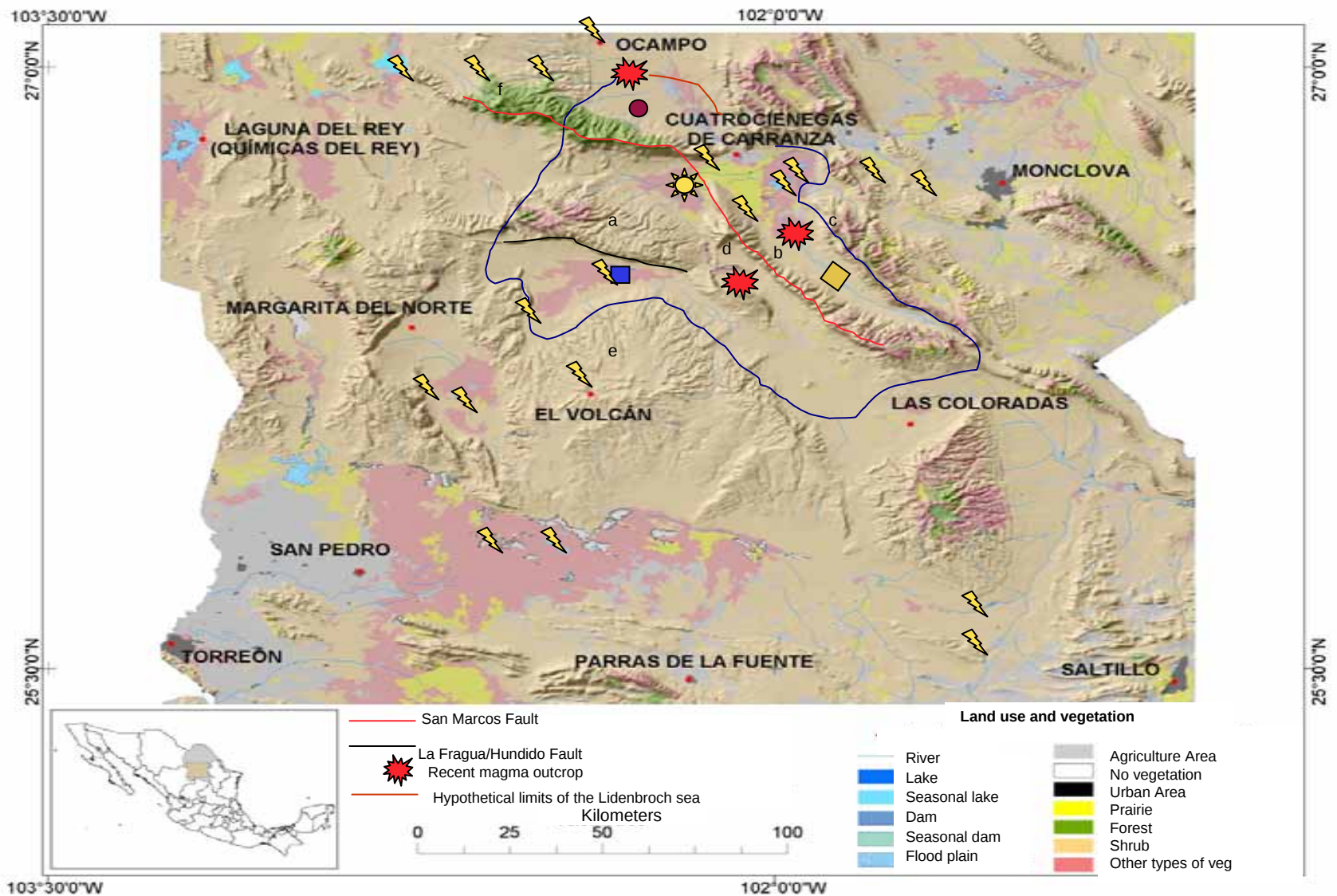






We are
looking for
the edge of
the sea

Sampling april 2004



Key questions:

Is every thing small
everywhere?

Is every thing small as
everybody else?

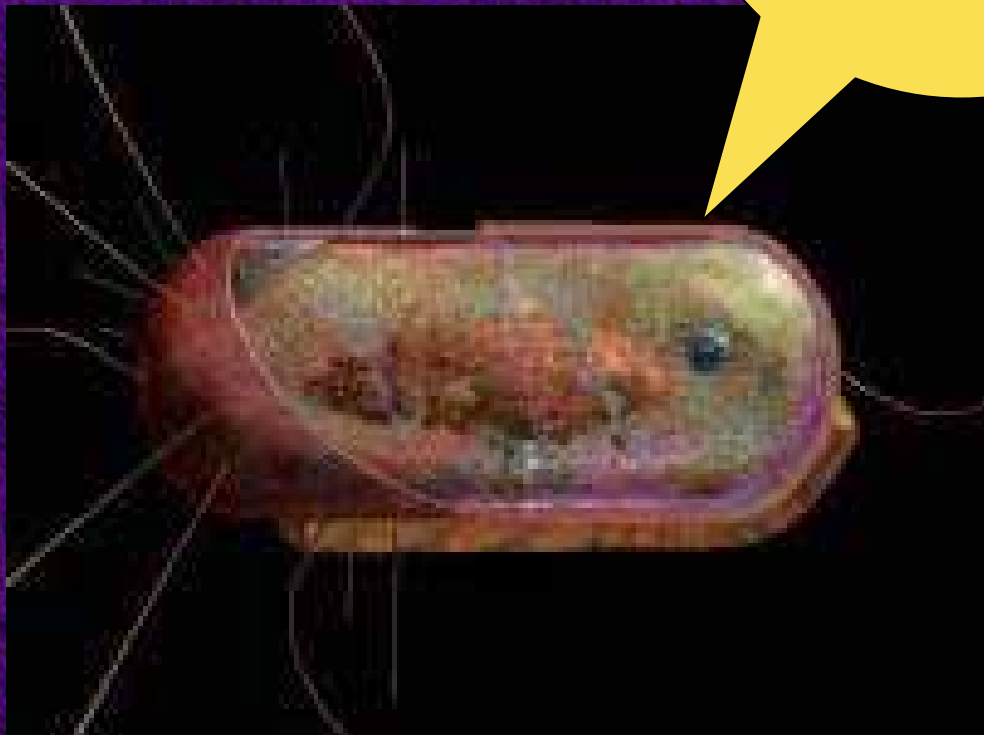
- Why we care?

- Because these are key questions in microbial ecology

- Because molecular data on the size of the aquifer is the main evidence to stop the agricultural development in the area.



I do not know
anything about
politics, and I
am an honest
creature



Thanks

NASA, UNAM, SEMARNAT, SEP,
CONACYT and CONABIO \$\$\$\$\$

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CARSON, JACK FARMER, CAROL TANG,
PETER ROOPARINE, ELVA ESCOBAR,
JAVIER ALCOCER, LUISA FALCON.

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