Protecting a window into the ancient Earth:

Towards a Precambrian Park at Cuatro Cienegas, Mexico.

Valeria Souza[1], Luisa I. Falcón[1], James J. Elser[2], and Luis E. Eguiarte[1]

[1] Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM AP 70-275 Coyoacán, Mexico D.F., 04510, Mexico.

[2] School of Life Sciences, Arizona State University, Tempe AZ, 85287, USA.

Conservation biology usually focuses on landscapes of scenic value or those containing particular endangered or charismatic macrobiota, usually animals and plants. However, what if conservationists had an opportunity not only to preserve an ecosystem that harbors not only rare species or valued scenery but also to protect a unique window into Earth's deep past? Such a situation is available in the Cuatro Cienegas basin (Coahuila, Mexico; "CCB" hereafter). In this paper we describe the opportunities and challenges associated with conservation of a habitat that presents a unique glimpse of a microbe-dominated world last seen in the Precambrian, one where a unique combination of chemical and physical conditions sustains ecosystems similar to those present when animal-dominated food webs first emerged in Earth's history. In doing so we make a case for expanded protection of this unique valley and call for the establishment of the "Cuatro Cienegas Precambrian Park" that will greatly increase protection of the water supply that sustains Cuatro Cienega's aquatic environments.

Charismatic landscapes and macrobiota:

The CCB is in central Mexico in the state of Coahuila (Figure 1). The valley is located at ~740 m above sea level and measures approximately 30 km by 40 km, surrounded by high mountains (>3000 m). It is an enclosed evaporitic basin that receives ~150 mm of annual precipitation. Despite the dry climate, the CCB harbors an extensive system of springs, streams, and pools ("pozas") interconnected with subsurface caverns, sinkholes and other limestone and evaporitic karst features of significant scientific interest (Minckley ADDIN1969, 1992). Radiocarbon dating of sediment cores indicates that some have existed for thousands of years, perhaps as long as 31,000 (Meyer 1973).

Older travertine hot-spring deposits and lower temperature tufa mounds are found in association with some active springs but also occur in the older, dry portions of the basin floor, indicating a long-term persistence of aquatic habitats in the basin (Minckley 1969). Although preliminary models have been proposed (Cortes et al. 2002, Johannesson et al. 2004), the hydrology of the region is still poorly understood and before recently, its microbiota have not been characterized, despite the fact that current evidence argues for a unique evolutionary history.

Documented biodiversity includes more than 70 endemic species of aquatic vertebrates, distributed among a wide variety of aquatic and terrestrial ecosystems. From this perspective, CCB is widely regarded as a biodiversity oasis within the Chihuahuan desert. In total, the combination of abundant water in an arid region along with long-term stability in this deep valley setting has created an array of unique habitats and plant and animal species that have already motivated important conservation efforts. Specifically, an 85,000-ha area is currently designated as a federal "Area for the Protection of Flora and Fauna" (Gomez-Pompa and Dirzo 1995), a designation that accommodates conservation of natural systems alongside sustainable development activities. The World Wildlife Federation, Mexico's National Commission on Biodiversity (CONABIO), and non-government organizations such as PRONATURA and the Nature Conservancy have all classified the Cuatro Cienegas valley as globally outstanding due to its high species endemism and recent history of evolutionary radiations. However, as will be discussed below, it is not clear that this level of protection is sufficient to preserve the ecological integrity of CCB in the long term.

Cryptic biodiversity in the microbiota: clues to an ancient marine influence?

The extensive biodiversity at CCB extends well beyond the macrobiota. Using modern techniques of molecular evolutionary biology, Souza and colleagues (Souza et al. 2006) have begun the daunting task of characterizing CCB's microbiological diversity. In building CCB's first clone library, they obtained a total of 98 16S rRNA partial gene sequences representing 38 unique phylotypes from 10 major lineages of Bacteria and one of Archaea (Figure 2). They were able to bring 350 strains into cultivation, of which were 250 distinct and diverse phylotypes with the most common ones being related to *Bacillus*, *Pseudomonas*, *Vibrio*, *Rhizobiace*, *Planctomyces*, γ-Proteobacteria, and Gram-positive bacteria (Figure 2). When these sequences were compared with published data on nine libraries prepared from aquatic or terrestrial environmental samples, the unique nature of the CCB microbiota became apparent. Specifically, it was found that nearly half of phylotypes from the CCB were closely related (90-99% sequence similarity) to organisms or cloned 16S rRNA gene sequences from marine environments. This finding was consistent with the identities of cultivated bacteria, which included strains 95%-98% similar to marine organisms such as *Bacillus aquamaris*, *Halomonas elongata*, and *Chromohalobacter canadensis*. These results yield the surprising conclusion that aquatic ecosystems at CCB harbor a diverse microbial community that includes organisms that are typically found in soil and fresh water environments but where a surprisingly large number of others are characteristic of marine environments, such as the cold northern Pacific, the Arctic, the Baltic sea, and marine hydrothermal vents.

This unexpected predominance of taxa with marine microbial affinities was surprising because the chemical composition of CCB's waters does not resemble seawater and the basin is located 800 km from the Gulf of Mexico. Souza and colleagues hypothesized that some portion of the biota and water of CCB may be derived from microbes and water entrapped when the Mesozoic strata that underlie the CCB were formed and which have been released more recently during active and ongoing subsurface karstification of limestones and evaporites. It is important to note that they have successfully cultured several of the taxa having marine affinities, indicating that their methods have detected live bacteria and not just fossilized DNA sequences. Supporting the inference of marine affiliation of the microbes of CCB, the results from 'nearest neighbor' analysis of data from published studies of microbial diversity in diverse environments showed that clones obtained from seawater samples were most closely related to known marine bacteria, while samples from continental freshwater contained non-marine prokaryotes, independent of their salinity. However, analysis of clones from CCB using the same procedure indicated that a large number of the bacteria from habitats in CCB were most closely related to marine bacteria.

How might marine-affiliated microbes have come to inhabit the springs at CCB? Souza et al. noted that, at the end of the Paleozoic, the supercontinent Pangea fragmented to form two great landmasses, Laurasia (north) and Gondwanaland (south). With the separation of Laurentia (North America) from Eurasia during the Jurassic, the North Atlantic and Gulf of Mexico began to open, eventually connecting to the ancient Tethys sea, through the Mediterranean to the Pacific (CENTENAL 1975). In the CCB region a regional uplift called the Coahuila Island was present throughout the late Jurassic (Tithonan) to early Cretaceous (Neocomian) periods. The sedimentary sequences exposed in the surrounding mountains represent fluvial and shallow marine sediments of limestones and sulfates, entrapping interstitial marine waters (CENTENAL 1975). Subsurface dissolution of these formations appears to be the source of high sulfate and bicarbonate concentrations of CCB's surface waters. Ancient water composition likely changed due to ionic exchange with the surrounding rocks, forcing the ancient marine microorganisms to adapt to this new environment and to diverge from their ancestors. We see the descendants of these marine microbes throughout the Cuatro Cienegas basin today.

Stromatolite-based food webs under severe P-stress:

One feature for which CCB is world-renowned and which offers yet another unique window into Earth's deep past is the presence of diverse, calcifying microbial mats that produce laminated structures in pozas and streams throughout the basin. These "living stromatolites" have been the focus of early studies (Winsborough 1990) and more recently have formed the focus of NASA-funded research on possible factors affecting the Precambrian-Cambrian transition. This has been possible because the stromatolites of CCB, unlike the more famous stromatolites of Shark's Bay (Australia), are unusual in supporting a metazoan-dominated food web in which the endemic snails *Nymphophilus minckleyi* and especially *Mexithauma quadripaludium* directly graze the stromatolite biomass. This offers a glimpse, perhaps, of what the earliest animal-dominated food webs on Earth may have been like. In developing this work, Elser (2003) proposed that, since nutrient-limited algae produce biomass that is of poor quality for animal growth, perhaps the early evolution of metazoans was delayed because early Earth was severely nutrient-limited. Might such "stoichiometric constraints" have limited the expansion and diversification of animals before 600 mya? Water sampling at CCB has revealed that concentrations of dissolved forms of nitrogen (e.g. NO_3) are generally very high but that concentrations of phosphorus are extremely low. These very high N:P ratios suggest that P is likely to be severely limiting to stromatolitic cyanobacteria and algae. This suggestion has been strongly supported by P enrichment experiments using oncoid stromatolites in the Rio Mesquites (Figure 3). The experiments demonstrated major changes in microbial community composition (large increases in diatom abundance), productivity, and C:P and N:P ratios after P enrichment (Elser et al. 2005a).

But how did P enrichment affect the performance of the grazing snails? In a series of experiments, it was shown that small P enrichments produced improvements in snail growth rates and survivorship, consistent with the hypothesis of stoichiometric constraints, but larger P enrichments resulted in decreased snail growth rate and increased mortality (Elser et al. 2005b, 2006), indicating that these snails have evolved under long-term conditions of low dietary P supply. These studies lend some credence to the proposal that P availability may have played a role in early evolution of metazoans. Importantly, they also call attention to the very high sensitivity of the ecosystems of CCB to nutrient enrichment, an important message for the conservation of these unique systems and the ecological interactions they support.

Threats from beyond and future prospects:

The work reviewed here illustrates the numerous ways in which the valley of Cuatro Cienegas is a kind of "lost world" where scientists and other visitors can catch a glimpse of life when microbes dominated the Earth. As noted above, these unique features have earned an important level of protection for CCB in the form of federal designation as a protected area. However, this protected area encompasses a list of protected animals and plants as well as parts of the valley floor containing the springs. It does not protect the water or the streams themselves. It does not involve the broader landscape that includes the subterranean aquifers, as yet unstudied, that bring water to the basin's many springs. As a result, it is now becoming apparent that the level of protection for CCB is insufficient. Similar to situations occurring with increasing frequency in various other arid regions of the world (5, 48, 49), agricultural development and associated water extraction in the region have placed new pressures on the ecological integrity of the unique ecosystems of Cuatro Cienegas. In 2001, ranchers associated with two dairy consortia abandoned operations near Torreón due to shortage of water and the presence of arsenic and heavy metals in the dwindling groundwaters. In late 2002, they began operations in the Valle del Hundido (Fig. 1b), close to the CCB Protected Area, proceeding without environmental impact assessments as required by Mexican law. Ten thousand hectares of alfalfa fields with 106 wells were established based on a claim that there was no relation between the aquifers of the CCB and El Hundido valleys. Observations during summer 2006 indicate that this has resulted in drastically lower water flows in the western basin of CCB, where 70% of wetlands within the Churince Spring drainage have been dried. Despite record rains during summer 2006, water levels in the Churince system have not yet recovered. Finally, water diversion in canals has also been increased in other areas of the basin while pressures to expand gypsum mining and unregulated recreational tourism appear to be building. Concern about the environmental impacts of this water extraction on the CCB protected area has attracted considerable media attention (numerous press releases and articles in La Jornada, Milenio Torreon, La Palabra, El Universal, Reforma and Excelsior during 2002-2007 as well as many TV reports in TV Azteca, Televisa and Canal 11 in late 2006). Consequently, for the first time in the history of Mexican environmental policy, legal injunctions halting this water use were issued. This has culminated in a presidential decree signed on February 24th, 2007 to protect CCB not only legally but with a considerable budget allocation to implement improved and sustainable irrigation systems, to convert alfalfa fields to cultivars that consume less water, to re-forest diminished mesquite populations, and to develop a plan for responsible ecotourism.

It is notable that disputes about the sources of water reaching the CCB basin have been at the center of this controversy but no comprehensive and scientifically independent assessment of the hydrology of CCB has yet been performed. However, molecular data collected by Souza et al. (2006) show a very high degree of similarity of lineages of Archaea and Bacteria between CCB and adjacent valleys: 90% of the numerically abundant phylotypes were common to all sites. These findings suggest that lineages were derived from a common source or there has been migration between the three valleys that share a deep aquifer. Preliminary geohydrological studies of the region do suggest that, while a considerable fraction of the CCB groundwater may originate from local recharge in mountains surrounding the valley, an additional fraction is derived from inter-basin flows of older water (K.H. Johannesson, pers. comm.; M.J.M. Rodriguez, pers. comm.). Nevertheless, the main producer of dairy products in the region has recently agreed to close its wells in the Hundido valley and cancel the contract with other alfalfa growers in the valley in order to protect the CCB oasis of diversity.

Our microbiological data, along with the low hydrologic recharge of the superficial aquifers and geologic structure of the region, indicate that serious concerns are warranted regarding the impacts of regional water extraction on the unique ecosystems in the Cuatro Cienegas basin and nearby valleys. Our results also highlight the need to incorporate a regional perspective in legal designations that seek ecosystem conservation (50), as the critical processes and environmental factors sustaining local habitats and biotas often can be distant and complex, a principle applicable to numerous aquatic systems worldwide.

We, the undersigned scientists and visitors to Cuatro Cienegas, wish to express our marvel at this extraordinary valley, and our profound desire to protect its irreplaceable natural environment for generations to come. We support all efforts to safeguard the dozens of species of flora and fauna that are unique to the area, sustained by the underground waterways. We also seek to safeguard the extraordinarily rare communities of microbes, which create the spectacular stromatolites that grow in the pozas and provide an amazing glimpse into some of Earth's earliest forms of life. And we seek to safeguard Cuatro Cienegas as a living laboratory for scientific discovery, as an educational space and as a living land for its residents, visitors, and this extraordinary wildlife.

Recent declarations by the Federal Government of Mexico suggest that the extraordinary importance of the Cuatro Cienegas ecosystem is beginning to be appreciated and that concrete financial, judicial and regulatory steps will be taken to stop water extraction in el Hundido in the immediate future. While we welcome those measures, we also think that Cuatro Cienegas cannot survive as a desert oasis without the abundant water circulation that created it. This vital and invaluable environment is now under grave threat from uncontrolled water use and intensive agriculture that are depleting its waters. We therefore ask the help of all people in the relevant governmental institutions, non-governmental agencies, commercial businesses, and local communities in securing the highest level of preservation of this unique valley along with its tiniest to largest inhabitants that all depend on its treasure of water.

Towards this end, we propose the creation of a national "Precambrian Park" that will extend conservation protection beyond the current federal protected area and that will include a prohibition on groundwater pumping in adjacent valleys along the fault system that communicates with underground hydrological systems. We also propose replacement of current inefficient irrigation practices in the valley and surrounding region with sustainable approaches, including drip irrigation and greenhouse-based hydroponics. If we start to acknowledge the environment as a natural water user, we can allocate all of the water saved by sustainable agricultural practices to replenish the basin's ecohydrological system. This will result in an improved quality of life for all of Cuatro Cienegas' residents, humans, fishes, and microbes.

Signed by

Name / email address / professional titles, affiliations (if relevant) / City, State, Country

[Note: Institutions listed below are for identification purposes only and do not constitute endorsement]

Richard E. Lenski / lenski@msu.edu / Hannah Professor of Microbial Ecology, Michigan State University; Member, National Academy of Sciences, USA / East Lansing, Michigan, USA.

Edward O. Wilson, Member, National Academy of Sciences, USA /Harvard University, Museum of Comparative Zoology

John C. Avise, Department of Ecology and Evolution, Distinguished Professor with special expertise in ecological and evolutionary genetics. Member, National Academy of Sciences, USA

Rodolfo Dirzo, Bing Professor in Ecological Sciences, Member, National Academy of Sciences, USA/ Stanford University. Conservation Ecology

Exequiel Ezcurra, Director of Biodiversity Research Center of the Californias, San Diego Natural History Museum. Conservation Ecology.

Forest Rohwer, forest@sunstroke.sdsu.edu, Assistant Professor, San Diego State University; AAAS Fellow and Gordon and Betty Moore Investigator/San Diego, CA UAS. Viral ecology.

Madeleine LENSKI / lenskiM@msu.edu / Academic Specialist, Department of Epidemiology, Michigan State University / East Lansing, MI. USA.

Brandon Gaut, Department head, Ecology and evolutionary Biology UCI. expert in molecular evolution of plants.

Susan V, Bryant, PhD / svbryant@uci.edu / Professor of Biological Sciences, University

of California, Irvine, Aldrich Hall 155 / Irvine, Califorinia 92697-3175, USA.
Dr. David Gardiner, dmgardin@uci.edu, Research Biologist, Department of Developmental and Cell Biology and The Developmental Biology Center, University of California Irvine
Rudi C. Berkelhamer, University of California at Irvine, Senior Lecturer in Ecology & Evolutionary Biology and Associate Dean of Undergraduate Education, PhD in Zoology with emphasis on Ecology & Evolutionary Biology.
Stephen G. Weller. Dept. of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697
Michael Travisano, University of Minnesota, Dept Ecology, Evolution, & Behavior. Expertise in experimental evolution and adaptation in microbes.
Jorge Soberon, Biodiversity Research Center, Universidad de Kansas. Biogeography and conservation.
Valeria Souza. Dept. Evolutionary Ecology, Instituto de Ecologia, UNAM, Mexico. Evolutionary microbial ecology.
Luis E. Eguiarte Dept. Evolutionary Ecology, Instituto de Ecologia, UNAM, Mexico. Evolutionary plant ecology.
Luisa Falcón, Dept. Evolutionary Ecology, Instituto de Ecologia, UNAM, Mexico. Cianobacterial Ecology, Nitrogen cycle.
Evan W. Carson, Postdoctoral Fellow in the Department of Biological Sciences at the University of South Carolina, expert in the fishes and snails of Cuatro Cienegas.
Jim Elser. School of Life Sciences, Box 874501 Arizona State University, expert in limnology and in the oligotrophic systems of Cuatro Cienegas

Thomas E. Dowling, School of Life Sciences, Box 874501 Arizona State University, expert in the fishes and snails of Cuatro Cienegas.

Mya Breitbart, College of Marine Science, University of South Florida, microbiologist expert on virus.

Paul Davies, Director, BEYOND: Center for Fundamental Concepts in Science, Arizona State University.

Maude Tenaillon, tenaillon@moulon.inra.fr, CNRS researcher, Gif sur Yvette, France. Molecular evolution of plants

Olivier Tenaillon, tenaillon@bichat.inserm.fr , INSERM, Université Paris 7, Paris, FRANCE, Microbial evolution and adaptation models.

Sergio Revah, Department head of Process Engineering, Universidad Autónoma Metropolitana- Cuajimalpa.

Gabriela Olmedo Alvarez, golmedo@ira.cinvestav.mx, Dept. Genetic Engineering, Cinvestav-Campus Guanajuato, Mexico.

Janet L Siefert, Faculty Fellow, Dept of Statistics, Rice University, Houston, TX, Evolutionary biologist.

Barbara Winsborough,Winsborough Consulting, Austin, Texas, USA.Aquatic ecologist and specialist in the taxonomy and evolution of the diatom flora of the Cuatro Cienegas Basin

Patricia Koleff Osorio, Directora Técnica de Análisis y Prioridades, CONABIO, Mexico

Roberto Salinas, Mexico DF.

Francisco Valdés Perezgasga, ONG Pro-Defensa del Nazas, A.C.Torreon, Coahuila, Mexico.

Ismael Cabral Cordero, icabralc@gmail.com, Desarrollo ConCiencia ONG, Nuevo León, México.

Mari Nieves Autrey, Historian, Diputada Federal plurinominal por el PAN, Director and editor of Arqueología Mexicana Member of several conservation ONGs

Federico Reyes Heroles, opinion leader, Mexico

Beatriz de Reyes Heroles, historian, Mexico

Sioban Odonogue, San Miguel Allende Mexico

Teresa Ludlow, Mexico DF.

Valente Souza. Mexico City.

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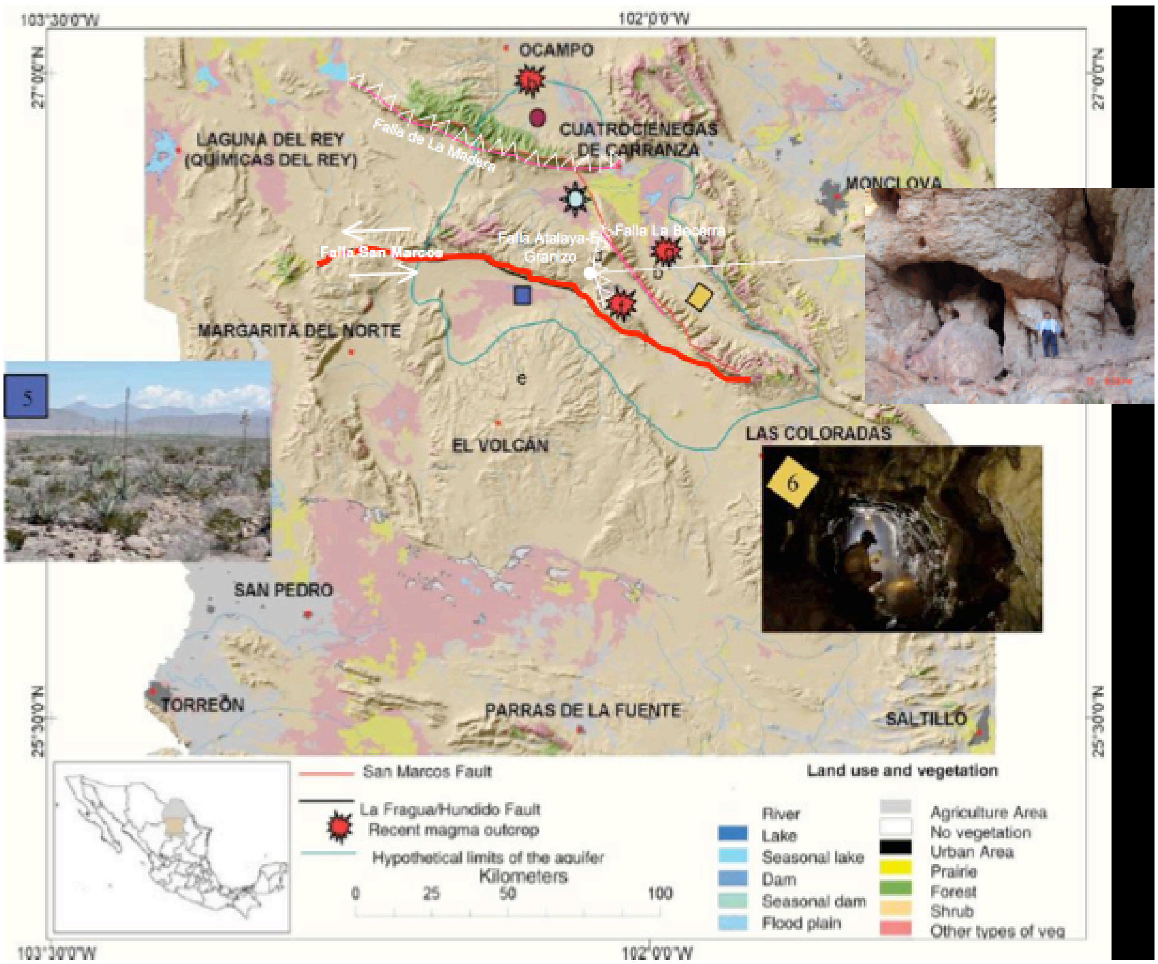
Figure 1. Map of the Cuatro Cienegas basin and surrounding region. Sampling sites for the study of Souza et al. (2006) are indicated by different symbols: Red circle - Calaveras valley, Star - CCB, Square – El Hundido valley, and Diamond - Rosario Mine. At the top and side of the figure are photographs of habitats at each locality: (1) new alfalfa field in Calaveras valley; (2) El Mojarral headspring in the CCB (photo by Paolo Petrignani, La Venta Exploring Team; www.laventa.it); (3) intermediate lagoon along a flow path from one of the CCB's surface springs (photo by J.J. Elser); (4) desiccation pond in the flats of the CCB; (5) native vegetation in El Hundido valley with an alfalfa field in the background (photo by Dean Hendrickson, University of Texas); 6) subsurface sampling site in the El Rosario mine (photo by Paolo Petrignani, La Venta Exploring Team; www.laventa.it). Major mountain ranges are also shown: a, La Fragua Mountains, b, San Marcos Mountains, c, Sierra la Purisima, d, Sierra el Granizo and e, Sierra de Australia (the "Island of Coahuila"). Recent magma outcrops in the vicinity of the CCB are indicated by a red "blast" symbol and identified as: f, El Jabali valley (Rodriguez-Martinez, personal. observation), g, San Marcos valley and h, Calaveras outcrop. The red and black lines indicate major active faults while the blue line delineates a hypothetical boundary for the hydrological system of Cuatro Cienegas.



103°30'0"W
102°0'0"W
27°0'0"N
25°30'0"N
OCAMPO
CUATROCIENEGAS DE CARRANZA
LAGUNA DEL REY (QUIMICAS DEL REY)
Falla de La Madera
MONCLOVA
Falla San Marcos
Falla La Becerra
MARGARITA DEL NORTE
EL VOLCÁN
LAS COLORADAS
SAN PEDRO
TORREÓN
PARRAS DE LA FUENTE
SALTILLO
5
6
San Marcos Fault
La Fragua/Hundido Fault
Recent magma outcrop
Hypothetical limits of the aquifer
Kilometers
0 25 50 100
Land use and vegetation
River
Lake
Seasonal lake
Dam
Seasonal dam
Flood plain
Agriculture Area
No vegetation
Urban Area
Prairie
Forest
Shrub
Other types of veg

Figure 2. Molecular data from rRNA gene sequences in samples from the region of Cuatro Cienegas (Souza et al. 2006). The pie diagram illustrates the relative proportion of habitat affiliation assignments for the sequences based on comparison of 16S rRNA clone library sequences for CCB microbes with the Ribosomal Database Project. The meaning of each color in the diagram is shown to the right.

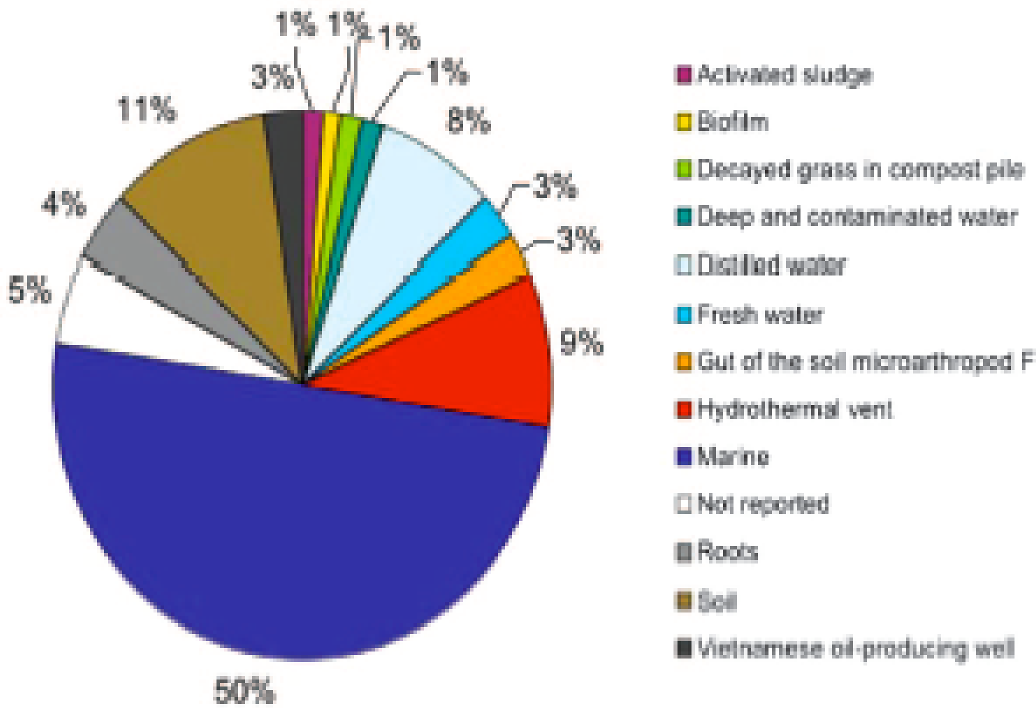


Figure 3. Phosphorus enrichment experiments at the Rio Mesquites (a) involving oncoid stromatolites ("microbialites") (b) and the snail *Mexithauma quadripaludium* (c). P enrichment greatly increased the relative abundance of diatoms in the stromatolite (d), lowered the C:P ratio of stromatolite biomass (e), and produced a unimodal response of snail production (f). In panel (d), diatoms are represented by shadings indicated by A-E at the bottom of each panel's stack; the other components are cyanobacteria. Figures are from Elser et al. (2006) except for panel (d), which is from Elser et al. (2005a)