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BIOSELEOLOGY

UNIQUE MICROBIAL DIVERSITY IN AN APHOTIC CAVE HOT SPRING

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Glenwood Hot Springs, Colorado, a sulfide-rich hot-spring system, issues from numerous springs before eventually flowing into a commercialized pool. To examine the effect of light energy on microbial community structure in such a sulfide-rich environment, we examined microbial communities from two of these springs; the photic 'drinking spring' and the aphotic 'dark-zone,' which flows through a cave. Both of these springs contain white, filamentous microbial communities, and the water flowing through them is chemically and physically identical. Molecular-phylogenetic analysis using 16S rRNA, revealed significant differences in community structure between the photic and aphotic filament communities. The filaments from the photic 'drinking spring' consisted predominantly of a single phylotype, an Epsilon-Proteobacteria related to a hydrothermal-vent symbiot. In the aphotic 'dark zone', the filamentous community displayed significant microbial diversity, with 58 phylotypes representing 7 domains of the Bacteria, including the Proteobacteria, Green non-sulfur and OP11 divisions. In addition, Archaeal species not previously identified under the conditions of temperature and pH found in the hot spring were identified only in the aphotic spring. The results suggest that the absence of light has a profound influence on microbial community structure, presumably through the development of complex metabolic networks required for chemolithotrophic energy conservation. This may suggest a higher degree of complexity in sulfur cycling by aphotic, subterranean communities.

TROGLOMORPHY IN THE CAVE FISH *POECILLIA MEXICANA*, FROM CUEVA DE VILLA LUZ, TABASCO, MEXICO

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The cave fish *Poecillia mexicana*, a type of molly, is found only in Cueva de Villa Luz (aka Cueva de las Sardinas) in Tabasco, Mexico. The cave has mixed energy inputs from sulfur, bats, and through multiple skylights. This rich food base supports an amazing density of the cave fish. Studies done in the 1950s and 1960s stated that the fish showed increasing troglomorphic adaptations and less hybridization with surface forms deeper into the cave. However, the previous data show considerable variation and overlap of characteristics by sample location, and no statistical analyses were done. We wanted to determine if fish in remote areas of the cave had smaller eyes than those in areas near the surface stream. We used a Polaroid camera to take pictures of captured fish for measurements and analysis. Our results show variation in troglomorphy, but no statistical differences in fish from any part of the cave. The rich food base in this cave may reduce pressure to develop troglomorphy.

METABOLIC AND ISOTOPIC DIVERSITY OF CHEMOAUTOTROPHIC SULFUR-OXIDIZING BACTERIA FROM LOWER KANE CAVE, WYOMING

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Microbial communities from Lower Kane Cave (Wyoming) were investigated using phylogenetic analyses of 16S rRNA gene sequences and detailed isotopic surveys. Microbial mats from three sulfidic spring locations were discretely sampled along flow transects, from anaerobic waters in the spring orifices through the aerobic discharge channels, with mats extending 10-15 m from the orifices. Dense mats were 3–10 cm thick, and had short (<1 cm) and long (>10 cm) white filaments interconnected with white web-like films on the surface, and a gray-brown gel of filaments underneath. Discontinuous patches of yellow biofilms also intermixed with short filaments. Most of the microorganisms identified from the mats were sulfur-oxidizing bacteria. *Thiobacillus* spp. were detected from yellow patches, and short filaments along the stream channels were closely related to *Thiothrix unzii*. The most abundant bacterial populations in all the filamentous samples belonged to an uncharacterized group of sulfur-oxidizing bacteria within the epsilon-Proteobacteria class. Similar organisms have been found in other sulfidic systems, including Cesspool Cave (VA) and Parker Cave (KY). Microbial mats from Lower Kane had an average $\delta^{13}C$ value of -36‰, demonstrating chemoautotrophic fractionation against ^{13}C from an inorganic carbon reservoir (cave water was -8.9‰). Each of the sulfur-oxidizing bacterial morphotypes, however, had distinct carbon isotope compositions, indicating that pathways for obtaining carbon may be slightly different. These complex populations provide energy for the cave ecosystem as chemoautotrophs, while driving speleogenesis due to sulfide oxidation and the production of sulfuric acid.

CAVE DIPLURA OF GEORGIA

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Examination of 27 collections of campodeid diplurans from 20 caves in northwestern Georgia revealed five distinct species belonging to the genus *Litocampa*. Each species belongs to a different species group. The *cookei* group is represented by the widely dispersed *L. cookei*, having been found in Howards Waterfall Cave in Dade County. The *henroti* species group is represented by an undescribed species known from caves in Dade and Walker counties, and from other caves in Tennessee and Alabama. The *valentinei* and *puckett* species groups are each represented by an undescribed species, having been found in caves in Chattooga County and Walker County, and Dade County and Walker County, respectively. Both of these species are also known from neighboring Alabama caves. Lastly, a new undescribed species belonging to the primitive *condei* species group was collected from a cave in Bartow County. The discovery of this new species offers further support for the hypothesis that the ancestral home for most of the cavernicolous species of *Litocampa* in the United States was the highlands of southeastern Tennessee.

ANOTHER CHAPTER IN THE BIOSPELEOLOGICAL INVENTORY OF VIRGINIA CAVES

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Since the publication of Holsinger & Culver's 1988 work on the cave invertebrates of Virginia and northeastern Tennessee, biospeleological inventories of cave invertebrates have continued. Their work included records of invertebrate fauna from 319 of 2377 Virginia caves known in 1988. From late 1989 to 2000, a total of 317 Virginia caves have yielded new records from identified collections of cave invertebrates in 25 of the 26 solutional cave-bearing counties in the Virginia Valley and Ridge province, and solutional caves in one county each in the Virginia Blue Ridge and Piedmont provinces. No new identifications were made in Frederick County. Records from caves in Bedford (Blue Ridge province), Clarke, and Loudoun (Piedmont province) are the first cave invertebrate records from these counties. Of the 317 caves reported in this study, 83 were included in the earlier study but have yielded addition records. The two studies have yielded identified cave invertebrates in 553 caves out of the 3884 Virginia caves known in 2001. Both studies include other caves from which no collections were made, or from which collections have not been identified.

In addition to a better understanding of the biological resources of Virginia caves, these studies have saved some caves from destruction or minimized impacts of roads and other land-use changes. Regrettably, Virginia caves continue to be affected by land-use changes. In some cases, we now know that biological resources have been lost by habitat destruction or cave destruction.

DISTRIBUTION AND ABUNDANCE OF THE MIDGE *GOELDICHIRONOMUS FULVIPILUS*, IN CUEVA DE VILLA LUZ, TABASCO, MEXICO

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The terrestrial and aquatic ecosystems in Cueva de Villa Luz (aka Cueva de las Sardinas) in southern Tabasco, Mexico, are based on energy inputs of both organic material and sulfur. Sulfur bacteria in the stream are a major source of food for the chiromomid larvae of *Goeldichironomus fulvipilus* (formerly *Tendipes*, then *Chironomus*). Several aspects of the life history of this midge have been studied. Adults lay abundant egg cases just at the waterline, which hatch into minute red larvae. The larvae live in cases, with the highest density found in rapidly flowing water. When they pupate, the adult midges emerge from the water. Adults do not feed, but often reach such high densities that they produce an audible buzzing sound, as noted in the naming of the Buzzing Passage and the Other Buzzing Passage. Adult midges are initially red from larval hemoglobin, but change to a green color within 24 hours. The ratio of red to green midges is significantly different in different parts of the cave. Capture of emerging midges also shows a variable distribution, with productivity ranging from < 1/m² per day in silt-bottomed areas of the cave, to several hundred per m² per day in riffle areas. The productivity determines the ratio of red to green adults. The midges are an important source of food for the cave fish in the stream and support large numbers of spiders in the terrestrial system.

A SYSTEMATIC APPROACH TO SAMPLING THE ARTHROPOD ASSEMBLAGE OF GREGORYS CAVE, GREAT SMOKY MOUNTAINS NATIONAL PARK

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Recent extensive bioinventories such as the All Taxa Biodiversity Inventory (ATBI) in progress in Great Smoky Mountains National Park (GSMNP) have spawned several studies both above and below ground. Though faunal inventories are common in biospeleology, quantitative sampling techniques for cave arthropod populations are few, and studies of this nature in GSMNP are non-existent. In this study, a systematic sampling approach with standardized, repeatable methods determined population sizes and spatial distributions of 11 arthropod taxa in Gregorys Cave, and a comprehensive species list was compilated. Monthly sampling trips, from May 2000 to April 2001, yielded 46 arthropod species representing 35 families, 16 orders, and 5 classes. Of these, 29 were new records for the cave, with two being undescribed species and at least one a new record for GSMNP. Of the 11 taxa studied in detail, all but two showed significant fluctuations, both of their monthly population and their distribution within the cave relative to the entrance. Aquatic arthropod populations were also monitored, and their presence and abundance compared to factors influencing the water table. Richness and dissimilarity indices were calculated for the 12 months of sampling. The greatest numbers of species were observed during November 2000, December 2000, and February 2001, and the peak changes in taxon composition occurred between May 2000 and June 2000. I propose that epigean weather parameters brought on by seasonal change greatly influence the arthropod community in Gregorys Cave.

POPULATION STUDIES OF THE AQUATIC SNAIL *PHYSA SPELUNCA* (GASTROPODA: PHYSIDAE) FROM LOWER KANE CAVE, WYOMING

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Most cave ecosystems are energetically limited, depending on the transport of organic materials into the system from the surface. In contrast, Lower Kane Cave, Wyoming, contains sulfidic waters that support abundant communities of sulfur-oxidizing microbes, which use chemical energy to produce food. Other cave systems containing comparable microbial communities have abundant and diverse invertebrate fauna associated with this type of sulfur-based production (e.g., Movile Cave, Romania; Frasassi Caves, Italy). While its invertebrate fauna assemblage is not as diverse as similar systems, Lower Kane Cave contains an extremely abundant aquatic snail, *Physa spelunca*. Stable isotope analyses show that *P. spelunca* sampled from within the cave feed on the microbial mats, with both $\delta^{13}N$ (7.5‰) and $\delta^{13}C$ (-36‰) values exhibiting typical trophic effects. In contrast, snails collected from the entrance of the cave had $\delta^{13}C$ values (–26‰) typical from terrestrial C3 photosynthesis, indicating a shift to surface productivity. The abundant microbial food source has led to extremely high population densities in the immediate vicinity of the mats, with estimates as high as 6800 individuals/m². Although *P. spelunca* was originally described as exhibiting classic troglomorphic features (i.e., eye reduction and pigment loss), observations of the Lower Kane Cave population indicate that there are at least two other color morphs present (red and black). Preliminary investigations into the genetics of this unusual cave population have been initiated based on internal transcribed spacer gene regions, in order to estimate genetic diversity and evaluate population structure.

WHEN CAVE FISH SEE THE LIGHT: REACTION NORM TO LIGHT EXPOSURE DURING DEVELOPMENT IN EPIGEAN, TROGLOMORPHIC, AND HYBRIDS OF *ASTYANAX FASCIATUS* (CHARACIDAE)

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The study of phenotypic plasticity among hypogean fauna has been virtually ignored. Anecdotal accounts suggested that the development of troglomorphic features such as blindness and depigmentation could be epigenetically influenced by exposure to light. We conducted a series of experiments to ascertain the reaction norm to light on eyes, pigmentation, and behavior among epigean (eyed, pigmented), troglomorphic (blind, depigmented), and hybrids (epigean X troglomorphic) individuals of *Astyanax fasciatus* (Pisces: Characidae). Results show that light (or its absence) can strongly influence the development of pigmentation in the regressed eye and swimming behavior of different stocks of this fish species. These results may have important implications in the understanding of the reduction or loss of features during evolution. The ability to respond to changes in light regimes may explain the different phenotypes among many taxa that can be found in the hypogean environment. Further, this phenotypic plasticity may be an adaptive feature on which natural selection acts to determine survivability in the cave environment.

PRELIMINARY REPORT ON INVESTIGATIONS OF RED IMPORTED FIRE ANT (*SOLENOPSIS INVICTA*) IMPACTS ON KARST INVERTEBRATE COMMUNITIES AT FORT HOOD, TEXAS

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Red Imported Fire Ant (RIFA), *Solenopsis invicta*, predation upon karst invertebrate communities in central Texas has been reported in studies by Elliott, Reddell, and Cokendolpher. We have begun a year-long study of 6 caves that seeks to quantify aspects of this phenomenon. The study sites are at Fort Hood (Bell and Coryell counties, Texas), near the northern limit of the Edwards Plateau. Caves there harbor a variety of troglobitic macroinvertebrates, including several narrowly endemic taxa. Above ground, we use timed bait censusing to measure RIFA foraging activity on a grid of points centered over cave entrances and conduct mound counts within the study plots. Inside the caves, timed RIFA bait traps are placed along an in-cave transect. Visual censusing in a 0.1 m² quadrate frame quantifies diversity and abundance of cavernicoles along the in-cave transect. Preliminary results corroborate earlier observations, in that RIFA mound density and foraging activity are higher at disturbed, open sites. RIFA foraging on the trogloxene *Ceuthophilus secretus* in and outside of caves suggests that the interactions between these two species could have a negative impact on cave communities. We have observed an active RIFA foraging trail in the dark zone of a cave (2 cm soil temperature 17.0°C) while epigean 2 cm soil temperatures were too low for surface foraging by RIFA (average 12.8°C), demonstrating that RIFA can use the cave community as a food source when temperatures near the surface are too low for foraging.