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Ecological evaluation of air and water habitats in the Great Cavern of Santo Tomás, Cuba

Evaluación ecológica de los hábitats de agua y aire en la Gran Caverna de Santo Tomás, Cuba

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Abstract. Air and water habitats in the Great Cavern of Santo Tomás were investigated using cultivable indicator microbial groups. In addition, cave pools were sampled to evaluate the biomass and diversity of aquatic meiofauna. In the climatically dynamic entrance part named Cueva de las Antorchas, concentrations of total cultivable airborne bacteria ranged from 2 to 15 CFU/20 cm^2/20 min, and of airborne yeasts and moulds from 2 to 23 CFU/20 cm^2/20 min. Different pools fed by constant percolation water or occasional underground floods were sampled. Microbial indicators of water habitats (total bacteria, coliforms, *Escherichia coli*, Enterobacteriaceae) showed that microbial load very likely depended on the sampling position in the vertical transection of the cave system and closeness to the entrance. During the underground flow of Santo Tomás Stream through the cave system, the concentration of isolates typical for *E. coli* was reduced from 56 CFU/ml at the ponor in Valle de Santo Tomás to a concentration below the detection limit at the spring in Valle de Quemado. In some cave pools, apart from microorganisms, aquatic fauna contribute highly to the total biomass. Investigation for aquatic meiofauna diversity in cave pools revealed relative high abundances of copepods and ostracods.

Keywords: monitoring, microbial indicators, underground water, meiofauna, aerobiology.

Resumen. Se investigaron los hábitats del aire y del agua de la Gran Caverna de Santo Tomás mediante indicadores de grupos microbianos cultivables. Además, las pozas de agua dentro de la cueva fueron muestreados para evaluar la biomasa y la diversidad de meiofauna acuática. La parte de la entrada climáticamente dinámica se llama la Cueva de las Antorchas y las concentraciones totales de bacterias cultivables en el aire de esta sección oscilaron entre 2 y 15 CFU/20 cm^2/20 min, y las levaduras y mohos medidos fueron de 2 a 23 CFU/20 cm^2/20 min. Se muestrearon diferentes pozas alimentadas por agua de infiltración constante o por inundaciones subterráneas ocasionales. Los indicadores microbianos de hábitats acuáticos (bacterias totales, coliformes, *Escherichia coli*, enterobacterias) mostraron que la carga microbiana muy probablemente depende de la posición de muestreo relativa al corte transversal vertical del sistema de la cueva y de la cercanía a la entrada. Durante el flujo subterráneo de la corriente de agua de Santo Tomás a través del sistema de la cueva, la concentración de los aislamientos típicos para *E. coli* se redujo de 56 CFU/ml en el ponor en Valle de Santo Tomás a una concentración por debajo del límite de detección en el ojo de agua de Valle de Quemado. En algunas pozas, además de los microorganismos, existe una contribución de la fauna acuática a la biomasa total. La investigación de la diversidad acuática de meiofauna en las pozas reveló una alta abundancia relativa de copépodos y ostrácodos.

Palabras clave: monitoreo, indicadores microbianos, agua subterránea, meiofauna, aerobiología.

Introduction

In Cuba, karst occupies around 66% of the land surface (Núñez-Jiménez, 1984; Molerio-León and Parise, 2009; Febles-González et al., 2012), and in some regions karst aquifers represent an important source of water for human consumption and agriculture (Molerio-León and Gutiérrez-Díaz, 1999). The semi-tropical climate of the Caribbean region, accompanied by high organic input in karst underground, is reflected in high turnover rate of organic matter. The entrance sections and occasionally deeper parts of such caves, frequently shelter different trogloxenic animals such as: snakes, scorpions, frogs, crabs, crickets and spiders. In the western part of Cuba, the

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Great Cavern of Santo Tomás is a complex cave system from which local people use water and other natural resources for their needs (Núñez-Jiménez, 1990) and it harbours an obligate subterranean biota (Pérez-González and Yager, 2001).

Air and water were sampled in The Great Cavern of Santo Tomás. Atmospheric conditions and airborne microbes were evaluated in the climatically dynamic entrance section (Cueva de las Antorchas and Cueva de la Incógnita). Cave aerobiology investigation was conducted in this cave system because air has been poorly investigated in the Caribbean region, to evaluate conditions and organic load in this habitat, and to emphasize air as a vehicle for transport of airborne microbial pathogens. In Cuban caves spores of the human and bat pathogen *Histoplasma capsulatum* are frequently encountered (Font D'Escoubet and Macola Olano, 1976; Fernández Andreu, 1988; Erkens et al., 2002; Craven, 2013).

The same microbial indicators used for the air study were applied for water habitats. Microbiological indicator groups, such as cultivable heterotrophic aerobic bacteria, *Escherichia coli*, coliform bacteria, enterobacteria, and fungi are of great help in assessing the environmental health of underground ecosystems (Mulec et al., 2012a). Information on these parameters is even more significant in warm caves in tropical and subtropical zones that are rich in organic material. Microbes related to the environmental health of underground habitats are airborne and originate from cave sediments, guano and external air, and waterborne which originate from surface/underground river and/or percolating water (Borda et al., in press; Mulec et al., 2012a). Water samples were collected from pools and active river flow to observe eventual differences related to the complex water pathway due to the multi-level nature of the Great Cavern of Santo Tomás. To get a better overview of biomass in pools containing captured water after floods and in pools with percolation water, in addition to concentration of bacterial indicators, the abundance of aquatic fauna was also estimated. In comparison to temperate caves, higher turnover rates of organic matter and more dynamic relations between biota are expected in tropical and subtropical caves due to generally higher temperatures. Results on water quality are important, as the local population uses water for drinking from karst underground. This study represents an incentive for wider use of cave monitoring in the Caribbean region, because it is generally poorly studied (Day and Koenig, 2002; Parise and Valdes-Suarez, 2005).

Material and methods

Description of the cave. The great cavern of Santo Tomás in Sierra de Quemado, Cuba (in Spanish literature found under the following names: Caverna de Santo Tomás, Cueva de Santo Tomás and La Gran Caverna de Santo Tomás, Fig. 1A), extends for approximately 46 km of dry and flooded subterranean passages at 7 main levels (I-VII) and 65 m of vertical distance in a mogote, a residual karst hill. Since 1994 a part of the cave system named Cueva de las Avispas and Cueva de las Perlas (VI level) was equipped for tourist visits, but without electric lighting. Local people use water from the cave for their domestic needs, and bat guano to fertilize tobacco fields in the foothills of surrounding mogotes. This complex karst system has been recognized as a Cuban National Monument due to its natural, historical, paleontological and archaeological importance (Núñez-Jiménez, 1990; Parise and Valdes-Suárez, 2005). Sampling was limited to the unrestricted part of the cave system.

Microbiological media. RIDA®COUNT plates were used to enumerate different microbial groups, RIDA®COUNT Total Aerobic Count for total heterotrophic aerobic bacteria, RIDA®COUNT *E. coli*/Coliform for total coliform bacteria with discernment of *E. coli* colonies, RIDA®COUNT Enterobacteriaceae for enterobacteria, and for yeasts and moulds RIDA®COUNT Yeast&Mold Rapid. For airborne microbiota, approximately 2 hours before caving, RIDA®COUNT media sheets were activated by adding 1 millilitre of sterile physiological saline (R-Biopharm AG, Germany). Isolates that exhibited ß-D-glucuronidase and ß-D-galactosidase biochemical activities on RIDA®COUNT *E. coli*/Coliform plates were considered indicative of *E. coli* (R-Biopharm AG, Germany). After inoculation of plates

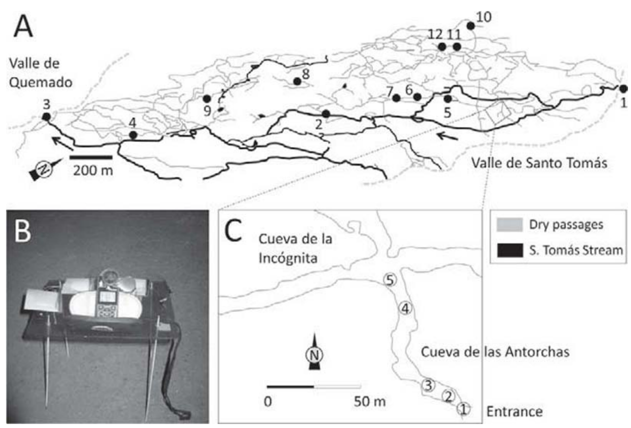

Figure 1. A, ground plan of The Great Cavern of Santo Tomás with designated positions for water (1-12, Table 1) and air samples; B, measuring of atmospheric parameters and sampling of airborne microbiota using gravity settling principle; C, entrance section of Cueva de las Antorchas with designated sites for air samples (after Núñez-Jiménez, 1990).