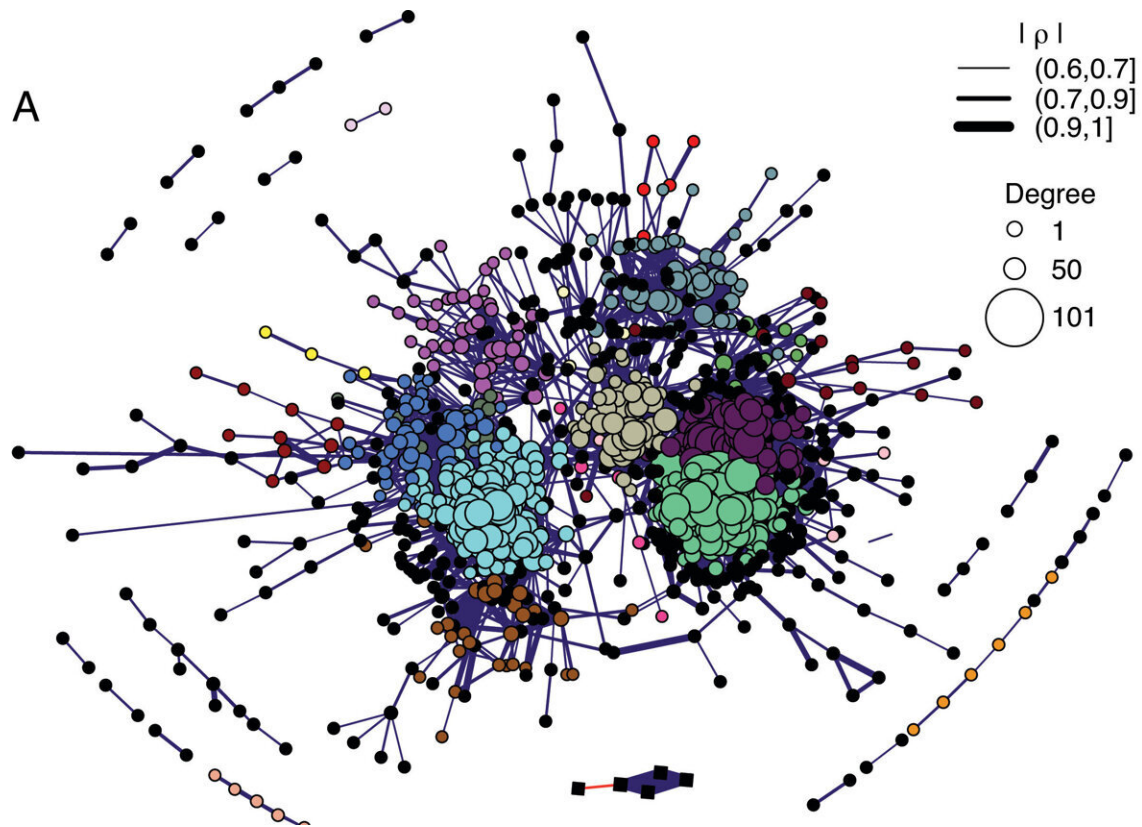


Yucatán's underwater caves host diverse microbial communities

November 10 2023, by Amanda Morris



Global co-occurrence network of bacterial and archaeal ASVs from the eastern Yucatán carbonate aquifer. (A) Network colored by ASV clusters. Nodes are sized by degree (total number of co-occurrences) while the width of each edge corresponds to the strength of the Spearman correlation coefficient (ρ). (B) Relative abundance of ASV clusters. Samples are ordered via ASV-level hierarchical clustering. Credit: *Applied and Environmental Microbiology* (2023). DOI: 10.1128/aem.01682-23

With help from an experienced underwater cave-diving team, Northwestern University researchers have constructed the most complete map to date of the microbial communities living in the submerged labyrinths beneath Mexico's Yucatán Peninsula.

Although previous researchers have collected [water](#) and [microbial samples](#) from the cave entrances and easily accessible sinkholes, the Northwestern-led team reached the deep, dark passageways of unlit waters to understand better what can survive inside this unique underground realm.

After analyzing the samples, the researchers noted a system rich with diversity, organized into distinct patterns. Similar to a stereotypical high school lunchroom, microbial communities within the cave system tend to cluster into well-defined cliques. But one family of bacteria (Comamonadaceae) acted as a popular social butterfly—appearing at nearly two-thirds of the "cafeteria tables." The findings hint that Comamonadaceae is the ecological linchpin of the broader community.

The [research was published](#) in the journal *Applied and Environmental Microbiology*.

"This is certainly the most expansive microbial survey across this part of the world," said Northwestern's Magdalena R. Osburn, who led the study. "These are incredibly special samples of underground rivers that are particularly difficult to obtain. From those samples, we were able to sequence the genes from microbial populations that live in these sites. This underground river system provides drinking water for millions of people. So, whatever happens with the microbial communities there has the potential to be felt by humans."

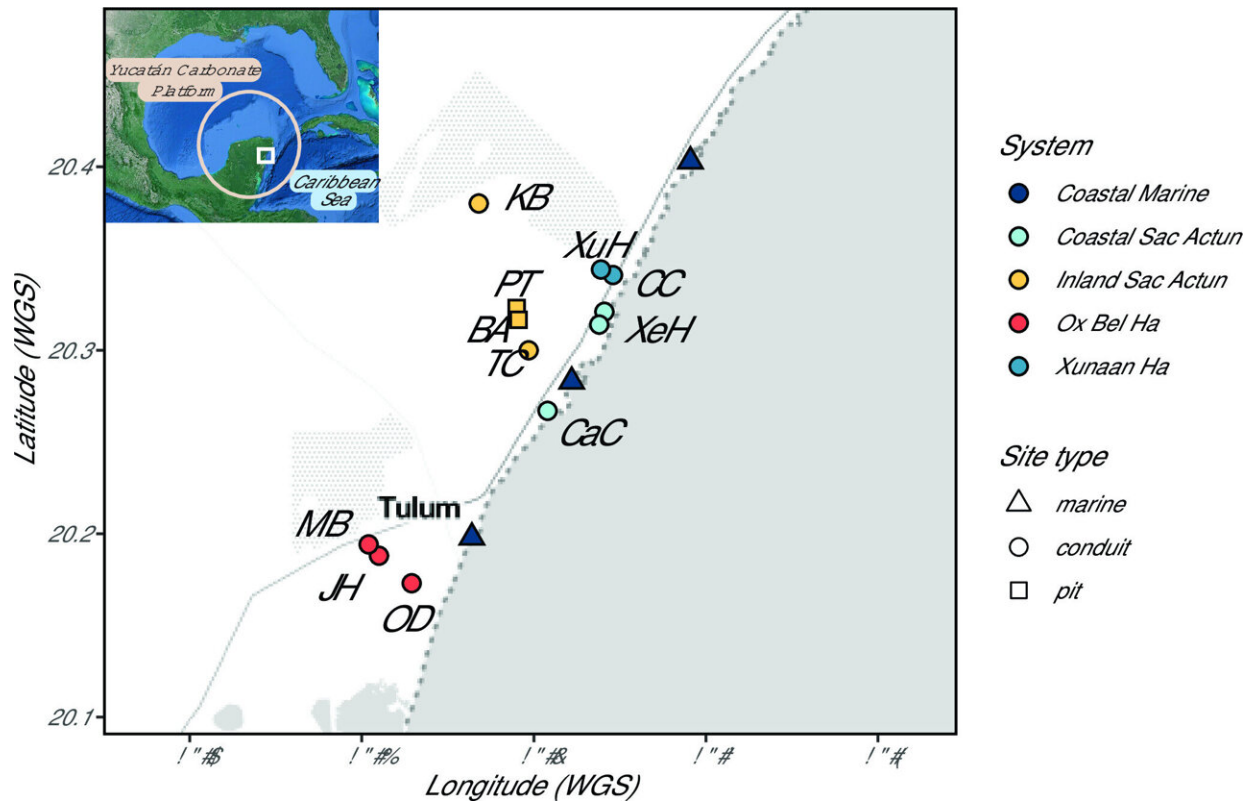
A geobiology expert, Osburn is an associate professor of Earth and planetary sciences at Northwestern's Weinberg College of Arts and

Sciences.

Northwestern alumnus Matthew Selensky led this project as a part of his dissertation when he was a graduate student in Osburn's laboratory. Study co-author Patricia Beddows, professor of Earth and planetary sciences at Weinberg, led the cave-diving expedition and leveraged her decades of experience working on these caves. Other Northwestern co-authors include Andrew Jacobson, professor of Earth and planetary sciences, and former graduate student Karyn DeFranco, who focused on the geochemistry.

Located primarily in southeastern Mexico, the extensive Yucatán carbonate aquifer is pockmarked by numerous sinkholes leading to a complex web of underwater caves. Hosting a diverse, yet understudied microbiome, the underwater network contains areas of freshwater, seawater and mixtures of both. The system also includes a variety of zones—from pitch-black, deep pits with no direct openings to the surface to shallower sinkholes sparkling with sunlight.

"The Yucatan platform is essentially a Swiss cheese of cave conduits," Osburn said. "We were curious which microbes are found together when we look across the whole system versus which microbes are found within one 'neighborhood'."



Study sites. Bacterial and archaeal communities from 66 water samples spanning the meteoric, halocline, and saline groundwater layers in the aquifer were analyzed in duplicate and compared to Caribbean seawater. Communities were sampled from 11 aquifer and 3 surface seawater sites near Tulum, Quintana Roo, Mexico. Sites are colored by cave systems according to previously mapped conduits. The meteoric water typically flows toward the coast, although decoupled saline groundwater may alternatively flow coastward and inland based on sea level (11). Refer to Table 1 in the main text for site descriptions and label IDs. Site Xel Ha (XeH) was sampled in two conduit branches. The base map was constructed using the R package ggmap (Kahle and Wickham, 2013). Credit: *Applied and Environmental Microbiology* (2023). DOI: 10.1128/aem.01682-23

To explore this question, a team of cave divers collected 78 [water samples](#) from 12 individual sites within the cave system near the Caribbean coast in Quintana Roo, Mexico. The sample collection

spanned from the Xunaan Ha system at the north end to inland and coastal portions of the Sac Actun system (including a distinctive, 60-meter-deep pit) to the Ox Bel Ha system to the south.

Back in a dive-shop-turned-science lab, researchers filtered cells out of each sample and analyzed its chemistry. Next, back at Northwestern, they identified microbial communities by sequencing their DNA. Then, Selensky developed a new computational program to perform network analysis on the data set.

The resulting networks showed which species tend to live together. For each site, the researchers considered the environmental context of each microbial community, including cave type (pit or conduit), cave system, distance from the Caribbean coast, geochemistry, and position in the water column.

Although water from the Gulf of Mexico flows into the Yucatán aquifer, the aquifer's microbiome varies substantially from the nearby sea, the researchers found. The microbiomes also vary throughout the cave system—from cave to [cave](#) and from shallow water to deep water.

"The microbial communities form distinct niches," Osburn said. "There is a varying cast of characters that seem to move around, depending on where you look. But when you look across the whole data set, there's a core set of organisms that seem to be performing key roles in each ecosystem."

Osburn and her team found that Comamonadaceae, a family of bacteria typically found in groundwater systems, lived in several niches. They also discovered that a deep, pit-like sinkhole with a surface opening (allowing sunlight to spill in) housed the most microbial communities—segregated into layers of distinct niches throughout the water column.

"It seems that Comamonadaceae performs slightly different roles in different parts of the aquifer, but it's always performing a major role," Osburn said. "Depending on the region, it has a different partner. Comamonadaceae and its partners probably have some mutualistic metabolism, maybe sharing food."

More information: Magdalena R. Osburn et al, Microbial biogeography of the eastern Yucatán carbonate aquifer, *Applied and Environmental Microbiology* (2023). [DOI: 10.1128/aem.01682-23](https://doi.org/10.1128/aem.01682-23)

Provided by Northwestern University

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