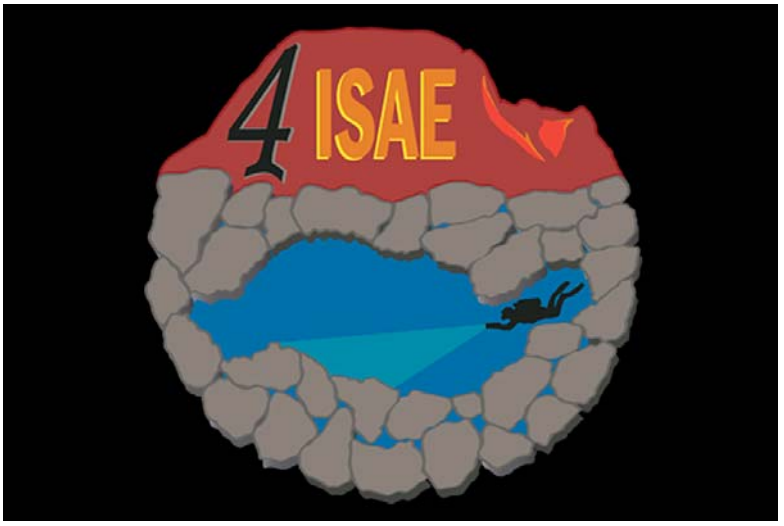
IV SIMPOSI INTERNACIONAL D'ECOSISTEMES ANQUIHALINS - 2018

Lanzarote, Illes Canàries

Los Jameos del Agua (Lanzarote, Canàries).
Entrada de llum pel Jameo Chico.



IV SIMPOSI INTERNACIONAL D'ECOSISTEMES ANQUIHALINS

Celebrat de l'1 al 5 d'octubre de 2018 a Lanzarote, Illes Canàries

Nota prèvia i recopilació a càrrec de Lluís Auroux

Què són els ecosistemes Anquihalins?

Les cavitats amb dipòsits aquàtics salobres que es desenvolupen prop del mar però sense una comunicació directa, es denominen Anquihalines. Aquestes aigües poden tenir diferents concentracions de sal, com a molt la del mar pròxim.

La fauna que colonitza aquests llacs, túnels i conductes es denomina Fauna Anquihalina i posseeix unes característiques particulars que la separa de la típica fauna aquàtica i/o marina.

Aquest simposi que va tenir lloc els dies 1 al 5 del passat octubre a l'illa de Lanzarote, ha reunit especialistes científics i estudiants de molts països el món, que treballen o estan interessats en el medi Anquihalí. Els camps tractats són: biologia, sistemàtica i evolució, biogeografia, geomicrobiologia, ecologia, geologia, hidrologia, paleontologia, arqueologia, exploració, immersió, etc.

S'han presentat una cinquantena treballs, vuit en la modalitat Pòster i una quarantena de ponències.

Fotos: Pere Oromí. Grup assistents al Simposi.

4TH INTERNATIONAL SYMPOSIUM ON ANCHIALINE ECOSYSTEMS

1 – 5 October 2018 Cabildo de Lanzarote, Canary Islands, Spain

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Organizer:

Alejandro Martínez García – CNR-ISE, Verbania (Italy)

Organization committee:

Elena Mateo Mederos – Lanzarote and Chinijo Islands UNESCO Geopark, Lanzarote (Spain)
Clara Bonilla Cabrera - Lanzarote and Chinijo Islands UNESCO Geopark, Lanzarote (Spain)
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Guillermo García Gómez – University of Liverpool (United Kingdom)
José Zamora Sánchez - Glaukos: Association for scientific divulgation and faunal studies, Madrid (Spain)

Scientific committee

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Dávid Brankovits – Woods Hole Oceanographic Institution, Massachusetts (USA)
Ester Eckert – CNR-ISE, Verbania (Italy)
Pete van Hengstrum – Texas A&M University, Texas (USA)
Tanja Shabarova - CAS
Thomas M. Iliffe – Texas A&M University, Texas (USA)
Vasilis Gerovasileiou – Hellenic Center for Marine Research, Crete (Greece)

PROGRAMA

1 d'octudre del 2018:

CERIMÒNIA D'INAUGURACIÓ:

Oromí, P. "The Canary Islands subterranean realm and its adapted fauna"

Mateo-Mederos, E. "Role of Geoparks in research"

Lamson, M. "Anchialine restoration in Hawai'i – One pool complex at a time"

Sedar, D. "Using carbon dioxide to remove invasive introduced fish in anchialine pools on Hawaii Island"

CIÈNCIES DE LA TERRA

-van Hengstrum, P. "Holocene Environmental Development in Karst Subterranean Estuaries"

-Creswell, J. "Modern Benthic Foraminifera in Palm Cave Bermuda: Impact of conduit morphology on fertilization of cave benthic habits"

-Ginés, A. "A Geological approach to the anchialine karst environments of Mallorca Island (Western Mediterranean)"

-Streker, U. "Entertainment talk: Lanzarote – Lava, sand, and Flower Power"

Dia 2:

BIOLOGIA EVOLUTIVA

Gonzalez, B. C. "Evolution and ecology of anchialine cave fauna"

Pisera, A. "Lithistid demosponges from submarine caves of Crete Island (Eastern Mediterranean Sea): is their occurrence controlled by water silicate content?"

Álvarez, F. "Isopods of the anchialine systems of the Yucatan Peninsula: Systematics and Distribution"

Khodami, S. "New insights to the phylogeny of Misophrioida (Copepoda) inferred from nuclear and mitochondrial DNA"

Wilkens, H. "Eye reduction and distribution of M. polymorpha (Galatheidae, Crustacea)"

Jurado-Rivera, J. "Mitochondrial phylogenomics and biogeographic patterns in anchialine cave shrimps"

Ballou, L. "From head to tail: investigating the morphological diversity within Remipedia"

Stemme, T. “Anatomy of the central nervous system in Remipedia – an evolutionary perspective”

Fontaneto, D. “Anchialine caves to understand evolutionary processes in the marine biota”

MACROECOLOGIA I GRANS BASES DE DADES

Gerovasileiou, V. “Three years with the World Register of marine Cave Species (WoRCS) initiative: current status and future perspectives”

Mercado-Salas, N. “Additions to the cave copepod diversity from the Yucatán Peninsula and Cuba”

Benítez, S. “How is the anchialine fauna of the Yucatan Peninsila distributed? Existence of hotspots of diversity and endemism”

Martínez, A. “What is anchialine anyways? A faunistic perspective”

Dia 3: Excursions

Dia 4:

MICROBIOLOGIA

Sharabova, T. “MISSING TITLE”

Cabello-Yeves, P. “Aquatic Genome-Resolved Metagenomics: An application to anchialine systems”

Davis, M. C. “Microbial community function within a stratified anchialine sinkhole: a window into the coastal mixing zone”

Tonolla, M. “Microbial functions in stratified waters through genomics and proteomics”

Snyder, M. D. “Characterization and analyses of the Crab Creek Cave biofilm community”

Panou. M. “Exploration of cyanobacteria microbial life in Mediterranean terrestrial caves”

Humphreys, W. F. “Metagenomics profile of Bundera Sinkhole, Western Australia”

Kumaresan, D. “Microbial life in a unique chemosynthesis-driven cave ecosystem”

BIOGEOQUÍMICA I HIDROGEOLOGIA

Brankovits, D. “Shaping anchialine ecosystem function: the role of hydro-biogeochemical processes in a karst subterranean estuary”

Scharping, R. J. “The fate of urban springs: Pumping-induced seawater intrusión in a coastal phreatic cave”

Calderon-Gutierrez, F. “Temperature stability and precipitation impacts of anchialine caves”

Dinon, C. “Stratification of a coastal anchialine sinkhole in Hudson, Florida”

Polhman, J. “The biogeochemistry of anchialine ecosystems: Progress and MORE possibilities”

TAULA RODONA: ECOSISTEMES ANQUHIALIN, PERSPECTIVES Y REFLEXIONS

DIA 5:

REPTES, EXPLORACIONS DE CAVITATS

Iliffe, T. M. “Diving Exploration and Discovery in Anchialine Caves: My Story”

Perello, M. A. “Liquid Caves: Cave exploration in Mallorca”

Kovacs, P. “Lithological and structural controls of cave development on Kornati Islands”

Farr, M. “The Darkess Beckons: The history and development of cave diving”

SESSIÓ DE CLAUSURA

Closure: Minutes, balance, anchialine association.

Consideració:

Podem afirmar que el Congrés va ser tot un èxit, tant per la bona organització com per l’assistència i treballs presentats. Feliçitats!

INFORMACIÓ OFICIAL DE L'ORGANITZACIÓ I RESUMS DELS TREBALLS PRESENTATS.

RESUMS DE LES PRESENTACIONS (ordenats per àmbits): ABSTRACTS

ÀMBIT: EVOLUTIONARY BIOLOGY

1- ISOPODS OF THE ANCHIALINE SYSTEMS OF THE YUCATAN PENINSULA: SYSTEMATICS AND DISTRIBUTION

Fernando Álvarez *, Brenda Durán and Sergio Benítez

Colección Nacional de Crustáceos, Instituto de Biología, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México

The stygobitic isopods of the anchialine systems of the Yucatan Peninsula, Mexico, are revised and a new species is described to bring the total number of species to seven. A new species of the genus *Curassanthura*, of the family Leptanthuridae is now being described, being the first species of the genus to be recorded from a continental site. It can be distinguished from the other known species in the genus by the shape of the head, wider than long, and the number of spines (30) on the palmar margin of propodus of pereiopod 1. All the available records of the other six known species were compiled and mapped for the first time. A key to all the anchialine species from the Yucatan Peninsula is provided, as well as photographs, except for *Haptolana bowmani*, to make their identification easier. The observed distribution pattern of all the seven species is discussed considering the geological history of the peninsula.

2- EXPLORING LOCAL-SCALE BIOGEOGRAPHY OF ANCHIALINE CAVE FAUNA USING THE GENUS *TYPHLATYA* (FAMILY: ATYIDAE) IN THE YUCATAN PENINSULA

Lauren Ballou (1*) Lexy Salinas (1) Shari Rohret (1) Thomas Iliffe (1) and Elisabeth Borda (2)

(1) Texas A&M University at Galveston, USA, Galveston, balloul@tamu.edu; lexysalinas11@tamu.edu; sharirohret96@tamu.edu; iliffet@tamug.edu (2) Texas A&M University San Antonio, USA, San Antonio, bordae@tamug.edu

Anchialine caves (stratified subterranean estuaries) are present throughout the world and host a diverse array of stygobitic fauna. The distribution and general ecology of anchialine fauna

remains largely unknown due to the limited accessibility of anchialine cave systems. Developing a better understanding of the distribution of taxa will provide insight into their evolutionary and biogeographic history. In this study, the shrimp genus *Typhlatya* was used as a model taxon for biogeographic analysis in the Yucatán Peninsula. Specimens were collected from seven cave systems in the Yucatán and were morphologically studied and molecularly identified via DNA barcoding of mitochondrial gene 16S. Genetic data was then phylogenetically assessed and compared to previously sequenced *Typhlatya* species in the Yucatán (*T. pearsei, T. campecheae*, *T. dzilamensis*, and *T. mitchelli*). Some species ranges show extensive expansion compared to previously recorded distribution patterns (particularly in the case of *T. dzilamensis*). Future work will seek to analyze the connectivity within *Typhlatya* populations of the Yucatán to better understand the dispersal capabilities and life history strategies of anchialine crustaceans.

3- FROM HEAD TO TAIL: INVESTIGATING THE MORPHOLOGICAL DIVERSITY WITHIN REMIPEDIA (CRUSTACEA)

Lauren Ballou (1*) Thomas Iliffe (1) and Jørgen Olesen (2)

1: Texas A&M University at Galveston, USA, Galveston, balloul@tamu.edu; iliffet@tamug.edu
2: University of Copenhagen, Denmark, Copenhagen, jolesen@snm.ku.dk

Remipedia, a crustacean class consisting of 29 species, are found in anchialine and marine caves throughout the world. Although remipedes are a subject of great interest in anchialine biology, little is understood about the relationships between species. The objective of this study is to examine and compare the morphologically diverse characters expressed within Remipedia to develop a better understanding of their evolutionary history. Organisms were collected via advanced cave diving techniques, preserved, and then transferred to the Zoological Museum of Copenhagen, Denmark. Specimens were then identified, catalogued, and photographed using light and scanning electron microscopy (SEM). Particular emphasis was placed on the feeding appendages of Remipedia, as these structures express some of the highest diversity amongst the taxa. A morphological matrix is being constructed to compare the characters between species. This matrix will expand upon Koenemann *et al*.'s (2007) previous work by incorporating more characters, species, and advanced microscopic technologies. This research will provide the framework for a phylogenetic reassessment of Remipedia. Future work will incorporate molecular techniques to further examine the relationships amongst this taxa.

4- HOW IS THE ANCHIHALINE FAUNA OF THE YUCATAN PENINSULA DISTRIBUTED? EXISTENCE OF HOTSPOTS OF DIVERSITY AND ENDEMISM

Sergio Abdiel Benítez León

Universidad Nacional Autónoma de México, Posgrado en Ciencias del Mar y Limnología, Circuito exterior s/n, México 04510, México. sabl290387@gmail.com

The Yucatan peninsula has a number of particular geological characteristics. One main characteristic is the presence of large systems of flooded caves, known as anchialine systems. The biodiversity found in them comprises 48 species of macro-crustaceans, two fishes, a gasteropod and an echinoderm. It is recognized that a considerable percentage of these species are of marine origin, and invaded the continental fresh waters using underground conduits. The relative isolation of the water bodies, their geological history and their geographical characteristics, allows many of the organisms that live in them to be endemic to very small areas, making an interesting question to investigate if there are hotspots of richness and endemism in these systems. The statistical analysis used is based on spatial distributions using cells of 0.125 degrees of latitude by 0.125 degrees of longitude creating a grid throughout the peninsula. It is used to analyze the relationship of each cell with the surrounding ones which if there is a spatial autocorrelation to distinguish a structured pattern from a random one. To carry out this study, a database was compiled with all the records available in the literature and in the National Collection of Crustaceans of the Institute of Biology, UNAM, in addition 38 samples were added with 31 new for a total of 650 records of 52 species in 220 locations. Other results are the finding of a new species of isopod. Preliminary results suggest the existence of two hotspots of richness and endemism in contrasting areas of the peninsula. and one hotspot for the corrected index of endemism.

5- ANATOMY OF THE CENTRAL NERVOUS SYSTEM IN REMIPEDIA – AN EVOLUTIONARY PERSPECTIVE

Torben Stemme

Institute of Neurobiology, Ulm University, Germany, 89081 Ulm,
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The enigmatic crustacean taxon Remipedia plays a key role in our understanding of arthropod evolution. Remipedia have been regarded as basal crustaceans in early taxonomic studies, but molecular investigations proposed a derived position, including a sister group relationship to Hexapoda. Because of these conflicting hypotheses independent data are crucial to gain new

insights on arthropod evolution. The anatomy of the nervous system has been proven to contain promising characters for such phylogenetic investigations – an uprising field termed neurophylogeny. Using antibody labelling against neurotransmitters and structural components as well as tracer injections, the morphology of the nervous system could be analyzed in considerable detail. Especially the description of individually identifiable neurons in the ventral nerve cord and the morphology of the olfactory pathway support a close relationship to the Hexapoda. However, no information on the nervous system development is available in Remipedia, but crucial for meaningful conclusions concerning homology of neuronal structures. Nevertheless, Remipedia can be of special interest in this context, as they are proposed to grow their entire life, adding functional body segments at a posterior growth zone. This is supported by different stages of swimming appendages at the posterior body region, reaching from a simple limb bud to fully developed swimming appendages. Thus, an investigation of neurogenesis in the adults provides insights into the mechanisms of nervous system ontogeny. In this contribution, I will summarize the current knowledge of the neuroanatomy in Remipedia and present first data on adult neurogenesis in this fascinating representatives inhabiting anchialine ecosystems.

6- EYE REDUCTION AND DISTRIBUTION OF MUNIDOPSIS POLYMORPHA (GALATHEIDAE, CRUSTACEA)

Horst Wilkens

The genus Munidopsis is represented in the Atlantic Ocean by more than 70 species. They are usually living deeper than 500 m and characteristically on the abyssal plain at > 2000 m. The only exception is M. polymorpha Koelbel, 1892, which occurs close below the water surface. It is exclusive to Lanzarote and was first sampled in the Jameos del Agua by the Austrian scientist Oskar Simony in 1888, but also exists in the marine groundwater filling the lava fissures of this volcanic island. M. polymorpha has lost its dark body pigmentation completely. The eyestalks are reduced in size and very small. They carry a translucent but non compartimented cornea. The crystalline lenses are rudimentary or even lost. Imaging is therefore not possible. Nonetheless, M. polymorpha is light sensitive, because rhabdomeric structures still exist. This is supported by negative phototactic reactions, most obviously demonstrated by the presence of higher numbers of specimens grazing algae at night than during the day in the lighted Jameos del Agua. Light perception is also corroborated by the existence of all three very optic ganglia and nervous structures connecting them with the rhabdom.

7- ANCHIALINE CAVES TO UNDERSTAND EVOLUTIONARY PROCESSES IN THE MARINE BIOTA

Diego Fontaneto

CNR-ISE, Verbania ITALY

Animal communities inhabiting anchialine environments can be used as a model to investigate the general and peculiar evolutionary patterns and processes in marine habitats. Anchialine environments represent world-wide distributed land-locked water bodies with marine origin. They are comparable to islands for terrestrial habitats as they encompass young, discrete habitats with comparable ecological conditions to the sea, thus providing independent replicates of comparable evolutionary processes. All anchialine habitats investigated so far harbour high level of endemics, disharmonic communities, species with unique set of troglomorphic features, and animal lineages interpreted as living fossils. During the presentation, we will review what has been done until now and what can be done to test each of these observations and the processes related to them. Our suggestion is to analyse large data sets with both macroecological and phylogenetic methods and a broad theoretical perspective from the field of evolutionary ecology, comparative evolution and island biogeography. This will facilitate to link the ecological and evolutionary processes observed in these habitats (easy to isolate and test due to the discrete and young nature of anchialine habitats) with those affecting oceanic ecosystems, providing a tool for a deeper understanding of the marine biota. Whereas similar approaches are well characterized in terrestrial environments (e.g. island), they are missing in the ocean, obscuring our understanding of the processes occurring there benthic assemblages. This preliminary biodiversity assessment provides a baseline for future monitoring and conservation initiatives.

8- EVOLUTION AND ECOLOGY OF MACELLICEPHALINAE SCALE WORMS (POLYNOIDAE, ANNELIDA)

Brett C. Gonzalez1,2,*, Katrine Worsaae2, Karen Osborn1

(1) Smithsonian Institution, National Museum of Natural History, Department of Invertebrate Zoology, MRC-163, P.O. Box 37012, Washington D.C., 20013, USA (2) University of Copenhagen, Marine Biological Section, Department of Biology, Universitetsparken 4, 1st Floor, 2100 Copenhagen Ø, Denmark

Scale worms (Aphroditiformia) are one of the most successful annelid radiations and are easily recognizable by their paired dorsal covering of segmental scales. Within Aphroditiformia, family Polynoidae is the largest and most diverse group, having colonized all marine habitats and ecological extremes. Due to restricted access to material, few studies have assessed their

evolution into (or origin within) these habitats. As a result, longstanding uncertainties persist in the interpretation of macellicephalinid evolution due to lack of phylogenetic resolution and representation. However, we recently managed to include two cave genera of Macellicephalinae (Pelagomacellicephala and Gesiella) from caves of different age, geology and opposing sides of the Atlantic, in a phylogenetic study on scale worms. For the first time, we showcase these rare cave genera, recovering them in a sister relationship and nested within a strictly deep-sea inhabiting clade. Divergence estimates suggest a single colonization into subterranean environments from the deep sea during late stage opening of the Atlantic. Phylogenetic comparative methods and ancestral character reconstructions further explored this deep sea-cave connection, examining behavioral and morphological traits between extreme and non-extreme scale worm taxa. Our results indicate that deep sea and cave taxa have similar morphological specializations, including eye loss and elongated sensory structures that evolved after initial colonization of the deep sea from a shallow water ancestor, becoming more pronounced in taxa occupying caves. Together, these systematic relationships and adaptations will inevitably help guide our ongoing phylogenomic investigations into the underlying functional and molecular mechanisms of adaptation in extreme environments.

9- MITOCHONDRIAL PHYLOGENOMICS AND BIOGEOGRAPHIC PATTERNS IN ANCHIALINE CAVE SHRIMPS

José Antonio Jurado-Rivera (1*) Joan Pons (2), Fernando Álvarez (3), Alejandro Botello (4), William F. Humphreys (5), Timothy J. Page (6), Thomas M. Iliffe (7), Endre Willassen (8), Kenneth Meland (9), Carlos Juan (1,2), Damià Jaume (2)

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We aim to test the relative contribution of historical vicariance and dispersal in the evolutionary history of a group of Atyidae cave shrimps (genera Typhlatya, Stygiocaris and Typhlopatsa) with highly disconnected distribution (Eastern Pacific, Caribbean, Atlantic, Mediterranean, Madagascar and Australia). Our study focuses on three main geological events: the opening of the Atlantic Ocean, the breakup of Gondwana, and the closure of the Tethys Seaway. We perform complete mitochondrial genome sequencing and analysis to reconstruct phylogenetic and biogeographic scenarios with fossil-based calibrations.

The inferred chronograms placed the origin of the group in the early Cretaceous. Their most recent common ancestor was inferred to live along the western shores of Tethys, in the area

corresponding to the Proto-Caribbean and the Proto-Atlantic, and there it diverged into two primary lineages: the western Atlantic/east African lineage diverging at ca. 119.2 Ma and the Australian/ Malagasy/ Caribbean lineage at ca. 118.5 Ma. The divergence between the Australian Stygiocaris and the Malagasy Typhlopatsa was dated to later in the Cretaceous at ca. 85.1 Ma, while the split between the Caribbean species T. monae and the Mediterranean species is estimated at ca 105.4 Ma. Our inferences show that the divergence time among sister-species on opposite shores of the Atlantic are highly consistent with a vicariance scenario. On the other hand, the Australia/Madagascar shrimp divergence postdates the Gondwanan breakup, thus suggesting the occurrence of dispersal processes. Finally, our results show that the Tethys closure appears not to have been influential in the biogeographic patterns observed in the group.

10- NEW INSIGHTS TO THE PHYLOGENY OF MISOPHRIOIDA (COPEPODA) INFERRED FROM NUCLEAR AND MITOCHONDRIAL DNA

Sahar Khodami1*, Nancy F. Mercado-Salas1, Alejandro Martínez2 and Pedro Martínez Arbizu1

(1) Senckenberg am Meer, German Centre for Marine Biodiversity Research, Südstrand 44, 26382 Wilhelmshaven, Germany (2) Institute for Ecosystem Studies, National Research Council of Italy

Familial level phylogenetic relationships between the three extant families of Misophrioida Gurney, 1933 have been investigated here for the first time using two nuclear genes, small- & large- subunits (18S & 28S) and one mitochondrial gene, cytochrome c oxidase subunit I (COI) performing Maximum likelihood and Bayesian analysis. Misophrioids as the earliest branch of Podoplea are notable for their success in invading subterranean aquatic habitats, such that the Palpophriidae Boxshall & Jaume, 2000 and most of the members of Speleophriidae Boxshall & Jaume, 2000 predominantly colonized anchialine caves. In this study representatives of the family Misophriidae Brady, 1878 including hyperbenthic genera Misophriella Boxshall, 1983, Misophria Boeck, 1865 Misophriopsis Boxshall, 1983 and bathypelagic Benthomisophria Sars G.O., 1909 recovered as a monophyletic group. The family Speleophriidae is represented here by five genera comprising the hyperbenthic Archimisophria Boxshall, 1983 from North Atlantic around Iceland and, subterranean inhabitants of the genera Expansophria Boxshall & Iliffe, 1987, Boxshallia Huys, 1988, Speleophriopsis Jaume & Boxshall, 1996, Speleophria Boxshall & Iliffe, 1986 from anchialine caves in Bermuda, Lanzarote and Mexico. The Palpophriidae is represented by its single genus Palpophria Boxshall & Iliffe, 1987 collected from stygofauna of

anchialine caves in Cuba and Lanzarote. A new phylogenetic system of Misophrioida has been provided here for the first time using molecular approaches focusing on the cave restricted inhabitants of this order and the importance of anchialine habitats as refuges for ancient lineages of copepods is stressed.

Keywords: Misophrioida, Molecular phylogeny, Anchialine caves.

PRESENTACIONS MODALITAT POSTER:

1- **LARVAL DEVELOPMENT OF THE STYGOBIOTIC SHRIMP CREASERIA MORLEYI (CREASER, 1936) (DECAPODA: PALAEMONIDAE), FROM THE YUCATAN PENINSULA, MEXICO.**

Sergio Abdiel Benítez León (1*) y Juan Salvador Martínez Cardenas (2)

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This study tries to describe the larval development of the palemonid *Creaseria morleyi*. It has been described that larval development for marine or brackish palemonids is widespread, while for strictly freshwater species it is abbreviated. There are numerous studies for the larval development of the family, but not for *Creaseria morleyi* which is a stygobiotic species. It is characterized by his size, being able to reach 70 mm, has a translucent to white coloration. It is endemic to the Yucatan peninsula and has been observed in areas of total darkness, as well as in areas that receive light. The samples were obtained within eight caves of the Ox Bel Ha system (SOBH), near Tulum, Quintana Roo, Mexico between 2013 and 2016. All the samples were collected by cave diving technique, each organism was collected individually. The larvae were sequenced and compared with the COI gene of adults to confirm the species of these. The results show six larval stages before reaching the stage of decapodid, this may indicate that *C. morleyi* adults prefer freshwater environments, although larval stages it is found in brackish water (5-10 ‰) presenting a "extended" development. It is believed that this shrimp derives from a marine ancestor stranded by sea level regressions in the early Pleistocene which may explain the type of development it presents. Important morphological changes in some stages suggest the existence of more larval stages. All the larvae stages are presented, described, measurement and illustrated.

2- HABITAT SELECTION OF ANCHIALINE FAUNA: REGIONAL AND LOCAL SCALES

Fernando Calderón-Gutiérrez1*, Lauren Ballou1, Fernando Álvarez2 and Thomas M. Iliffe1

(1*) Department of Marine Biology, Texas A&M University at Galveston, U.S.A., Galveston, fercg12@tamu.edu; balloul@tamu.edu; iliffet@tamug.edu
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The aim of the present work is to unveil a first approach of the anchialine fauna habitat preferences on regional and local scales. The habitat selection is here discussed by studying fauna inhabiting anchialine caves, principally along the Yucatan Peninsula, but also using marine and freshwater caves records, and other locations when pertinent. The high endemism rate of anchialine fauna, and the patchy distribution resulting in highly structured microhabitats; along with the distribution patterns of microorganisms and food sources, are key discoveries to understand the habitat selection of the anchialine fauna. Certain species have proven the dispersion capabilities, as the mysid Antromysis cenotensis, that has been recorded in a third of the Mexican anchialine caves, or the mysid Stygiomysis cokei inhabiting caves as far as 205 km; while the shrimp Typhlatya pearsei is in both, anchialine and freshwater caves. Nevertheless, other species as the sea star Copidaster cavernicola or the gasteropod Teinostoma brankovitsi, are found in a single cave, most likely as a result of highly specific necessities of biotic-abiotic conditions. Furthermore, anchialine fauna also exhibits a strong preference for just a small area of the caves. The main challenge to understand the habitat selection is the poor availability of information restricted to a small fraction of the anchialine caves, and practically inexistent for microorganisms, ecological function, and food sources.

3- TANAIDACEOS

Alvaro García-Herrero….

Colección Nacional de Crustáceos, Instituto de Biología, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México

The stygobitic isopods of the anchialine systems of the Yucatan Peninsula, Mexico, are revised and a new species is described to bring the total number of species to seven. A new species of the genus Curassanthura, of the family Leptanthuridae is now being described, being the first

species of the genus to be recorded from a continental site. It can be distinguished from the other known species in the genus by the shape of the head, wider than long, and the number of spines (30) on the palmar margin of propodus of pereiopod 1. All the available records of the other six known species were compiled and mapped for the first time. A key to all the anchialine species from the Yucatan Peninsula is provided, as well as photographs, except for Haptolana bowmani, to make their identification easier. The observed distribution pattern of all the seven species is discussed considering the geological history of the peninsula.

4- ANCHIALINE CAVE-DWELLING SPONGE FAUNA (DEMOSPONGIAE) FROM LA QUEBRADA SYSTEM, MEXICO, WITH THE DESCRIPTION OF NEW SPECIES

Patricia Gómez 1* and Fernando Calderón-Gutiérrez 2

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The sponge community has been ignored in most of the anchialine study sites, therefore, anchialine caves of Cozumel Island, especially La Quebrada system (Chankanaab Cenote), has not been studied, in terms of sponge biodiversity, despite being the richest group, and the second in density. The aim of the present work is to describe the new sponge species living in this cave, together with two new records for Mexico from open waters but also inhabiting the cave. Samples were collected by hand using cave diving techniques, and performed in the dark area, searching samples extending along the main line until reaching at least 100 m from the nearest entrance. The six new species reported belong to troglobite populations, from genera: Calix, Haliclona, Neosiphonia, Lithobactrum, and Diplastrella. In addition, three troglophile species that co-exist but are open water's ihabitants and first time recorded from anchialine ecosystems are Discodermia adhaerens, Geodia neptuni, and Cinachyrella kuekenthali. The latter two recorded at Veracruz coral reefs, eastern Gulf of Mexico, and at Cozumel Island coral reefs. This is the only inventory of Porifera in Mexican caves up to now. Not feasible to find more species, since the number of dives and observation time is apparently enough for same and constant environmental conditions. These Porifera findings make up an endemic community living in anchialine caves, not recorded elsewhere, despite the high research effort in Cozumel Island and great Caribbean reefs. Natural conservation programs are needed to preserve this unique and special ecosystem.

5- PRELIMINARY RESULT OF RESEARCH IN "TOMINA JAMA" AN ANCHIALINE CAVE, NERETVA RIVER ESTUARY, CROATIA

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Tomina jama cave was explored and preliminary research conducted in 2016 and 2017. Research was conducted by Baštinik from the city of Metković in collaboration with the Croatian Biospeleological Society, Ruđer Bošković Institute, Pennsylvania State University and Society for karst research- Phreatic. Tomina jama is currently the only known anchialine cave in the Neretva estuary. At this time, the cave has been explored up to 58 m with the last 23 m submerged. Tomina jama is a typical anchialine cave system with a freshwater lens at the surface and seawater layer at the bottom. Salinity in the cave increased from 0 at the surface to 33 at a depth of 22m as dissolved oxygen decreased from 9.1 mg/L to anoxic conditions at depth. The hypoxic/anoxic zone extended for more than 10 m. Water temperature ranged from 14.8°C at the surface to 16.2°C at the bottom while pH decreased with depth from 7.74 to 6.86. Redox sensitive trace metals (i.e. Mo and U) deviated from salinity driven conservative mixing behavior. The upper freshwater layer was characterized by low Mo and U concentrations, 6.5 and 2.1 nmol/L, respectively. As salinity increased with depth, Mo and U concentration also increased (65.0 and 8.1 nmol/L, respectively). Of interest however, as depth continued to increase to the bottom salt layer (salinity 33) the Mo and U concentrations were significantly lower than for open ocean (~115 and ~13.5 nmol/L, respectively) implying removal process of Mo and U within the anoxic bottom waters. Further investigation of possible Mo and U enrichment within the cave sediment and porewaters is needed in order to establish possible linkages between the water and porewater chemistry (i.e. especially sulphide concentration, a trigger for Mo water column exhaustion) and the removal processes of Mo and U within the water column. Within the anoxic bottom waters, increased concentrations of Fe and Mn were observed indicating the formation of reduced Fe and Mn minerals within. Several stygobionts were discovered in the freshwater layer including Serpulids Marifugia sp. and the crustaceans Troglocaris sp. and Nifargus sp. Most notable is the finding of the endemic serpulids in an anchialine cave. Prior to this discovery, Marifugia have only been found in freshwater caves

from Dinaric karst region. Preliminary DNA results indicate that this species is not Marifugia cavatica and may be a newly discovered species. Below the halocline, the cave walls and bottom were lined with a significant bacterial mat. Samples were collected for future microbiome analyses. This cave system is the farthest inland of any Croatian anchialine cave with significant communities in both the fresh and saltwater layers. The discovery of Marifugia sp. populations and the significant bacteria mats require further detailed research. Moreover, the cave may serve as a natural laboratory for investigation of redox sensitive trace metals biogeochemistry.

6- HUI LOKO – HOW A NETWORK OF ANCHIALINE POOL AND FISHPOND MANAGERS HAVE COLLABORATED TO RESTORE ECOSYSTEMS IN HAWAIʻI

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Hui Loko, or “pond group” in the Hawaiian language, is an informal network of 23 organizations (government and non-government), private landowners and Hawaiian cultural practitioners who collaborate to protect and restore traditional fishponds and anchialine ecosystems. This group is based on the West side of Hawaiʻi Island and is a subset of the statewide fishpond network called Hui Mālama Loko Iʻa (group to care for fishponds). The mission of Hui Loko is to “continue to preserve and restore loko (pond) culture and native Hawaiian ecosystems by providing a space for connection and exchange of ideas, resources, and encouragement among pond managers to perpetuate the traditional Hawaiian practice of mālama i kou wahi (care for your place).”

To achieve these objectives, Hui Loko members meet quarterly at a rotating local fishpond or anchialine pool location to discuss updates, share successes and challenges they face at their particular site. Working together, these caretakers and managers share resources, identify common goals as well as challenges, brainstorm solutions, and effectively communicate the importance of these wahi pana (sacred places) to other residents and island visitors.
In the past year alone, Hui Loko members collectively managed over 80 hectares of coastal brackish habitats, and successfully restored anchialine pools by removing invasive fish, resulting in the return of a native invertebrate species. We hope this poster helps to demonstrate the work of Hui Loko to the broader anchialine community worldwide, in the hopes to inspire further participation and collaboration in future International Anchialine Ecosystems Symposia.

7- DUNGEONS AND DRAGONS: KYNORHYNCHA IN CENOTES AND LAVA CAVES

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Mud dragons belong to Kinorhycha, a phylum of minute free-living marine ecdysozoans rarely longer than 0.5 mm. Although kinorhynchs appear from the intertidal to the deep-sea bottom all over the world in various benthic environments, most of the 250 described species are recorded from muddy shallow water marine sediments. Despite the significant advances in the study of kinorhynch diversity during the last years, cave dwelling kinorhynch fauna remains unexplored. Mud dragons are frequently recorded in submarine caves but rarely identified to species level, except for records of Echinoderes dujardinii and Meristoderes macracanthus in the Mediterranean Sea, Echinoderes cavernus from Queensland (Australia) and, Ryuguderes iejimaensis from Ryukyus Islands (Japan). Whereas E. dujardinii and M. macracanthus are widely distributed in marine environments, E. cavernus and R. iejimaensis exclusively known from single caves and considered as stygobitic.

We here present five new records of cave kinorhynch from a marine cave in the Canary Islands (Cueva de Punta Prieta, Tenerife) and an anchialine cave in the Yucatan Peninsula (Casa Cenote, Sistema Ox Bel Ha). Cueva de Punta Prieta is a fully marine lava cave with a direct connection to the ocean, while Casa Cenote opens at the end of a mangrove area and exhibits water stratification with both brackish and fully marine water bodies. Five species of mud dragons were identified belonging to three families: Centroderes barbanigra (Centroderidae), Paracentrophyes (Paracentrophyeidae, indeterminate species) and Fujuriphyes sp1 (Pycnophyidae) were recovered from Casa Cenote; Pycnophyes cf. zelinkaei and Leiocanthus sp1 (Pycnophyidae) from Punta Prieta. Two of the discovered taxa, Fujuriphyes sp1 and Leiocanthus sp1 can be considered exclusive cave-dwelling kinorhynchs, both species new for science and belonging to the family Pycnophyidae, one from Punta Prieta and one from Casa Cenote. Strange morphological characters are present in the pycnophyid specimens of Casa Cenote, with an anterior ornamentation never seen before in kinorhynchs. This feature may represent an adaptation to the life conditions of anchialine systems.

8- SUBSURFACE LASER SCANNING AND PHOTOGRAMMETRY IN THE CORONA LAVA TUBE SYSTEM, LANZAROTE, SPAIN

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The Corona lava tube (Lanzarote, Canary Island, Spain) is one of the world's largest volcanic cave complex, with a total length of about 8km, comprising both dry and submerged sections. The 6 km long terrestrial portion of the tube is open to the surface through a series of skylights, in the local language called “jameos”, that are aligned along the cave pathway. The underground passages are mainly sinuous, partly braided and multilevel, horizontal tunnels of variable width from 2m up to over 25m (5-7m on average), laying from few meters to a maximum of 30 meters from the surface. Although the first explorations and mapping campaigns of the lava tube have started already in the seventies, until now a clear view on the development and morphometry of this subterranean conduit was elusive. With three surveying campaigns carried out between February and November 2017, about 5 km of cave passages were mapped using a Leica P40 laser scanner. A total of 28 working days and over 440 scans were necessary to map this lava tube system, which is composed by different sections connected to each other through external collapses. The main path of the entire cave system was mapped with an unprecedented resolution of few centimetres, including the most relevant upper levels as the Jameos Cumplidos, the two tourist parts of the Cueva de Los Verdes and Jameos del Agua and the partly flooded part of Cueva de los Lagos.

The surveys realized inside the cave with the laser scanner were georeferenced on the surface through differential GPS, LIDAR (from the Spanish Geological Service) and photogrammetry data obtained through flights realized with unmanned aerial vehicles on the external collapses located along the cave path.

The dataset gathered by the detailed 3D model of the cave system have three main purposes: 1) create a virtual and analogic model of the cave to be used for outreach and didactic purposes in the touristic centre of Casa de Los Volcanes of the Lanzarote Geopark; 2) provide

a detailed map of the tube and its relative depths from the surface, identifying critical zones for potential collapses, in order to allow local institutions to develop a protection plan for this subterranean environment in relationship to the actual different land use of the overlying surface; 3) provide to scientist quantitative morphometric data (volumes, morphologies, surface roughness, etc.) to develop detailed studies on the tube genesis and peculiar morphologies. These latter studies are on-going and will allow for the first time a detailed comparison between this exceptional example of terrestrial lava tube and similar features observed on the Moon and Mars

ÀMBIT: BIOGEOCHEMISTRY AND HYDROGEOLOGY

1-SHAPING ANCHIALINE ECOSYSTEM FUNCTION: THE ROLE OF HYDRO-BIOGEOCHEMICAL PROCESSES IN A KARST SUBTERRANEAN ESTUARY

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Karst subterranean estuaries (KSEs) extend into carbonate platforms along 12% of all coastlines and provide an essential habitat for globally dispersed anchialine fauna. A recent investigation found that microbial methane consumption is an important component of the carbon cycle and food web dynamics within flooded caves that permeate a KSE in the Yucatán Peninsula – a region known for its extensive cave systems inhabited by the anchialine fauna. Nevertheless, basic questions about seasonal and hydrological effects on methane production, consumption and transport within such coastal karst aquifers are unresolved. In this study, we obtained high-resolution (2.5-day) temporal records of dissolved methane concentrations and its stable isotopic content ($\delta^{13}C$) to evaluate how regional meteorology and hydrology control methane dynamics in the KSE. Our records suggest methane pulses observed in the hypoxic brackish water are driven by precipitation-induced hydrologic events, which is consistent with less methane accumulated in the anoxic freshwater during the wet season (4,361 ± 89 nM) than during the dry season (5,949 ± 132 nM). The $\delta^{13}C$ of hypoxic brackish water methane (−38.1 ± 1.7‰) was consistently more ^{13}C-enriched than freshwater methane (−65.4 ± 0.4‰),

implying methane oxidation is a persistent process in the caves. This study has recently been expanded with continuous dissolved oxygen measurements collected from the dry to wet season that suggest seasonal differences in the availability of this electron acceptor. Using a hydrologically based mass balance model, we calculate the regional methane consumption during the dry season within the approx. 100 km^2 catchment basin of the cave passage investigated. The approx. 1 ton of methane consumed during the dry season within the catchment area constrains the availability of a critical resource that sustains a widely distributed subterranean ecosystem.

2- TEMPERATURE STABILITY AND PRECIPITATION IMPACTS OF ANCHIALINE CAVES

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Interannual thermal stability and precipitation disturbances on anchialine caves were evaluated. This study was conducted in Cozumel Island, Mexico, in two anchialine caves lacking direct connection with the sea (Tres Potrillos; Bambú), and two with direct connection (El Aerolito; La Quebrada). Temperature and precipitation were measured at 5 and 10 minute intervals from January 2015 to July 2016. Caves without direct connections exhibited a stable temperature, with an interannual variation of ≈0.5°C. Caves with direct connections had a strong seasonality with a variation of ≈5.5°C. In contrast, the water temperature at the furthest point from the coastline at La Quebrada, variated only 0.7°C; the cave morphology creates a “two cave” system in terms of temperature stability. The halocline is absent between “caves” (i.e. sections), therefore, the absent water layer diffuses through the rock. Temperature dropped along the year in the same magnitude than the interannual variation, corresponding to >30mm precipitations. In El Aerolito, a day after a storm, a 5m deeper mixing zone was present, as opposed to a marked halocline. Consequently, organisms died or were weak due to osmotic and thermal shock. Is possible to conclude that: a) Temperature stability depends on the type of connection with the sea and cave morphology; b) Single events, such storms can affect the anchialine fauna; and c) Depth of the halocline/bottom may have an important role in the anchialine biodiversity. Therefore, the risk for species inhabiting caves with haloclines close the bottom is magnified, and should be considering for anchialine fauna conservation.

3- STRATIFICATION OF A COASTAL ANCHIALINE SINKHOLE IN HUDSON, FLORIDA

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Hudson Grotto is a water-filled sinkhole located in northern Pasco County, Florida and is located less than a mile from the Gulf of Mexico. The sinkhole is approximately 46 meters and intersects the superficial Tampa Limestone down to the Suwannee limestone, and possibly into the Ocala limestone formations. Multiple scuba dives were conducted over a ten-year period to collect salinity, dissolved oxygen, temperature, and pH data via hydrolab. Each layer was subjected to water chemistry tests to measure the following: total organic carbon, phosphorous, ammonia, nitrite, nitrate, sulfide, sulfate, and alkalinity. The bottom most layer roughly begins at 30-35 meters. It lacks dissolved oxygen and has higher salinity than the other layers. The mid-water column layer and the upper most layer can be most differentiated during the wet season. The mid-water column layer has more dissolved oxygen than the bottom layer, and less than the upper most layer. When present, the upper most layer has the lowest salinity and highest dissolved oxygen levels. Hudson Grotto's water column has two to three distinct layers that can vary with seasonality, tidal cycles, and possibly major weather events. We are currently evaluating the microbial communities that thrive within the system to see if and how they are affected by the changes in the water column.

4- RAPID ASSESSMENT OF SESSILE BENTHOS IN MARINE CAVES OF THE ISLAND-DOMINATED AEGEAN SEA (EASTERN MEDITERRANEAN SEA)

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Marine caves constitute an iconic feature of the Mediterranean rocky coastline. In the eastern basin, the Aegean Sea encompasses a large number of marine caves that are mainly situated along the coasts of islands where limestone rocks prevail. However, only a small number of caves has yet been quantitatively assessed regarding sessile benthic communities. In this work, nine marine caves from different Greek islands were studied for the first time by means of SCUBA diving and non-destructive sampling in the framework of a rapid assessment initiative. In each cave, 16 photoquadrats (25 x 25 cm) were randomly obtained from the vertical walls. Sessile taxa were identified to the lowest possible taxonomic level and their spatial coverage

was estimated by using a dedicated seabed image processing software (photoQuad). A total of 70 taxa were identified, belonging to 9 major taxonomic groups. Sponges were the dominant group in terms of species number in all studied caves. Although statistical multivariate analysis revealed significant differences between all examined sites, they were consistently grouped in three main clusters: a) deeper and large caves (submerged and semi-submerged), b) shallow semi-submerged caves, and c) one semi-submerged cave with internal freshwater springs that was markedly differentiated from all others. The results highlight that in addition to a high level of individuality, different geomorphological types of caves also harbor distinct benthic assemblages. This preliminary biodiversity assessment provides a baseline for future monitoring and conservation initiatives.

5- METAGENOMICS PROFILE OF BUNDERA SINKHOLE, WESTERN AUSTRALIA

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Bundera sinkhole is a highly stratified anchialine system in which water quality profiles and stable isotopes suggest it is partly supported by chemolithotrophy. We investigated the composition and function of the microbial community between 2 and 28 m depth. Water quality was profiled using a sonde. Samples were pumped from ten depths to determine water chemistry and microbiology. Microbial densities were enumerated using flow cytometry and the composition and function of the microbial communities were investigated using 16S rRNA amplicon and shotgun metagenomic sequencing respectively. Polymodal profiles through depth existed for several water quality parameters and cell densities. DOC and CH4 were at extremely low concentrations and DIC at high concentrations throughout the profile. The photic zone was dominated by diatoms, algae, Gammaproteobacteria and Spongibacter. Below 8 m the microbial assemblage was dominated by Gammaproteobacteria and the ammonia-oxidizing archaea Nitrosopumilus. Metagenome-derived population genomes were recovered for more than 100 organisms, many of highly novel microbial taxa. Throughout the water column a high abundance of CO2-fixing genes coincided with a high concentration of DIC. At 2 m depth the CO2-fixing genes were largely associated with photosynthesis, however, at lower depths autotrophic carbon fixation appears to have been coupled with sulfide and ammonia oxidation.

These trends suggest that "dark carbon dioxide fixation" is the primary process fueling this ecosystem and that methane cycling plays a minor role.

6- THE BIOGEOCHEMISTRY OF ANCHIALINE ECOSYSTEMS: PROGRESS AND MORE POSSIBILITIES

John W. Pohlman David Brankovits

Anchialine ecosystems found within caves of karst subterranean estuaries (KSEs) are reservoirs for biodiversity and a groundwater resource for coastal populations throughout the world. Animals inhabiting KSE caves are specifically adapted to the dark and generally stable conditions of the caves, yet their survival depends on energy originating from either the sun or the center of the Earth. To transfer that energy to cave animals, plants and microbes must convert chemical and light energy into organic matter (OM) by photosynthesis and chemosynthesis. In comparison to photosynthetic material inputs, chemosynthetic production fueled by geothermal energy is likely rare in KSEs. However, that is not to say anchialine ecosystems are simply fueled by plant debris washed into the cave, especially in remote cave passages distant from sinkholes where limited particulate OM is found in the water column or sediments.

For the past several years, we have investigated KSE caves in the northeastern (NE) Yucatan Peninsula to identify the sources and forms of carbon and energy that sustain the community of cave-adapted crustaceans and fish found in areas outside the influence of the OM-rich cenotes. We chose to investigate pristine cave passages isolated from large sinkholes because they are the most prevalent KSE habitat for this region, and, most likely, for coastal karst globally.
We found that dissolved organic carbon (DOC) and methane are the dominant forms of OM in all the KSE cave passages we investigated. The carbon source for DOC and methane is surface vegetation that decomposes in the shallow anoxic vadose zone. The methane is both a product of fermentation and carbonate reduction; the latter a chemosynthetic process that is at least partially responsible for generating the unusual stable carbon isotope values Pohlman et al. (1997) reported from anchialine crustaceans decades ago. Subsequent mixing of DOC and methane into a micro-oxic water mass in the caves allows heterotrophic microbes to grow by assimilating the DOC and methane. In turn, the microbes support the higher trophic levels of the food web. The structure of the anchialine ecosystem food web is analogous to the oceanic microbial loop, but distinct in that methane is a substantial (25%, on average) part of the DOC assimilated by the food web.

A simple, but important, finding of our work is that stratification features typically not considered in conventional coastal aquifer models can be of crucial ecological importance. For example,

the NE Yucatan KSE is partitioned into three distinct layers of differing salinity and oxygen content (Brankovits et al., 2017), rather than it being a bi-layer of meteoric (or limnic) and marine water separated by a thin mixing zone, or halocline (See Bishop et al., 2015). The meteoric layer is separated into a fresh layer typically found near the ceiling of some domed passages and an underlying brackish layer. The fresh layer is both anoxic and rich with DOC and methane, whereas the underlying brackish water is slightly (but not always) hypoxic and contains far less DOC and methane. The fresh water is where organic matter from the overlying vegetation rots and decomposes. The interface between the fresh layer and the brackish layer is where the OM is oxidized. The juxtaposition of DOC-rich fresh water and hypoxic brackish water within the meteoric lens of KSEs is the key physical feature that facilitates the transfer of DOC into the food web.

Understanding what factors control the production, distribution and cycling of DOC in caves is the subject of our ongoing and future research. We have recently found that the intensity of precipitation between the wet and dry seasons in the dry tropical forests of the northeastern Yucatan Peninsula controls the dynamics of methane consumption in the caves, and that the biogeochemical reactions converting dissolved compounds into biomass occur within the cm-scale interface between the stratified water masses in the caves.

Although these contributions have greatly advanced our understanding for how such an abundance of life exists in anchialine caves, new questions raised vastly outnumber the answers obtained. We have identified that microbes known to utilize a range of electron acceptors are present, but we have not targeted specific pathways or directly measured rates of microbial activity. We have not analyzed the molecular composition of the dissolved organic matter to evaluate what compounds are most critical for supporting the food web. We have not established how quickly carbon moves through the food web. We do not understand how the biogeochemistry affects faunal biogeography. And, most important, our observations are largely limited to the NE Yucatan KSE, and only where the overlying vegetation is dry tropical forest and human impacts are not evident. We seek (and encourage others) to investigate settings where external OM inputs differ and where the hydrology alters the transport and mixing of chemicals that support life in anchialine ecosystems.

The globally dispersed nature of anchialine ecosystems and their association with expanding human populations reliant of the pristine condition of their waters alone merits further investigation of these systems. However, our observations have logical relationships with

processes that are central also to the operation of the carbon cycle of the oceans, and perhaps extraterrestrial bodies. Anchialine ecosystems are an excellent analog for the oceanic microbial loop within the expanding oxygen minimum zones. Processes occurring at the anoxic-hypoxic interface in KSEs might reveal what will occur as oxygen minimum zones in the oceans become more pervasive. Even clues related to possibility for the existence of methane-based life in the oceans of Saturn's moon Enceladus could be lurking deep within the caves of KSEs.
In this presentation, we will review the state of our knowledge for anchialine biogeochemistry, present updated models for how anchialine ecosystems function (based on recent findings), and predict how perturbations related to direct and indirect human activities may affect the viability of the waters and lifeforms within KSEs. Management and protection of this biological refuge and water supply rests on understanding how the ecosystems within these coastal aquifers operate in their natural, unaltered form. Biogeochemical studies investigating how chemical, physical, biological and geological processes within the KSE, surface biosphere and oceans are intertwined have the potential to provide the insight required to protect and preserve this unique and sensitive habitat, and to delineate how reactions within the KSE alter the chemical content of groundwater discharged into coastal oceans bordering karst coastlines.

7- THE FATE OF URBAN SPRINGS: PUMPING-INDUCED SEAWATER INTRUSION IN A COASTAL PHREATIC CAVE

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The objective of this study was to characterize the hydrogeological and ecological impacts that urbanization might have on Sulphur Springs Cave, an extensive phreatic cavity that produces a large spring in metropolitan Tampa, Florida, USA. To address potential mechanisms of seawater intrusion at this site, we consolidated historical and current hydrogeological data, and supplemented these data with our own collected on scientific dives. To identify ecosystem impacts, we sampled biofilms growing in Sulphur Springs Cave for analysis using fluorescence microscopy, carbon stable isotopes, and DNA sequencing and collected cave water for geochemical analysis. Groundwater extraction at this site has occurred for many decades, but intensified in the early 2000s, corresponding to a rapid increase in spring water salinity. Numerous vents in the cave issue saline, slightly-thermal, sulfidic water, and are likely connected to bedrock fractures which provide preferential flow-paths for deep-sourced

anchialine water. Cave-water salinity increased during dry-season pumping activity but also throughout wet seasons, likely because recharge to regional topographic highs disproportionately pressurized deep saline water. Saltwater vents hosted white, filamentous biofilms which exhibited δ13C values lower than those of sediment organics collected from a nearby, hydraulically-connected sinkhole (-35‰ and -28‰, respectively). Brown biofilms colonizing the remainder of the cave consisted of a mixed community exhibiting δ13C values matching those of surface sediment organics (-28‰). As groundwater extraction and seawater intrusion at this site continues, the cave ecosystem will probably start to favor the colonization of white biofilms, and the spring may become unable to support low-salinity surface habitats.

ÀMBIT: MICROBIOLOGY

1- AQUATIC GENOME-RESOLVED METAGENOMICS: AN APPLICATION TO ANCHIALINE SYSTEMS

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Thanks to the “next-generation” of high-throughput deep sequencing techniques (NGS), microbiology has entered a new dimension based on genome-resolved metagenomics and single cell genomics. Now it is possible to study the majority of the existing microbes in nature, which were hidden by many other approaches due to the intrinsic difficulty of obtaining them in pure culture. Furthermore, nowadays it is becoming relatively straightforward to obtain thousands of nearly completely assembled genomes from uncultured microbes of great ecological relevance coming from many different environmental samples (Albertsen *et al.*, 2013; Anantharaman *et al.*, 2016; Parks *et al.*, 2017). At present, many studies have focused on genome-resolved metagenomics in aquatic ecosystems, particularly from oceans, seas, freshwater lakes and reservoirs, soils or wastewater systems. We have applied the state of the art of metagenomics to provide nearly complete metagenome assembled genomes (MAGs) and phages/viruses from various environments such as salterns (Legault *et al.*, 2006), Mediterranean Sea (Haro-Moreno *et al.*, 2018), Caspian Sea (Mehrshad *et al.*, 2016), Amazon River (Ghai *et al.*, 2011), Lake Baikal (Cabello-Yeves *et al.*, 2018) and freshwater reservoirs (Cabello-Yeves *et al.*, 2017). Recent studies from karst subterranean estuaries in Mexico (Yucatan) have revealed methane sink and a great contribution of microbes involved in methanotrophy, heterotrophy, and chemoautotrophy, based on characterization by 16S rRNA gene amplicon sequencing (Brankovits *et al.*, 2017). However, anchialine systems and landlocked water bodies with subterranean connections to the ocean remain unstudied at this

high resolution genomic level. The study of the microbial communities from these systems could be of great interest to infer their contribution to the carbon cycle, to reveal novel and unstudied ecologically relevant microbes and to study the freshwater-brackish-marine microbial transitions of these coastal aquifers, which are density stratified, with the water near the surface being fresh or brackish, and saline water intruding from the coast below at some depth.

2- MICROBIAL MAT PROFILING IN A SHALLOW MARINE CAVE OF THE AEGEAN SEA (EASTERN MEDITERRANEAN)

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Cave-dwelling microbial mats are complex microecosystems, encompassing diverse bacterial phylotypes, while a correlation has been suggested between the main biochemical processes occurring in caves and the microorganisms they harbour. This study aims to examine the profile of a microbial mat in a shallow Aegean marine cave (Sithonia, Chalkidiki Peninsula, Greece) and to assess the potential ecological functions these organisms might perform. Parts of the microbial mat were collected, environmental DNA was extracted and the bacterial 16S rRNA gene was amplified by PCR and sequenced via Illumina MiSeq 2x300 bp. The recovered sequences were analyzed using Qiime 1.9. software. The results revealed dominance of the phylum Proteobacteria (51%), followed by Bacteroidetes (14%), Spirochaetes (10%), Firmicutes (5%), Deferribacteres (3%), Chlorobi (2%), Fusobacteria (1%), and Acidobacteria (1%). The orders Desulfobacterales and Desulfomonadales (Deltaproteobacteria), Campylobacterales (Epsilonproteobacteria), Rhodospiralles and Rhodobacterales (Alphaproteobacteria), Clostridiales, Spirochaetales, Deferribacterales, and Sphingobacteriales made up 55.3% of the bacterial community. The families Campylobacteraceae, Desulfobulbaceae, Desulfobacteraceae and Desulfuromonadaceae, strongly represented in the community, include genera known for sulfate reducing and sulfur oxidizing bacteria. Deferribacteraceae that have been found in deep-sea hydrothermal vents are also known for ferric iron, nitrate and sulfur reducing properties. Rhodospirillaceae and Rhodobacteraceae have metabolic potential of hydrocarbon degradation, denitrification and sulfur oxidation, whereas within

Spirocheataceae, halophilic and anaerobic strains are very common. The detection of these groups in the studied cave indicates the use of inorganic sulfur and nitrogen compounds as energy sources by members of the microbial mat community, although further research is needed to assess the involved biogeochemical processes.

3- MICROBIAL COMMUNITY FUNCTION WITHIN A STRATIFIED ANCHIALINE SINKHOLE: A WINDOW INTO THE COASTAL MIXING ZONE

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Anchialine sinkholes provide insight into coastal aquifer systems to understand groundwater ecosystem function. Aquifer microbial community function is usually inferred from geochemical information and there are few direct studies of microbial communities in the Floridan Aquifer. Hospital Hole is a 43m deep stratified anchialine sinkhole under the Weeki Wachee River, FL, with three distinct brackish layers; a hypoxic layer just below the river, a chemocline, and a deeper sulfidic anoxic layer. These three layers appear to be stable in the wet and dry seasons because of the Weeki Wachee River flowing over the sinkhole. Illumina sequencing and bioinformatic tools were used to reconstruct the potential metabolic functions and interactions of microbial communities in each layer. Each layer had a distinct microbial community and unique functions, which were influenced by the respective geochemistry. Sulfate oxidation and nitrate reduction appear to be the most abundant functions. Syntrophy between methane oxidizers, methanogens, and sulfate reducers is present in the anoxic layer. Similarities between the geochemistry and observed connectivity of Hospital Hole and the Upper Floridan Aquifer suggest that microbial communities of Hospital Hole can be used as a surrogate for the aquifer in the absence of direct studies of aquifer. Understanding the function of groundwater microbial communities within the Floridan Aquifer might be useful in predicting how the Floridan aquifer might react to anthropogenic change.

4- MICROBIAL LIFE IN A UNIQUE CHEMOSYNTHESIS-DRIVEN CAVE ECOSYSTEM

Deepak Kumaresan

Despite the lack of photosynthetically-fixed carbon, the Movile Cave ecosystem (located near Mangalia, Romania and situated ~25m below ground) hosts a diverse range of invertebrates

(worms, insects, spiders and crustaceans) that are adapted to life in the dark. This hypogenic cave ecosystem, devoid of any inputs from the surface due to layers of impermeable clays and loess, has been estimated to be isolated from the surface for ~5.5 million years. Majority of the invertebrate species present are endemic to Movile Cave and surrounding aquifers. Life within this ecosystem is supported by chemoautotrophic primary producers that derive energy from the oxidation of inorganic compounds (such as hydrogen sulfide) and methane that enter the cave with thermal fluids, analogous to deep-sea hydrothermal vents. More importantly methylotrophic and autotrophic bacteria fix carbon within this ecosystem. I will present our recent work on the microbiology of the Movile Cave ecosystem, with particular emphasis on functional diversity of bacteria involved in one-carbon metabolism (methane & methylated amines; methylotrophy).

5- MICROBIAL FUNCTIONS IN STRATIFIED WATERS THROUGH GENOMICS AND PROTEOMICS

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The meromictic lake Cadagno is characterized by high influx of sulfate, magnesium and calcium in the euxinic monimolimnion that generates a steep chemocline around 12 m depth. Within this zone a dense population (up to 107 cells per ml in summer) of mainly anaerobic phototrophic purple sulfur bacteria (PSB) of the genera Chromatium, Lamprocystis, Thiodictyon, Thiocystis, and the green sulfur bacteria (GSB) Chlorobium. Candidatus “Thiodictyon syntrophicum” strain Cad16T was shown to be highly productive in CO2 fixation, both in light and darkness. As PSB are typically characterized as phototrophic organisms, an important step is to understand also their dark CO2 fixation metabolism. Preliminary laboratory tests using 2D gels highlighted the presence of 17 protein spots that were more abundant in the dark, including three enzymes that

are part of energy pathway, such as the tricarboxylic acid cycle and β oxidation of fatty acids (polyhydroxybutyrate).

In this study we present a further proteomic analysis of Cad16T cultures incubated directly in situ within the chemocline of Lake Cadagno at a depth of 12 m using dialysis bags.

The incubation in their natural environmental produced different results compared to previous experiment carried on in laboratory. Moreover, the complete genome annotation of Candidatus "T. syntrophicum" strain Cad16T and PSB Chromatium okenii, also able to fix CO2 in the dark, permitted us to look for conserved metabolic pathway in similar microorganisms. In parallel, physical and chemical parameters were measured in situ daily. Therefore, we can constantly monitor the conditions that apply for the incubated cultures. To have also an understanding on the short term gene regulation, Cad16T mRNA levels were compared in a parallel. Additionally, CO2-reduction rates were measured using 14C scintillation protocol.

6- EXPLORATION OF CYANOBACTERIA MICROBIAL LIFE IN MEDITERRANEAN TERRESTRIAL CAVES

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Recognition of microorganisms in geologi¬cal processes in caves altered our perception of cave eco¬systems. Cyanobacteria and algae frequently play a key role in the trophic networks and colonization processes of rocky habitats, producing colourful patches on cave walls. Aerophytic cyanobacteria are easily observed in the entrance of caves, illuminated by direct or indirect sunlight, and in show caves equipped with arti¬ficial illumination, as part of the lampenflora community. Despite of the extended karstic environment in Greece with a high number of cave formations, few biological inventories have been compiled. In this context, we examined cyanobacteria populations in three unexplored caves of NW Greece, two in Corfu Island (Klimatia and Grava Caves) left in their natural state and one show cave, Perama Cave, in Ioannina. Cyanobacteria, growing on cave walls and around natural and artificial light sources, were scraped off with a scalpel or else a piece of rock was collected in order to preserve the natural form of the community. Cyanobacteria populations were examined microscopically and isolation efforts took place. We identified 54 cyanobacteria taxa, representing 34 cyanobacteria genera; we also isolated and characterised >30 cyanobacteria strains with respect to their morphology and phylogeny. Scytonema, Cyanosarcina, Nostoc

Chlorogloea and Chroococcus were the most common genera amongst the caves examined, whilst Perama cave had the greatest representation of cyanobacteria species, probably due to the presence of artificial light and underground water. The cyanobacterium Geitleria calcarea is reported for the first time Greece. The analysis of cyanobacteria populations in three Mediterranean terrestrial caves suggest a high degree of biodiversity, whilst molecular data from strains may provide new information for cyanobacteria diversity examined, until recently, only by traditional morphology-based approaches.

7- NOVEL CYANOBACTERIA DIVERSITY IN CAVES? INDICATIONS FROM CHIOS ISLAND, GREECE

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Caves are unique habitats supporting distinctive biofilm communities including cyanobacteria, chlorophytes, diatoms, dinophytes, fungi, and lichens. These phototrophic biofilms are adapted to all types of stress imposed by growth at air-rock interface and have developed the capacity to tolerate extreme near starvation conditions. Motivated by investigating the underexplored diversity of cyanobacteria in caves, we sampled two terrestrial caves (Agio Galas and Olympon caves) in Chios Island, located in Aegean Sea, E. Greece. Sample from Agio Galas cave was collected from a rock near artificial light, whereas in Olympon cave sample was collected from the wall side of roof hole open to sunlight. We isolated four strains belonging to three genera (Komarekiella, Phormidium, Leptolyngbya), and one Chroococcaceae strain, whose taxonomic status requires further investigation. Strains were characterised based on their morphological features, whilst their phylogeny was investigated by 16S rRNA and phycocyanin operon sequences. This work reports the occurrence of a taxon belonging to the newly established genus of Komarekiella, after it was first described in 2017 from the Hawaii region (Komarekiella atlantica gen. et sp. nov.). However, phylogeny results suggest that Komarekiella strains from this study may belong to a different species. Moreover, based on phylogenetic markers, our Chroococcoceae strain formed a clade distinct from other unidentified coccoid cyanobacteria, indicating new biodiversity of cyanobacteria from caves. We demonstrate the isolation and characterisation of five strains from two caves of a previously unexplored area, providing a missing study material for cave habitats ecology, concerning rock-dwelling cyanobacteria.

8- CHARACTERIZATION AND ANALYSIS OF THE CRAB CREEK CAVE BIOFILM COMMUNITY

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Crab Creek Cave is a coastal submerged cave in west-central Florida which forms a spring-head tributary in the Chassahowitzka River. This spring is unique from others in this watershed because it discharges brackish water and expels bright orange biofilm. Observations by divers have confirmed orange biofilm growing along the cave walls and a rare brackish-water population of Crangonyx hobbsi, a troglobitic amphipod. The purpose of our study is to characterize the orange biofilm microbial community and determine its role in the trophic structure of this anchialine cave using scientific cave diving techniques to collect hydrochemical, biological, and stable isotope samples. The cave water varies in salinity (2.8-5.8 ppt) in response to Florida's wet season, but maintains an average of 0.40 mg/L of total organic carbon. Nitrate and phosphorus levels remain at an average of 0.63 mg/L and 0.06 mg/L respectively. This indicates that this environment is oligotrophic, despite the presence of microbial life. Through fluorescence microscopy, we have identified sheaths characteristic of some chemolithoautotrophic iron-oxidizing bacteria. Future stable carbon and nitrogen isotope analyses will identify the trophic role of the Crab Creek Cave biofilms, and metagenomic 16S rRNA gene analyses will define the biofilm community structure and function. This study will (1) expand our understanding of how anchialine cave ecosystems differ from nearby freshwater ecosystems, (2) address the knowledge gap of primary production via iron cycling in underwater caves, and (3) define a pristine habitat that serves as a model to compare to local anchialine springs influenced by urbanization.

ÀMBIT: EARTH SCIENCE

1- MODERN BENTHIC FORAMINIFERA IN PALM CAVE BERMUDA: IMPACT OF CONDUIT MORPHOLOGY ON FERTILIZATION OF CAVE BENTHIC HABITS

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The main island of Bermuda is well renowned for its karst subterranean estuaries, which are innervated with flooded cave systems that are populated with anchialine ecosystems. In

general, Bermuda's cave systems can be considered flooded by the lower subterranean estuary, and the commonly investigated systems (e.g., Walsingham Cave, Green Bay Cave, Palm Cave) currently have well oxygenated water columns. These environmental conditions are well suited for using benthic foraminifera (shelled protists) to evaluated benthic habitat variability in these types of karst subterranean estuaries. The Palm Cave System is entirely flooded by well-oxygenated saline groundwater (no significant meteoric lens development), and has both limited subaerial and submarine karst windows. The purpose of this study was to investigate the distribution of benthic foraminifera in the Palm Cave System (PCS) in twenty-eight surface (<3 cm depth) sediment samples, with respect to several environmental characteristics of the benthic habitat (water depth, texture, organic matter content, C:N, $\delta^{13}C_{org}$). Q-mode cluster analysis on the resultant benthic foraminifera identified three distinct assemblages in the cave. The most diverse assemblage (Fisher alpha of 9.1) associated with passages between entrances is represented by *Spirophthalmidium emaciatum* and *Spirillina* spp. Passageways are characterized by 10% organic matter content and 204 mg/cc coarse sediment fraction (>63 μm). The second most diverse assemblage (Fisher alpha of 8.7) is proximal to submarine karst windows and is represented by *Rosalina* spp. and *Triloculina* spp. Benthic habitats near submarine karst windows contain ~5% organic matter content and 605 mg/cc coarse sediment fraction (>63 μm). However, these assemblages differ from caves with larger submarine karst windows (e.g., Green Bay Cave). The least diverse assemblage (Fisher alpha of 7.4) was more proximal to subaerial karst windows and was dominated by *Patellina* spp., *Spirillina* spp. and *Spiropthalmidium emaciatum*. Benthic habitats near subaerial entrances are characterized by ~10% organic matter content and 95 mg/cc coarse sediment fraction (>63 μm), suggesting limited terrestrial sediment flux compared to caves elsewhere. The C:N and $\delta^{13}C_{org}$ values were consistent with marine conditions throughout all environments. These results indicate that sediment source (i.e., subaerial vs. submarine) and size of the conduit morphology impact the types of benthic environments present within the karst subterranean estuary.

2- A GEOLOGICAL APPROACH TO THE ANCHIALINE KARST ENVIRONMENTS OF MALLORCA ISLAND (WESTERN MEDITERRANEAN)

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Anchialine environments are widespread along the coast of Mallorca island, owing to the abundance of carbonate rock outcrops which show both kind of karst, telogenetic and eogenetic. Anchialine pools are a quite common feature in the littoral caves of Mallorca, as a

result of the Holocene sea level rise and the subsequent adjustment of the coastal water-tables around the island. These pools, affected by tidal movements, hamper to the conventional "dry" cavers the access to the lower part of the cave systems, but at the same time it opens to scuba-diver cavers an impressive amount of potential underwater exploration. From the nineties, more than 45 km of drowned passages have been explored and carefully mapped by Majorcan scuba-diver cavers in the eogenetic karst region of Migjorn. The plan patterns of many Migjorn caves are rather chaotic, being mostly the result of breakdown collapse chambers connected more or less randomly, but also demonstrates the presence of ancient phreatic conduits related to former lower levels of speleogenesis or promoted by specific lithological or hydrogeological conditions.

The speleogenesis and morphological evolution of Mallorca's coastal karst were mainly controlled by sea-level fluctuations along the Pliocene and Quaternary times, including the presence of several types of detritic cave sediments and conspicuous Phreatic Overgrowths on Speleothems (POS) which record Mediterranean sea-stands. POS are powerful tools for sea-level change reconstruction, because they record with an excellent accuracy the elevation and fluctuation range of the sea surface at the time of their deposition. Furthermore, interesting paleontological and archeological data have been supplied by some coastal caves of the island spanning from the Pliocene to the Middle Ages.

3- HOLOCENE ENVIRONMENTAL DEVELOPMENT IN KARST SUBTERRANEAN ESTUARIES

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Karst Subterranean Estuaries (KSE) have a worldwide distribution on carbonate landscapes and they are populated by endemic anchialine ecosystems. Modern KSEs can be characterized by an upper meteoric lens (varying salinity, oxygenation) buoyed on saline groundwater intruding from the ocean below (varying oxygenation), and these water bodies increasingly mix and destabilize in the subsurface towards the coastal zone. These hydrographic conditions impose boundary conditions for the ecological requirements of different anchialine organisms, and on Holocene timescales, migrate in time and space in response to sea level change. Here we present a multi-proxy stratigraphic analysis of 14 sediment cores that were collected from 2 to 20 m below groundwater level in the Palm Cave System, Bermuda (e.g., textural analysis, x-radiographs, microfossil analysis, radiocarbon dating). The rate of deposition at each core site

was variable during the Holocene, and was dependent upon the proximity of core sample locations to cave openings ('karst windows') and conduit geometry. The oldest recovered sediment was Pleistocene-aged, terra-rosa soil deposits that predate the Holocene inundation of the cave by the coastal aquifer. By ~9500 Cal yrs BP, deposition in the cave was dominated by an organic-rich sapropel facies containing low brackish foraminifera (Trochammina, Polysaccammina) and bivalves, which indicates that a meteoric lens (low salinity, oligohaline) of the coastal aquifer flooded the cave benthos by ~9200 Cal yrs BP. A coherent pattern of Holocene sea-level rise in Bermuda emerges when the sedimentary results from the Palm Cave System, which is a minimum sea-level indicator, is compared to other Bermudian sea-level indicators (marsh deposits, cave deposits elsewhere). A system-wide shift to carbonate deposition occurred in the Palm Cave System at ~6500 Cal yrs BP, which indicates that the onset of oxygenated marine water entering the cave and development of a marine-dominated (i.e., submarine) cave environments. These results indicate that sea level exerts a primary control on environmental conditions in any individual cave passage through its tight coupling to groundwater in the Karst Subterranean Estuary. In addition, these results indicate that hydrographic conditions in the Karst Subterranean Estuary are going to be linked to the interaction between coastal circulation and available karst windows. Lastly, given that these results document environmental conditions not currently known in Bermuda, these results attest to the ability for anchialine ecosystems to rapidly migrate in the subsurface to locations (cave passages) with more suitable habitat.

ÀMBIT: MACROECOLOGY AND LARGE DATABASES

1- THREE YEARS WITH THE WORLD REGISTER OF MARINE CAVE SPECIES (WORCS) INITIATIVE: CURRENT STATUS AND FUTURE PERSPECTIVES

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The World Register of marine Cave Species (WoRCS) constitutes a Thematic Species Database of the World Register of Marine Species (WoRMS). It was launched in 2016 by 8 founding Thematic Editors and the WoRMS Data Management Team with the aim to create a comprehensive taxonomic and ecological database of species known from marine caves and anchialine environments worldwide. Currently the WoRCS database includes 1396 marine and 237 non-marine accepted species which belong to 19 phyla (17 Animalia and 2 Chromista) as well as 575 relevant literature sources. The database is an open source and is available online through a dynamic webpage hosted under the webpage of WoRMS (http://www.marinespecies.org/worcs/). The WoRCS Editor Group consists of 12 Thematic Editors with different roles in terms of taxonomy, management, types of environments and geographic areas covered. The International Symposium for Anchialine Ecosystems provides an excellent opportunity for co-organizing a parallel special “WoRCShop” where WoRCS Editorial Team, data managers and collaborators will be able to present new databases, review the WoRCS 5-year Strategic Plan and to develop a new work plan for 2018-2020.

2- WHAT IS ANCHIALINE ANYWAY?

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Initially defined as land-locked water bodies with marine origin partially isolated from the sea harboring specific assemblages of animals never found in the open ocean, anchialine habitats are island-like habitats spread all over the world often consisting of many access points linked by a common geological history but displaying different ecological parameters (e.g. distance to the sea, topography, depth). Ranging from tidally-influenced shallow ponds to 269-km-long subterranean labyrinths, they comprise numerous, relatively discrete, and varied entities that facilitate independent colonization events by epigean and hypogean biota, providing many replicates of comparable evolutionary processes. The data produced over the past 100 years of research collectively indicates that subterranean aquatic systems harbor a high percentage of local endemism and disharmonic animal communities when comparing them to surrounding epigean environments. However, it remains unclear whether those are general trends, or whether confounding effects such as sampling bias may produce misleading results.

We here discuss patterns of diversity in anchialine systems based on a global dataset of ca. 52,000 records for ca. 4,000 species in 6,000 aquatic caves, wells and springs with two goals. (1) We explore for common patterns in species composition and function across so-called anchialine ecosystems across the world; (2) We account for the influence of sampling intensity on the analyses by including the number of samples and number of published papers explicitly in the analyses. The highest diversity of species and habitats was found in the Caribbean and Mediterranean basins: two areas with many caves covering a wide range of ecological conditions, as both basins include marine, anchialine and freshwater subterranean habitats. However, the number of species in the Indopacific is comparatively high, despite the significantly lower number of papers published in this area. Whereas most of the recorded species correspond to crustaceans, we use the case of study of La Corona lava tube to discuss whether this dominance is due to the higher interest that these animals have historically awoken amongst cave biologist or, instead, reflects the adaptability of the groups in this specific type of environments. Our conclusions are briefly discussed based on published and ongoing phylogenetic studies and comparative methods; as well as the broader view of subterranean aquatic environments provided by classic literature.

3- A NEW LOOK TO STYGOFAUNA MUNDI: DATASET FOR AQUATIC FAUNA IN SUBTERRANEAN ENVIRONMENTS USEFUL TO UNDERSTAND DIVERSITY IN ANCHIALINE ECOSYSTEMS.

Alejandro Martínez *1, Nikoleta Anicic 1, Salvatore Calvaruso 1, Nuria Sánchez 2, Laura Puppieni 1, Tommaso Sforzi 1, Silva Zaupa 1, Rayanne Setubal 3, Fernando Calderón 4, Fernando Álvarez 5, David Bránkovits 6, Ludwik Gąsiorowski 7, Vasilis Gerovasileiou 8, Brett C. Gonzalez 9, William F. Humphreys 10, Alexandra Petrunina11, Nabil Majdi12, Thomas M. Iliffe4, Katrine Worsaae13, Nicolas Bailly 14, Diego Fontaneto 1

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The potential of subterranean environments as models to address major evolutionary and ecological questions has been highlighted in the literature. They represent partially isolated, discrete units offering several replicates of the same evolutionary processes. Amongst them,

anchialine habitats are represented by tidally influenced subterranean estuaries hosting particular assemblages of species with diverse origins and kinships. Species occurrence data of these environments is abundant, although sparse in the literature or gathered in databases established according to regional, taxonomical, or ecological criteria. We here present our efforts to assemble a new dataset consisting of records of aquatic animals in all types of caves or wells from all over the world. Literature sources were gathered from Google Scholar by independently searching for each metazoan phylum/arthropod order, as well as the key words “cave”, “groundwater”, “well”, or “stygobite”, in English, Galician, Spanish, Portuguese, Catalonian, French, Italian, Hungarian, Greek, German, Polish, Russian, and Serbo-Croatian. The relevance of each source was confirmed after checking the title and the abstract. For each selected source, we examined its reference list in order to identify studies that were not published in journals indexed in the databases we searched. From the 6886 selected references, we manually extracted all records that concerned either (1) occurrence of a species in a given geographical area or (2) occurrence of any taxon in a particular cave or well. Records were classified as primary or secondary, depending on whether they provided new information or referred to already published records, allowing us to identify redundant information in posterior analyses. Information for each access point was organized in as a gazetteer, including synonym names, geographical, ecological, and geological information. Following this strategy, we have obtained 52,485 records (23,513, primary) from 2020 references checked so far. Most records are amongst fish and crustaceans. In contrast, few data exist for other groups that are comparatively diverse outside caves, such as Nematoda. Relevant information will be included in World Register of Marine Cave Species.

4- ANTIBIOTIC RESISTANCE GENES

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5- ADDITIONS TO THE CAVE COPEPOD DIVERSITY FROM THE YUCATÁN PENINSULA AND CUBA

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The Yucatán Peninsula (YP) is a large karstic Neotropical system in south-eastern Mexico which includes a variety of epigean and subterranean aquatic ecosystems hosting a highly diverse crustacean fauna. In this area extensive networks of submerged subterranean cave passages extend from the cenotes along the central part of the Caribbean coast, where more than 1000 km of underwater cave passages have been surveyed. Several of such systems, even those fair inland have a surface layer of fresh water underlain by sea water. In the last decades special attention has been paid in the study of such anchialine ecosystems and its associated fauna. Herein we present additional records of copepods from the YP, especially from systems located within the corridor Tulum-Puerto Morelos, an area under high touristic pressure which has been pointed out as a hot spot for cavernicolous fauna worldwide. Twenty new records of copepods including cyclopoids, harpacticoids, calanoids and misophrioids are added to the vast diversity of the YP, while five records are reported for Cuba. One of the most interesting discoveries is a new species of the genus Speleoithona from the Akumal area (YP), which also represents the first record of the genus outside of Bermuda, where the three species of genus are distributed. The family Halicyclopinae presented the highest diversity of cyclopoid species, of which two are new species of the genus Neocyclops and one new species of Halicyclops. Among misophrioids a new species belonging to the Boxshallia-complex and a probable new species of Speleophria are reported. In the Cuban caves the discovery of a new species of genus Palpophria represents the first record of the genus outside of its type locality (Jameos del Agua, Lanzarote). A complete analysis of the cave copepod fauna and the conservation strategies of anchialine ecosystems in the YP and Cuba is provided.

6- THE CANARY ISLANDS SUBTERRANEAN REALM AND ITS ADAPTED FAUNA

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The origin and geological evolution of the Canary archipelago is analyzed, pointing out its old age with a continuous volcanic activity until present, and its proximity to a continent in

comparison with other oceanic archipelagos. All this features combined with a high ecological diversity has led to a rich biota plenty of plant and animal endemisms.
An introduction to the different subterranean environments is presented, emphasizing on those particular to volcanic terrains, the only ones present in the archipelago besides a few quaternary sediments. The terrestrial underground includes a high variety of habitats, depending on the different kind of lavas and their structures, and on the shallow subsurface layers as colluvial, alluvial and pyroclastic deposits and different types of soils. All these subterranean habitats hold an adapted, troglobiont fauna whenever they have enough humidity and some income of organic matter. The inhabitable deep underground is represented by the macrocaverns (> 20 cm) which in volcanic terrains are mainly lava tube caves, and less frequently volcanic pits and emptied dykes; and the network of interconnected mesocaverns (1 to 20 cm) and microcaverns (< 1 cm) from the bedrock, which have turned out to be even more suitable for troglobionts than the caves themselves. The most abundant shallow subsurface habitat is the volcanic MSS (mesovoid shallow substratum), made up by the lava clinker of moderately recent lavas. In older terrains there are other habitats like the weathering MSS (i.e. the deep C1 layer of the soil), the colluvial MSS or scree usually accumulated at the base of rocky cliffs, or the alluvial MSS under the bed of dry ravines; all these MSS's are usually protected against moister loss by soil or gravel lying upon. Another subsurface, very shallow subterranean habitat are the exposed pyroclastic fields that in spite of lacking any protection on their surface, they hold a varied and abundant adapted fauna thanks to the abundance of micro and mesocaverns and their capacity of moisture retention. As many as 170 troglobiont species have been described from these terrestrial habitats, all of them being endemic to the archipelago, each one exclusive to a single island having originated by local evolution, with no chance to colonize other islands. The faunistic differences found among the seven main islands are interpreted according to their geological and ecological conditions, and some examples are commented as interpreted to have originated by any of the current accepted theories for the evolution of terrestrial cave animals. Rainfall on the Canary Islands is not very abundant, which combined to the high porosity of the terrains derive to a scarce and deep phreatic level, so lava tubes and pits never have permanent streams or ponds inside. Thus, very few freshwater stygobionts are known, namely two amphipods and two nereid polychaetes, usually living in the tap water beneath ravine beds. There is a moderately richer stygobiont fauna in interstitial waters close to the seashore, especially amphipod species which are endemic to the different islands. But the most remarkable adapted fauna is found in anchialine environments, present in several islands but especially in Lanzarote, from where more than 60% of the 75 so far know Canary stygobionts are found. Most of the anchialine stygobionts occur in the flooded parts of the Corona lava tube system (Atlantida Tunnel, Jameos del Agua and Jameo de los Lagos),

though some of them are also occurring in interstitial waters from the nearby. The origin of the stygobiont fauna has been largely discussed, with some groups clearly related to other continental relatives, either Palearctic or amphiatlantic, but many others derived from deep sea ancestors which colonized the shallow anchialine habitats from Lanzarote.

7- LITHISTID DEMOSPONGES FROM SUBMARINE CAVES OF CRETE ISLAND (EASTERN MEDITERRANEAN SEA): IS THEIR OCCURRENCE CONTROLLED BY WATER SILICATE CONTENT?

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Lithistid demosponges (formerly order Lithistida), also known as “rock sponges”, exhibit a preference for deep waters and dark marine caves in tropical and warm seas. Specifically, 70% of the Mediterranean lithistid species have been recorded in marine caves, mostly in the Western Mediterranean basin. During the last decade, several marine caves of the Aegean ecoregion were surveyed with SCUBA diving in the framework of a broader scientific study on Eastern Mediterranean marine cave biodiversity. Large lithistids were recorded in three marine caves with internal freshwater springs in Crete Island. Sponge samples were collected for taxonomic identification (morphological and SEM examination) and water silicate concentration was measured in search of potential correlation with the lithistid distribution patterns. Surprisingly, sample examination allowed for their attribution to the corallistid species Neophrissospongia endoumensis Pisera and Vacelet, 2011 which was so far known from only one submarine cave in Marseille area (France, Western Mediterranean). Water silicate concentration in the cave zones where lithistids were found was 8-11 times higher (due to freshwater influx) than at the cave entrance. Lithistids are heavily silicified sponges in comparison with other demosponge taxa. Thus, we believe that apart from darkness and oligotrophy, which are similar in marine cave and deep-sea habitats, it is this high silicate concentration that allows for larval colonization and development in the studied marine caves. Further research in deep waters and marine caves with similar features in the Eastern Mediterranean Sea is expected to increase our understanding of the lithistid ecology and diversity.

ÀMBIT: CAVE DIVING AND EXPLORATION

1- DIVING EXPLORATION AND DISCOVERY IN ANCHIALINE CAVES: MY STORY

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My story began in 1977 with my first job as a Research Scientist at the Bermuda Biological Station. Arriving on the isolated mid-Atlantic island of Bermuda and beginning to scout around in my spare time, I was surprised to find more than a hundred limestone caves within a 10-minute ride from my home. Since Bermuda is a small oceanic island and nowhere is far from the sea, most of these inland caves soon descended to clear blue brackish water lakes that rose and fell with the tides. My curiosity was further peaked when I learned that no one had seriously looked for marine life in these subterranean environs. After inviting a cave diving instructor friend from Florida to come teach a course to myself and several local friends, we began serious diving explorations every chance we had. Before long, we found that Bermuda's underwater caves were even larger, better decorated, and extended much farther than the dry caves above. Below a depth of several meters, cave water reached ocean level salinity and a great variety of cave-adapted crustaceans were found. Shockingly these animals were not just new species, but instead represented new orders, families and genera. Often they had close ties to the deep sea or to cave locations in the Caribbean or Mediterranean.

After leading diving and collecting trips to caves in the Bahamas and Yucatan Peninsula, I was intrigued to see what might be found in a large submarine lava tube cave I heard about in the Canary Islands. This trip was followed by expeditions across the Pacific Ocean, first to Palau and the Philippines, and later, a yearlong island-hopping jaunt from Tahiti, to the Cook Islands, Tonga, Fiji, Western Samoa, Niue, New Caledonia, the Loyalty Islands, Australia, Tasmania, New Zealand, and Vanuatu. Decades later, I'm still not ready to stop, although most work now is closer to home in Yucatan, the Bahamas or Bermuda. To date, I have found and helped describe more than 300 new species of cave animals, including 70 new genera, 14 new families and 3 new orders. Nevertheless, I'm betting there are still many more discoveries out there, waiting to be made.

ÀMBIT: CAVE EXPLORATION

1- LIQUID CAVES: CAVE EXPLORATION IN MALLORCA

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In recent years, the exploration of underwater anchialine caves, of the Balearic Islands, has grown immensely due to new discoveries. The improvement of both diving techniques in itself, and that of the instruments used in filming, photography and illumination have allowed us to access and document newly discovered areas. The documentary offers, as well as images of cavities, statements of both speleologists and scientists. The discoveries shown, give new clues about global warming, and their answers have lead us to conclusions over the different climate changes that have occurred since before the Quartenary. To the present day, the exceptional conditions of conservation that the island's varied cavities present, have allowed for the majority of speleothems to be conserved in magnificent conditions. The paleolevels, a particular type of speleothem, give us information about the different levels the sea has had during these last five million years. This information will allow us to, knowledgeably, deal with the upcoming challenges of climate changes. Caves systems, such as those of 'Sa Gleda-Camp des Pou', 'Sa Cova des Pas de Vallgornera' are genuine treasures, measuring more than 30 Km. of submerged galleries between them both. We have only found such morphological similarities of our cavities in the Yucatan peninsula in Mexico, which is cited in the video report. The documentary refers to the findings of fossils, abundant and varied in some areas. Its study allows us to recreate the fauna of the region. On the other hand, the deposits of ceramics found, provide us information about the different cultures that settled on our islands. Many of the images are unpublished. The difficulty in obtaining them is primarily due to the complexity of the development of our cavities, and to filming and lighting in a hostile environment. The handling of the material up to and through the cavity as well as passing through siphons and submerged sections, make a day's work, for obtaining just a few images, to be insufficient. The improvement that has taken place in the materials and technique progression in cave-diving, has allowed us to document places up to now inaccessible. Improved side-mount systems and propellers of great autonomy have been essential for discovery, exploration, topography, and for the research of new galleries. Another great advance has been the technological revolution that has occurred in the world of illumination. The use of led technology, with more power to less consumption, and the new batteries, have contributed immensely to having great autonomy of lighting that, never before was imagined. The unquestionable improvement in lighting has not only allowed us to film our underwater caves with great amplitude, but has also been decisive for exploration, being able to observe every corner of our complicated galleries.

On the other hand, the latest models of cameras and underwater casings, as well as the large filming formats, have made it possible for us to show, in more detail, this tough environment.

LITHOLOGICAL AND STRUCTURAL CONTROLS OF CAVE DEVELOPMENT ON KORNATI ISLANDS

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Kornati Islands archipelago encompasses 91 carbonate islands and islet situated in the central part of eastern Adriatic coast in Croatia. They are predominantly composed of deformed Upper Cretaceous to Eocene pre-orogenic carbonates and stretch parallel with the coast in NW-SE, i.e. Dinaric direction.In order to contribute to the knowledge of the speleogenesis of the Kornati Islands caves, 45 caves (42 pits and 3 caves) have been identified and analysed during a three year research project of cave inventarization. In this research we aim to identify if the “inception horizon” exist and to connect cave forming to lithological or chemical deviation from the predominant carbonate facies within the surrounding sequence or to structural and tectonic elements. Geological structures above and within the caves were measured. Petrographic analysis of geological samples taken in the cave and on the surface were done in order to determinate inception horizons and how they influence cave geometry. Based on collected data a GIS analysis on cave density, distribution, vertical position has been performed. Spatial analysis of the cave distribution show higher density of the in the west coast of Kornati islands between 1 and 60 m a.s.l. while some of the cave extend below sea-level up to 45 m of depth. Based on the hydrological characteristics nine caves have no water and 36 caves are partially (22 anchialine caves) or completely submerged (10 sea caves). Analyses of the cave passage orientation indicate that most of the analysed caves are dissolution caves with dominant NW-SE orientation. This reveals dominant influence of neotectonic stress related to the Adria microplate underthrusting the Southern Alps. The intensive folding of the Islands and several identified faults caused development of secondary porosity that significantly influenced speleogenisis of Kornati islands caves. Results show existence of a favourable structures, unrelated to today’s surface topography, where a more intensive dissolution processes have occurred. This sequence extends approximately 60 m a.s.l. and 50 m below today’s sea level. This karstification processes probably began along expansion fracture in zone of axial surface and subvertical bedding planes.

ÀMBIT: OTHER

1- ANCHIALINE RESTORATION IN HAWAIʻI – ONE POOL COMPLEX AT A TIME

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Coastal development and introductions of invasive plant and animal species have significantly reduced the quantity and quality of anchialine pools throughout Hawai i. Hawai i Wildlife Fund worked with government agencies to designate a 538-hectare property in Hawai i into Forest Reserve to enable conservation of its anchialine ecosystems, native plants, and cultural resources. Perimeters of two large pools were overgrown with invasive plants (christmasberry, sourbush, mesquite), and both had a deep sediment layer. One pool had impenetrable growth of seashore paspalum and the other had Mozambique tilapia.

Native pool shrimp were never seen in the pool with tilapia but were previously observed in the pool choked with paspalum, and smaller pools within the complex. Species inhabiting this region include Halocaridina rubra, Metabetaeus lohena, and Palaemon debilis; H. rubra exhibit unique haplotypes and are genetically distinct from other nearby pools.

Restoration of these anchialine ecosystems began in 2009 with the hand removal of the non-native vegetation around the pools, followed by selective stump treatment with herbicide. Sediment removal was accomplished with a trash pump. Certain water quality parameters and relative shrimp abundance were monitored before, during and after restoration work.

Currently, the woody invasive vegetation is gone and pool peripheries abound with native plants. The sediment sites are also supporting native vegetation. A low level of effort is being expended quarterly to prevent invasive grasses from reinvading. Pool shrimp are now abundant in the pool where paspalum was removed. Tilapia eradication with rotenone is planned for the second pool in late 2018.

2- RESTORATION OF HAWAIIAN ANCHIALINE POOL ECOSYSTEMS ON HAWAII ISLAND: USING CARBON DIOXIDE TO REMOVE INVASIVE INTRODUCED FISH IN ANCHIALINE POOLS ON HAWAII ISLAND

Dena Sedar (1), Barbara Seidel (2), and Anne Brasher (3)

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Hawaii is the only state in the United States in which anchialine pools are found. Hawaii Island has the highest concentration of anchialine pools in the state, with over 650 recorded pools.

Unfortunately, many of these pools are degraded due to the presence of non-native fish. Efforts are being undertaken to restore the ecosystems through the removal of non-native fish using an experimental carbon dioxide technique. CO2 is pumped through diffusers into a pool at low tide to drop the pH level of the water, which slows the movement of the fish, making them easier to catch. This method has been used in multiple pools on Hawaii Island with varying success rates.

This restoration work is being done within two Hawaii State Parks, Kekaha Kai State Park and Kiholo State Park Preserve. 4,500 guppies (*Poecilia reticulata)* were removed from the Kekaha Kai pool over the course of four CO2 treatments. Over 5,000 guppies, 100 molllies (*Poecilia* complex), and 500 tilapia (*Oreochromis* sp.) were removed from the Kiholo pool during six treatments. The CO2 fish removal method has been shown to be successful at reducing the numbers of non-native fish within a pool. It is not without its drawbacks, as the effectiveness of the method is influenced by physical characteristics of individual pools; however, it can currently be considered the most effective fish removal technique for small anchialine pools. Further research is being conducted to refine the CO2 application method, which shows great promise as a tool for anchialine pool restoration.

LIST OF PARTICIPANTS

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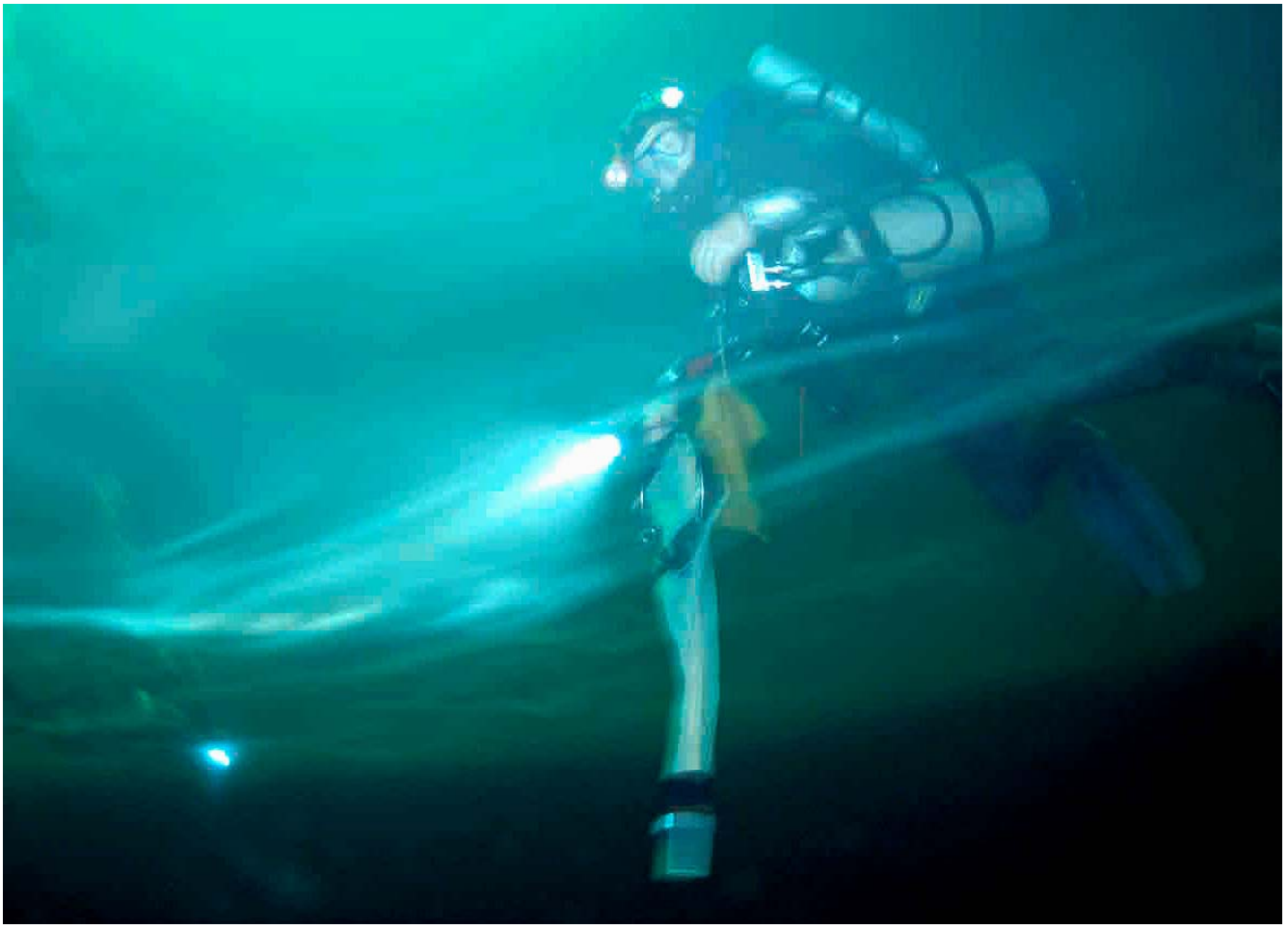
49. **Van Hengstrum, Pete**
50. **Wilkens, Horst**
51. **Zamora Sánchez, Jose Luis**

De dalt a baix: Túnel de la Atlántida, zona de la entrada. Foto: Brett C. Gonzalez . La Montaña de arena. Túnel de la Atlántida. Entremig dels grans de sorra hi ha un grup de fauna ben característic que colonitza aquest biotop. Foto: Jill Heinerth. El primer llac de la Cueva de los Lagos. Foto: Suso Fontes Esquerra: Túnel de la Atlántida. Foto: Juan Valenciano.



La varietat de colors indica les capes amb salinitat diferent. Les de color verdós (on veiem el bussejador) es deuen a aigua amb poca salinitat amb presència d'algues. Les de color més fosc, del fons, corresponen a aigua salada. El color blanquinós són capes amb contingut de sulfur d'hidrogen. Aquesta separació per densitats té lloc en llacs i galeries subterrànies d'aigües sense turbulències i amb una certa (poca) comunicació amb el mar. Foto: Tamara Thomsen.

Remipedia. Morlockia atlantida. Túnel de la Atlántida i Cueva de los Lagos. Mida 15 a 20 mm.

Decapoda. Munidopsis polymorpha amb una moneda de les llencades pels visitants. Los Jameos del Agua. Mida: 40 a 60 mm. Foto: Juan Valenciano.

Amphipoda. Spelaeonicippe buchi. Túnel de la Atlántida. 6 a 8 mm. Foto: Juan Valenciano.

Mollusca. Moluscs intersticials que habiten entre els grans de sorra de la Montaña de Arena, Túnel de la Atlántida. Foto: Emilio Rolán. Esquerra: Caecum clarkii, Centre: Pseudobis jameoensis. Dreta: Cima minima.

Annelida. Gesiella jameensis del Túnel de la Atlántida. Mida: 10 a 40 mm. Foto: Juan Valenciano.

Annelida: Speleobregma lanzaroteum del Túnel de la Atlántida. Mida: 20 a 30 mm. Foto: Alejandro Martínez.

Detall fotogràfic del cap de l'Annelida Gesiella jameensis. Foto: Brett C. Gonzalez.

Amphipoda. Liagoceradocus acutus. Mida: 7 a 10 mm. Túnel de la Atlántida i Cueva de los Lagos. Foto: Juan Valenciano.