

Biology and ecology of anchialine environments: a review

Biología y ecología de ambientes anquihalinos: una revisión

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INTRODUCTION

The term anchialine (from the Greek *anchihalos*, or “near the sea”) was initially proposed to describe open saltwater or brackish pools, both of volcanic and karstic origin, without surface connection to the sea, which fluctuates with the tides (Holthuis 1973). However, with the discovery of similar pools within caves, a more rigorous definition of the term was suggested, referring to haline water bodies with limited exposure to air and with underground networks that connect with seawater, presenting influence both of the marine and terrestrial environment, but with attenuated effect (Stock *et al.* 1986). Recently,

Bishop *et al.* (2015) unified the knowledge of this ecosystem, redefining this term more broadly as, a subterranean estuary or zone of encounter and interaction of the freshwater and seawater in submerged caves or galleries, where the only connection with the surface is through small holes which provide portals into these systems.

Anchialine environments are distributed in peninsulas and oceanic islands throughout the world. Although these ecosystems have a worldwide distribution, habitats that fit this definition are considered relatively rare. They are found mainly in the Caribbean, but also in the Bahamas, Bermuda, Hawaii, Mediterranean coast, Canary Islands, and Galapagos Islands (fig. 1) (Ilfie 2000). A variety of

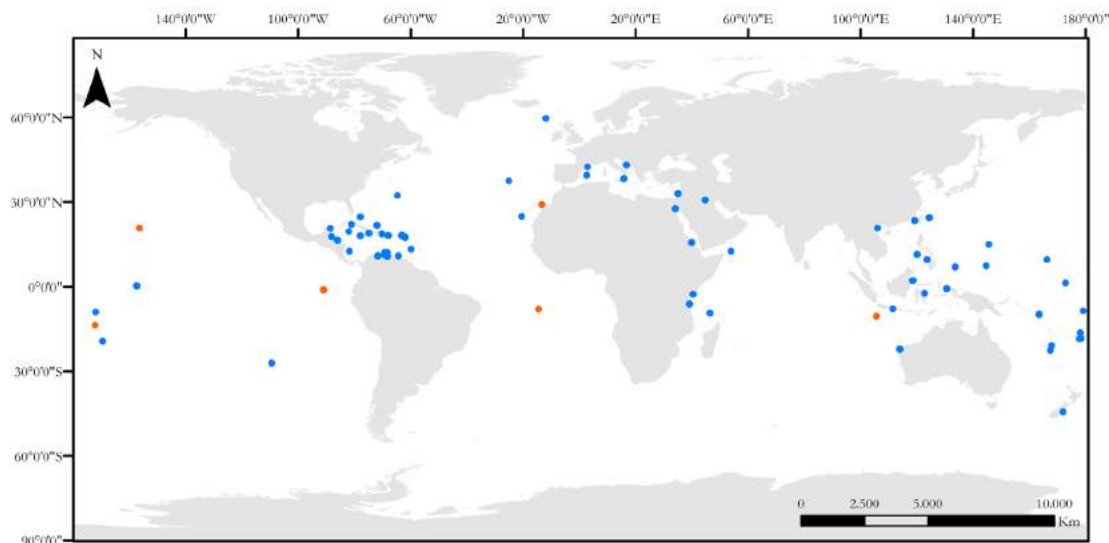


Figure 1. Distribution of anchialine ecosystems in the world. Blue dots indicate limestone anchialine caves and red dots volcanic anchialine caves.

terms have been used to define these systems. They are locally known as “blue holes” in the Bahamas and Belize, “Jameos de agua” in the Canary Islands, “tectonic cracks” in the Galapagos Islands and Iceland, “Cenotes” in the Yucatan Peninsula, or “sinkholes” in Australia; all of them with different names for the same technical description of a karst window (Ilfiffe & Kornicker 2009).

Anchialine habitats are protected against surface environmental changes. In contrast to most epigeic ecosystems that are short-lived (wetlands, rivers, or forests), these environments, like other subterranean ecosystems may persist relatively unchanged for millions of years (Gibert & Deharveng 2002). Therefore, anchialine parameters present a different dynamic to that observed in the epigeic environments (Cigna 2004), providing unique characteristics that define the establishment, adaptation, and survival of the fauna that inhabits it (Rodríguez-Noriega 2013). Waters are generally stratified as a result of a density gradient that involves a top layer of freshwater separated from the underlying seawater by a halocline or mixing zone, and that can vary from centimeters to meters (Beddows 2004). These gradients determine which species dwell in the different water layers. Cave-dwelling organisms of anchialine habitats are known as stygobionts, which by definition, are species restricted to subterranean waters that have adapted to narrow physicochemical parameters and similarly biological selection pressures (Holsinger 1991). For example, many amphipods of the genus *Pseudoniphargus* Chevreux, 1901, and the decapod *Creaseria morleyi* Creaser, 1936 (Caridea:

Palaemonidae) are only found in the meteoric waters above the halocline (van Hengstum *et al.* 2019), while the crustacean class Remipedia is limited to the hypoxic seawater layer underlying these systems (Yager 1994, Moritsch *et al.* 2014). Despite this segregation, anchialine fauna is present in both water masses, such as hadziid amphipods (Angyal *et al.* 2018) and some atyid shrimps (Moritsch *et al.* 2014).

In some cases, more than one halocline may be present, separating the water column into multiple layers within these caves, each of which has distinctive chemical and biological characteristics (Ilfiffe & Álvarez 2018). In these haloclines, dissolved oxygen levels are low or absent, and the water may be enriched with hydrogen sulfide (Sket 1996), making this interface a potential habitat for the development of chemosynthetic bacteria (Engel 2007). The structure and organization of the geochemical gradients that determine whether chemosynthesis can occur in anchialine caves depend on the oxygen balance of the water bodies, the availability of dissolved organic matter, and the thickness of the mixing zone. However, these factors are influenced by precipitation patterns, tidal regime, water residence time, aquifer hydrology, organic matter sources and transport mechanisms, and cave topography, but with attenuated effect (Pohlman 2011). The only stable and predictable factor in the groundwater environment is darkness. The absence of light prevents photosynthesis and, therefore, the existence of plants and algae (Gibert & Deharveng, 2002), generating profound implications in the adaptation of its inhabitants (Sket 1981).

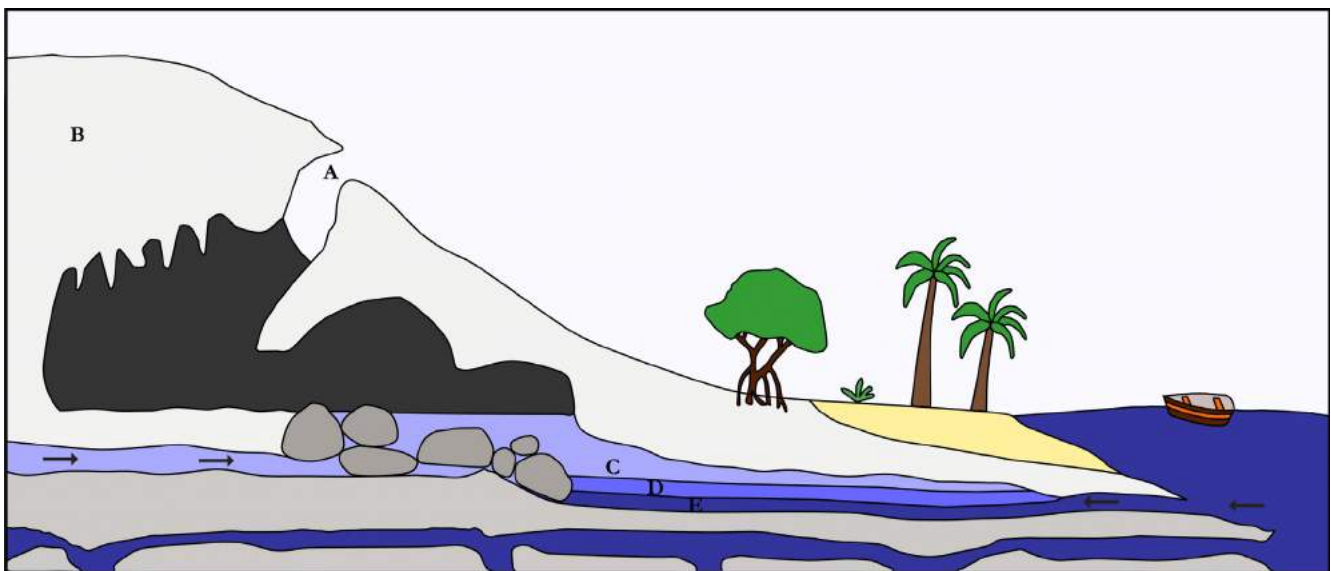


Figure 2. Diagram of anchialine environment in the coastal karst of Mallorquines, Morrocoy peninsula, Falcón state, Venezuela. (A) cave entrance; (B) Karst limestone matrix; (C) freshwater; (D) Halocline or mixing zone; (E) Seawater.

TROPHIC NETWORKS AS A DETERMINING FACTOR IN THE DIVERSITY OF SPECIES IN ANCHIALINE ENVIRONMENTS

As a result of the absence of autotrophic primary producers, the trophic structure of anchialine environments is relatively simple (Gilbert & Deharveng 2002). Pohlman (2011) suggested three trophic levels for these systems: 1) chemosynthetic producers, 2) primary or detritivorous consumers, and 3) opportunistic or generalist predators. Primary consumers are represented by crustaceans that have specialized strategies to filter small particles found in the benthos or water column (Mejía-Ortíz *et al.* 2013). For example, the mysid *Antromysis cenotensis* Creaser, 1936, and the thermosbaenacean *Tulumella unidens* Bowman & Iliffe, 1988 filter organic matter and bacteria suspended in the water column. Others as the atyid shrimp *Typhlatya*, filter particles in the water column and have sediment scraping strategies (Mejía-Ortíz *et al.* 2006).

Predators reduce their importance or may be absent in these environments with low nutrient density. Predatory behavior has been observed in the shrimp *Creaseria morleyi* (Creaser, 1936), which is considered the blind hunter of the Yucatan anchialine cenotes, Mexico (Chávez-Solís *et al.* 2017). The findings show that *C. morleyi* can hunt a variety of prey as *Typhlatya* sp. and *A. cenotensis*, including its own species. Also, *C. morleyi* has been observed feeding on bat excrement and even feeds on the cavefish *Typhlasina pearsei* (C. L. Hubbs, 1938) (E. Sosa, pers. com.), although it is not clear if the fish was captured alive. Similar behavior has been reported for remipedians. These organisms have a system of poisonous glands with paralyzing neurotoxins and specialized mouthpieces for the capture of prey (von Reumont *et al.* 2013). However, laboratory observations suggest that remipedians are not strict carnivores, because most of the time they filter small particles present in the water column, while the ingestion of large prey has occurred occasionally and selectively (Koenemann *et al.* 2007). These findings show that these organisms, with a tendency to opportunistic or generalist strategies, may have evolved to deal in extreme environments with limited resources such as the anchialine (Gibert & Deharveng 2002).

The low availability of food in these environments has been recognized as a limiting factor concerning the diversity and density of organisms, but this premise is not entirely true. Studies in the Bundera sinkhole, in northeastern Australia, suggested that higher trophic level invertebrates use chemoautotrophic products generated by organic matter degradation (Humphreys 1999). Similarly, recent evidence has suggested that trophic networks of anchialine

environments in the Yucatan Peninsula partially depend on chemosynthetic production as an energy source. The Cenote Crustacea is distinguished by its relatively high density and diversity of crustaceans. In this cave system, the dominant role of Proteobacteria with chemosynthetic metabolism has been demonstrated (Pakes 2013). These findings support the possibility that the macrofauna of these ecosystems depends on a source of chemosynthetic carbon (Pohlman *et al.* 1997).

Research studies carried out in other anchialine caves in Yucatan have shown symbiotic relationships between macroinvertebrates and chemosynthetic bacteria. The remipedian *Xibalbanus tulumensis* Yager, 1987 harbors ectosymbiotic chemosynthetic bacteria that allow it to absorb inorganic carbon as part of its diet (Pakes & Mejía-Ortíz 2014). Similarly, other studies have described the first case of chemosynthetic intracellular endosymbiosis in arthropods. This unusual form of symbiosis was found in *Typhlatya pearsei*, which also has adaptations to tolerate sulfur toxicity in the anchialine environment (Pakes *et al.* 2014). Although there is evidence of chemosynthesis in some anchialine caves, the knowledge of the carbon cycle in these aquifers is limited, and therefore more studies are needed to reach a better understanding of the trophic dynamics in these ecosystems.

BIODIVERSITY IN ANCHIALINE CAVES

Anchialine habitats harbor a considerable and underappreciated biodiversity with high levels of endemism (Sket 1996; Iliffe 2004). Stygobitic fish (fig. 3A) of the orders Ophidiiformes, Synbranchiformes and Perciformes can be found in these systems (Humphreys 2001, Wilkens 2001, Larson *et al.* 2013). However, these environments are dominated by invertebrates of various taxa such as sponges, annelids, molluscs, echinoderms, and crustaceans; while nematodes, cnidarians, echinoderms, and insects, have been collected occasionally and accidentally (Iliffe & Álvarez 2018).

Globally, crustaceans represent the most diversified group of anchialine fauna, with at least 500 species described, belonging to 4 classes, 16 orders, and 57 families (Iliffe & Bishop 2007, Gerovasileiou *et al.* 2016). Several taxonomic groups of crustaceans (see fig. 3), including the class Remipedia (fig. 3B) (Yager, 1981), the peracararides of the orders Mictacea (Bowman & Iliffe 1985 *in* Bowman *et al.* 1985) and Bochsacea (Gutu & Iliffe 1998), and the copepods of the order Platycopioidea (Fosshagen & Iliffe 1985) have been designated from specimens collected in this medium and are found exclusively in groundwater. The reason for the high diversity of crustaceans, endemism

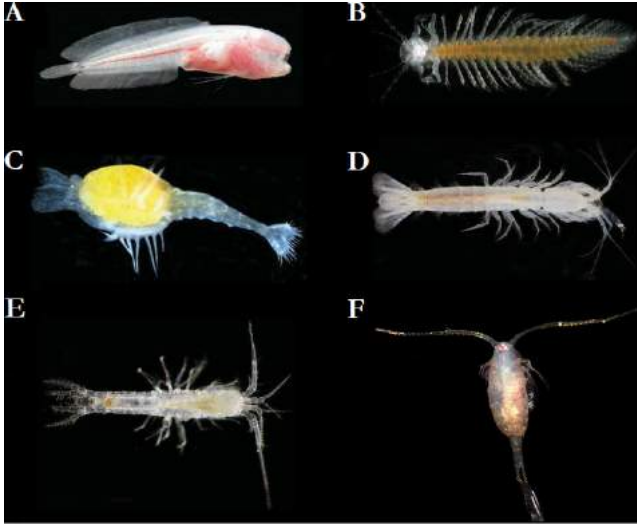


Figure 3. Representative anchialine taxa. (A) *Typhliasina pearsei* (C. L. Hubbs, 1938) (Ophiidiphormes) (B) *Cryptocorynetes longulus* Wollermann, Koenemann & Iliffe, 2007 (Remipedia) (C) *Tulumella unidens* Bowman & Iliffe, 1988 (Thermosbaena) (D) *Stygiomysis* sp. (Stygiomysidae) (E) *Mictocaris balope* Bowman & Iliffe, 1985 (Mictacea) (F) *Ridgewayia* sp. (Calanoida) (taken and modified from Pérez-Moreno *et al.* 2016; Iliffe & Álvarez 2018).

at high taxonomic levels and their prevalence over other higher taxa is not well known (Stoch 1995, Sket, 1999), but some authors suggest that this may be related to their basically omnivorous eating habits, which are suitable for these environments, and by the wide adaptability of their serial mouthparts (Por 2008).

Groundwater has long been considered as an unfavorable environment with low biodiversity because of its extreme environmental conditions. However, this paradigm is far from being true (Danielopol 1992). The Caribbean region and the Canary Islands have a high biological diversity (Iliffe & Álvarez 2018). Lanzarote is one of the seven most important volcanic islands of the Canary Islands and harbors the most diverse anchialine ecosystems of the Eastern Atlantic. A total of 77 species belonging to 10 taxonomic groups have been reported for the Corona lava tube, near the northern tip of Lanzarote. Of these groups recorded, Crustacea shows the highest diversity, with 31 species, and the highest degree of endemism (90%) (García *et al.* 2009). The Corona lava tube, like many other anchialine systems, is considered a detritus-dependent system (Venarsky & Huntsman 2018), where particulate organic matter is introduced by tidal exchange or infiltration through rocks. The presence of organic matter suspended in the water column serves as a food source for diverse endemic species dominated by crustaceans, such as thermosbaenaceans, mysids, and amphipods. In contrast, Ca-

ribbean anchialine systems are known to exhibit chemosynthetic processes from low concentrations of dissolved inorganic compounds (Pohlman *et al.* 1997, Brankovits *et al.* 2017), but it is not known whether these processes can occur in the remote sections of La Corona lava tubes (Martínez & González, 2018).

In Mexico, anchialine caves are only known in the Yucatan Peninsula, with over 2,241 cenotes recorded (Torres-Talamante *et al.* 2012, Gulden 2016). Half of these cave systems have records for only one to three species, five caves with more than ten records, while only three anchialine systems possess representatives of more than two phyla. Despite an apparently low diversity in this region, Calderón-Gutiérrez *et al.* (2017) performed an exhaustive diversity survey at El Aerolito cenote, which extends for 18 km, and reaches a maximum depth of 27 m. They reported that El Aerolito cave system holds 100 species (53 as new records, and 47 previously reported), grouped within 10 phyla (Porifera, Cnidaria, Platyhelminthes, Nemertea, Sipuncula, Annelida, Arthropoda, Mollusca, Echinodermata, and Chordata). These authors pointed out that El Aerolito is the richest and foremost biodiverse anchialine system of the world, and it could be a result of the energy input to the system by mangroves in the main entrance, and the direct connection with the sea, together with the chemosynthesis processes that occur there.

The increment of the species richness of anchialine fauna in some localities is evidence of the efforts to know these ecosystems. However, in many areas, this diversity is highly underestimated because of the lack of sampling of these environments. Venezuela is one of these cases, where anchialine environments are poorly known. So far, only two endemic anchialine species, *Cyathura univam* (Isopoda: Anthuridea) (Botosaneanu 1983) and *Metaniphargus venezuelanus* (Amphipoda: Hadziidae) (Stock & Botosaneanu 1983), have been reported, and they were described from specimens collected in a cave not yet officially recorded by professional speleologists in Falcon state, specifically in the Mallorquines karst (Botosaneanu's Cave, Morrocoy peninsula). These two species are the only representatives of their respective groups in South America and they were the result of the 1982 Amsterdam Expedition to the Venezuelan Islands and other localities on the mainland. Since then, this cave had remained unexplored until 2015, when it was located again, after several failed attempts. This system remains in a state of pristine conservation, and has shown signs of being inhabited by a relatively high density of crustaceans (fig. 3). Therefore, it is predictable that this cave could harbor a diversity greater than previously known. Further efforts by the present author and collaborators have been made to explore this anchialine system.



Figure 4. Anchialine system in the Mallorquines karst, Falcon state, Venezuela. (A) Karst platform besides Mallorquines Beach, (B) Botosaneanu's Cave entrance, (C) Visual collection of crustaceans in a section of the cave, (D) Anchialine pool (Photos by R. Carreño).

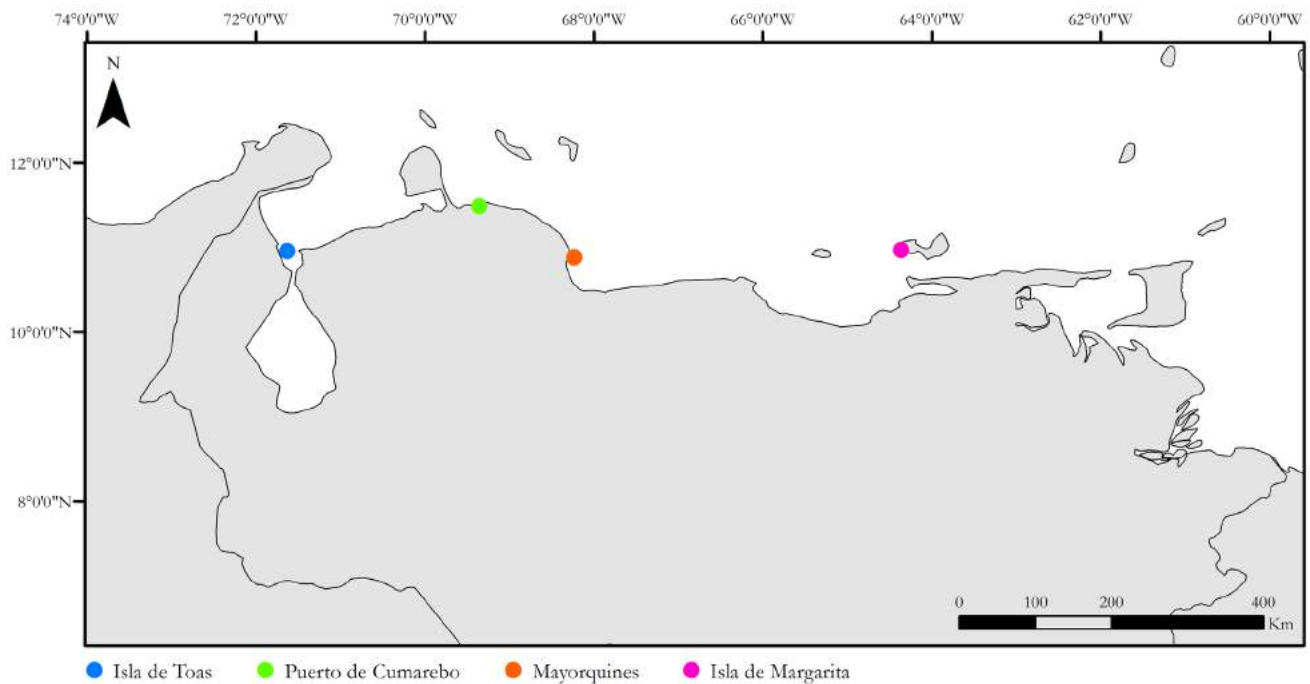


Figure 5. Distribution map of known anchialine caves or ecosystems in Venezuela.

Likewise, a few other anchialine environments have been detected in different locations in Venezuela, such as Isla de Toas, in Zulia state, Cumarebo, in Falcon state, and in Laguna Boca Chica, in Margarita Island (A. L. Vilorio, pers. com.). These areas have not been appropriately explored, but it is believed that they can provide valuable information concerning the diversity of stygobiont species, and given their location in the geographical context, it is expected that these systems are comparable to other anchialine caves studied in the Caribbean.

ADAPTATIONS OF ANCHIALINE FAUNA

As other organisms inhabiting subterranean environments, anchialine fauna exhibits morphological, physiological, and behavioral adaptations that are convergent and arose independently in different taxa (Bishop & Iliffe 2012). Darkness is their most obvious sensory limitation. Representatives of the stygofauna may be recognized by their regressive characters related to the reduction or loss of skin pigmentation and eyes in response to aphotic conditions (Sket 1996, Bishop *et al.* 2004). Some crustaceans have eye structures as embryos or young (Howarth & Moldovan 2018), and all blind cavefish exhibit initial eye development, but they subsequently degenerate and sink into the orbits (Jeffery 2009, Jeffery & Strickler 2010). In surface-dwelling animals, pigmentation is used to protect from sunlight, camouflage, mimicry, and species and sex recognition (Protas *et al.* 2011), but all of which are irrelevant in the dark cave environment. Many cavefish lineages are not pigmented. This is a result of the reduction or complete loss of melanin pigmentation (Niemiller & Soares 2015). Crustaceans may be transparent (*Troglocaris*), white or opaque, with their integument impregnated with calcium salts (e.g., *Cambarus*, *Niphargus*, *Proasellus*) (Ginet & Decou 1977).

Stygobionts often show constructive characters as an increase in tactile and chemical sensitivity, which is associated with the reduction or complete lack of sight (Hobbs 2005). In crustaceans, the appendages are usually elongated and numerous, with receptors highly developed to detect food in a highly heterogeneous environment (Gibert *et al.* 1994, Mejía-Ortíz *et al.* 2013). Cave fishes present elongated fins and barbels to avoid obstacles, and they use this tactile information to detect their surroundings (Windsor *et al.* 2008). The lateral line system in fishes like amblyopsids presents more neuromasts than their surface counterparts do (Montgomery *et al.* 2001, Yoshizawa *et al.* 2010). Neuromasts allow detecting vibrations of moving prey at a greater distance compared to similar epigeal species (Poulson 1963, Niemiller & Poulson 2010). More-

over, chemical sensory adaptations may have evolved so that, anchialine fauna avoids the high sulfur content of microhabitats in this environment, and detect changes in salinity and oxygen levels (Dattagupta *et al.* 2009).

Aquatic cave organisms have reduced metabolic rates compared to their surface relatives (Poulson 1963, Mejía-Ortíz & López-Mejía 2005), which is thought to be an adaptation to limited food and lower levels of oxygen (Hervant & Renault 2002, Bishop & Iliffe 2012). In that sense, stygobiont crustaceans possess elevated levels of enzymes associated with the anaerobic metabolism (Bishop & Iliffe 2012), allowing some species to survive in anoxic conditions for hours, or even days (Hervant *et al.* 1999, Issartel *et al.* 2009). However, exceptions are usual, and ground-water caves with higher nutrient inputs or well-oxygenated waters harbor crustaceans with metabolic rates similar to their epigeal relatives.

Bishop & Iliffe (2009) compared metabolic rates of organisms from an oxic anchialine cave system in Lanzarote (Canary Islands), with the metabolic rates of organisms from anchialine systems with anoxic levels in the Bahamas, and they found that in Lanzarote invertebrates, oxygen consumption was significantly greater. In addition, amphipods of the genus *Spelaeonicippe* (family Pardaliscidae) present at both locations, were compared based upon mass, with representatives from Lanzarote being significantly larger than those present in the Bahamas. In this sense, they suggested that the selective pressure of living in a reduced oxygen environment as Bahamas anchialine caves favors organisms with low metabolic rates, leading their populations to a smaller size. In contrast, selective pressure of low oxygen levels has been removed in Lanzarote, allowing for greater body mass and increased body size.

In the anchialine environment, food may be scarce or distributed in patches; and the stygofauna need to deal with food availability and tolerate long periods without feeding (Hervant *et al.* 1999). For example, the epigeal fish *Chologaster cornuta* dies after 45 days deprived of food, whereas cave-dwelling *Troglichthys rosae* can survive up to 20 months without eating (Niemiller & Poulson 2010). Starvation tolerance can be achieved by the following mechanisms: 1) low levels of activity and metabolism; 2) ingesting large amounts of food during sporadic and infrequent events; and 3) increased storage of fat (Hüppop 2000).

Stygobionts are characterized by replacing protein mass for lipid stores, but this does not occur in their epigeal congeners (Iliffe & Bishop 2007). Microscopic studies have revealed hundreds of small drops of transparent lipids along the trunks of two Remipedia species,

Godzilliognomus frondosus (Yager, 1989) and *Speleonectes benjamini* (Koenemann *et al.*, 2007). Similarly, hypogean isopods and many species of heptapterine cavefishes store lipids in digestive glands and in adipocytes distributed throughout the body (Wägele 1992, Trajano 2001). A lipid source provides neutral buoyancy without energy expenditure and serves as an energy reserve when food is scarce (Hervant & Renault 2002). The combination of lower metabolic rates and lower energy density allows stygofauna to destine a larger fraction of their energy to growth, like most deep-sea pelagic fishes (Childress & Nygaard 1974).

The stages of the development cycle of hypogean crustacean are longer than in related surface animals, as was demonstrated for amphipods and harpacticoids (Ginet & Decou 1977, Rouch 1968). The hypogean amphipod *Niphargus* lives for over 35 years (Turquin & Barthelemy 1985), whereas copepods harpacticoids live for more than 2 years (Ginet & Decou 1977). In contrast, epigeal crustaceans complete their life cycles in a few months or a single year (Howarth & Moldovan 2018). Furthermore, stygobionts stand out by their low fertility rates. This trend is clearly identifiable in *Niphargus*, in which females lay larger eggs than their surface relatives (Fišer *et al.* 2012) but invest less in a single progeny, similarly to cavefish from different genera (Poulson 1963, Niemiller & Poulson 2010). Larger eggs contain larger larvae that may have better starvation tolerance and greater swimming ability to avoid predators (Niemiller & Poulson 2010).

BIOGEOGRAPHY OF ANCHIALINE FAUNA

Anchialine fauna exhibit a disjunctive global distribution pattern at the genus level (Holsinger 1986, Wägele 1990), but at the species level, they present relatively small distribution ranges and high levels of endemism. These distribution patterns are common to a wide variety of taxonomic groups of crustaceans, including remipedians, atyid shrimps, thermosbaenaceans, hadziid amphipods, thaumatocypridid ostracodes, cirrolanid isopods and calanoid copepods (Neiber *et al.* 2011). Traits of the biology of stygobiont crustaceans suggest that their dispersal abilities to cross open water are limited (Jaume & Boxshall 1996, Neiber *et al.* 2011). Therefore, most authors attribute these distribution patterns to vicariance events (Holsinger 1991), resulting from tectonic plate movements that subdivided ancestral populations widely distributed along the margins of the Tethys Sea during the Late Mesozoic, which yielded the evolution of species pairs on the Atlantic and Pacific sides (Stock 1993, Jaume & Christenson 2001, Holsinger 2005, Culver *et al.* 2009). Subsequently,

multiple marine ancestors invaded cave systems, and their isolation and low dispersal capacity facilitated speciation that ultimately gave rise to the cave species known today (Trontelj *et al.* 2009), although their non-cavenicolous marine relatives are either extinct or inhabiting the deep sea (Juan *et al.* 2010).

Many anchialine taxa never found in the open ocean are exclusively limited to anchialine caves on the margins of the ancient Tethys (Jaume 2008, Neiber *et al.* 2011), but they are absent in the Mediterranean basin (e.g., remipedians and thermosbaenaceans). Although there is no evidence, these organisms could have existed in the Mediterranean and got extinct in the course of severe geological changes associated with the Messinian salinity crisis (6 Ma). In this event, the Strait of Gibraltar got closed and, the Mediterranean became progressively isolated from the Atlantic, causing a massive accumulation of salt on its bottom and a dramatic lowering of sea level due to evaporation (Krijgsman *et al.* 1999; García-Castellanos & Villaseñor 2011). Notwithstanding the crisis extirpated most of the Mediterranean fauna, some anchialine taxa remain represented there.

The genera of speleophriid copepods (Speleophriidae) have only been found in anchialine environments, and their species occur on both sides of the Atlantic, the Mediterranean (the Balearic Islands and Sardinia), the Pacific and the Caribbean. Boxshall & Jaume (2000) inferred that it is feasible that these speleophriids and their ancestors could be able to migrate to freshwaters in the face of the total desiccation of the surrounding sea, considering that anchialine habitats are also an extension of the subterranean freshwater environment. An alternative interpretation was presented by Sonnenfeld (1985), who postulated that the deposition of salts on the floor of the Mediterranean basin was possible without resorting to a model of total desiccation. In this case, it is possible that anchialine environments with near marine salinities were available from Tethyan to recent times, served as refuges during the Quaternary glaciations (Jaume & Boxshall 1996).

Although the vicariance hypothesis has not been validated by molecular phylogenetics, mitochondrial and nuclear sequences have been used to study the endemic decapod *Stygiocaris* of eastern Australia, and its relationship with other Australian species. The results show that the closest relative to *Stygiocaris* belongs to the genus *Typhlatya*, found in Yucatan, Mexico. Surprisingly, molecular data suggest that these two sister groups derived from an ancient vicariant event, supporting the hypothesis that they may have descended from a common ancestor that lived in the coastal marine habitat of the ancient Tethys Sea. If true, this confirms that the long-term stability

of these environments have allowed and still allows them to act as refuges to preserve many relict taxa not known anywhere else on Earth (Page *et al.* 2008). However, the existence of anchialine fauna as decapods of the genus *Procaris*, which are only found on islands in the middle of the ocean (Christmas Island, Ascension Island, Bermuda and Hawaii), which have never been part of or close to a continent, cannot be explained by the vicariance model. In these cases, dispersal model invoking ocean currents or even dispersion from the deep sea may have influenced this distribution (Stock 1993, Bruce & Davie 2006).

Some authors have suggested that the deep sea plays an essential role in some anchialine taxa that strictly inhabit the salt layers of these cave systems and have close relatives in the deep ocean (Hart *et al.* 1985, Boxshall & Jaume 2000). Galapagos Islands have never had shallow water connections, but some anchialine species are found in more than one island. *Tiphlatya galapagensis* Monod & Cals, 1970, *Galapsiellus leleuporum* (Monod, 1970) and *Kareloecia woutersi* Maddocks, 1991, inhabit both Santa Cruz and Isabela, while *Enantiosis galapagensis* Fosshagen, Boxshall & Iliffe, 2001, is present in Santa Cruz and Floreana (Iliffe 1991). The presence of the same species in islands comparatively distant, provided indirect evidence for connectivity among the island's anchialine habitats. This places the basis for theories of dispersal between caves through continuous crevicular systems, extending from shallow to deep habitats (Rondé-Broekhuizen & Stock 1987, Iliffe 1990).

On the other hand, many stygobionts found in anchialine volcanic caves, as those in Lanzarote, have shown an affinity with groups of animals only found in deep-seas (García *et al.* 2009, Gonzalez *et al.* 2017). Examples include the squat lobster *Munidopsis polymorpha* Koelbel, 1892, the thaumatocyprid ostracods, the annelids *Speleobregma lanzaroteum* Bertelsen, 1986, and *Gesiella jameensis* (Hartmann-Schröder, 1974), and several species of misophrioid copepods (Boxshall & Jaume 1999, Núñez *et al.* 1997). Although a deep-sea affinity not necessarily suggests a deep-sea origin (Martínez *et al.* 2013), phylogenetic analyses have shown that the ancestors for the cave lineages of the scale worm *Gesiella jameensis* and the squat *Munidopsis polymorpha*, in fact, originated in the deep sea (Ahyong *et al.* 2011, González *et al.* 2018).

Finally, the biogeography of anchialine fauna is a discipline full of problems and controversies. The debate on the relative role of different biogeographic models is still ongoing and far from being resolved. However, contemporary patterns of anchialine fauna involve both vicariance and dispersal processes (Lefébure *et al.* 2006), and the rela-

tive importance of each will probably vary from region to region (Culver *et al.* 2009).

IMPLICATIONS FOR CONSERVATION AND CONCLUSIONS

The study of anchialine environments is far from having reached a plateau and represents a frontier of exploration, in which many species and biological processes have yet to be discovered. In the last three decades, over 400 species of anchialine fauna have been described, and most of them are only known from a single cave system or even from very specific sections of these caves (Iliffe & Kornicker 2009). It is well known that adjacent ecosystems interact with anchialine habitats, determining the quantity and quality of organic matter entering those systems. This renders them particularly vulnerable to any anthropogenic changes (Pohlman *et al.* 1997, Schmitter-Soto *et al.* 2002, Jaume & Boxhall 2009).

The future for anchialine fauna is uncertain. Due to the connectivity of the subterranean realm, many anchialine ecosystems have experienced negative effects associated with activities related to land use and subsurface contaminants, even to several kilometers away from anchialine caves (Sket 1996, Iliffe 2002, Santos 2006). Despite the scarcity of information on the subject, twelve anchialine species endemic to the Yucatan Peninsula have been included in some category of the IUCN red list (most of them are in the critically threatened ones) (Calderón-Gutiérrez *et al.* 2017), while at least twenty five species from Bermuda are on the IUCN Red List of Endangered Species (Iliffe & Kornicker 2009).

Most insular aquatic caves are being greatly affected by the extraction of fresh water to supply the industry of tourism (Jaume & Boxhall 2009). This ecosystem is among the most threatened in Hawaii (Santos 2006). It is estimated that more than 90 per cent of Hawaii's anchialine habitats have been lost or degraded by anthropogenic activities, like coastal expansion and introduction of exotic species (Maciolek & Brock 1974, Brock & Bailey-Brock 1998). The close link that keeps the balance within the caves with what happens in the epigeal environment demands special protection for these fragile, vulnerable and poorly documented ecosystems. Therefore, considering the potential for regional endemism along with the ongoing alteration of anchialine habitats worldwide, it is necessary to increase research into these unique environments and their fauna to develop a broader understanding of the biodiversity, since such knowledge plays critical roles to develop conservation strategies.

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