



Smithsonian Institution  
Scholarly Press

SMITHSONIAN CONTRIBUTIONS TO THE MARINE SCIENCES • NUMBER 38



# Proceedings of the Smithsonian Marine Science Symposium

*Edited by  
Michael A. Lang,  
Ian G. Macintyre, and Klaus Rützler*

# Worldwide Diving Discoveries of Living Fossil Animals from the Depths of Anchialine and Marine Caves

*Thomas M. Iliffe and Louis S. Kornicker*

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**ABSTRACT.** Inland (anchialine) and offshore submarine caves in limestone and volcanic bedrock are extreme environments inhabited by endemic, cave-adapted (typically eye- and pigment-reduced) fauna. Specialized cave diving technology is essential for investigating this habitat. A number of new higher taxa are represented herein, including closely related species inhabiting caves on opposite sides of the Earth, thus suggesting an ancient common ancestry. Because many of these species are known from only a single cave, pollution or destruction of caves will result in their extinction.

## INTRODUCTION

### DEFINITION OF ANCHIALINE AND MARINE CAVES

Anchialine caves are partially or totally submerged caves situated within a few kilometers inland from the coast in volcanic or karstic limestone terrain. Tidal marine waters in these caves have a long residence time, of months to years. Such caves are locally termed “cenotes” in the Yucatan Peninsula of Mexico, “blue holes” in the Bahamas and Belize, and “grietas” in the Galapagos Islands. The caves typically possess a highly stratified water column, with surface layers of freshwater or brackish water, separated by a thermo-chemocline from underlying fully marine waters low in dissolved oxygen (Iliffe, 2000). Animals that are restricted to the anchialine habitat and show pronounced morphological, physiological, biochemical, and behavioral adaptations are termed stygofauna or stygobites. In some areas such as Yucatan, freshwater and marine stygobites inhabit their respective water masses within the same caves.

In contrast to anchialine caves, marine caves are located either directly on the coastline (e.g., tidal springs) or are wholly submerged beneath the seafloor (e.g., offshore blue holes) and contain marine waters that freely exchange with the sea on each tidal cycle. The stygophilic fauna of marine caves can also be found in suitable and similar habitats outside of caves (e.g., under rocks or in crevices within the reef) and lack specialized adaptations for subterranean life.

Moderate to strong tidal currents are present in many marine caves. As a result, encrusting and low-growing, filter-feeding animals such as sponges, hydroids, anemones, tube worms, and even some corals may completely cover all

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*Thomas M. Iliffe, Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77553-1675, USA. Louis S. Kornicker, Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163, USA. Corresponding author: T. Iliffe (iliffet@tamug.edu). Manuscript received 9 June 2008; accepted 20 April 2009.*

hard surfaces. Other organisms are swept into caves by tidal currents but can only survive there for short periods of time and are termed accidentals. Some species of fishes, lobster, and mysidaceans seek shelter within marine caves but must venture out into open waters to feed and are classified as stygoxenes.

Some extensive marine caves extend far or deep enough so that a more or less gradual transition to long water residence times takes place and conversion to a true anchialine habitat occurs. Similarly, a number of inland anchialine systems have submerged entrances in the sea, with significant water exchange occurring in the entrance sections but with a transition to anchialine characteristics and fauna taking place as distance from the sea increases and the magnitude and impact of tidally exchanging water decline.

#### *Biological Significance*

Anchialine caves contain a rich and diverse, endemic stygobitic fauna (Sket, 1996; Iliffe, 2000, 2004) but, because of the specialized technological demands and potential dangers of cave diving, are relatively unstudied. These habitats serve as refuges to “living fossil” organisms, for instance, members of remiped crustaceans, and to animals closely related to deep-sea species, such as the galatheid crab *Munidopsis polymorpha*. Such stygobites typically possess regressed features including loss of eyes and body pigmentation. For reasons that remain unclear, the invertebrate fauna is dominated by crustaceans and includes the new class Remipedia, plus three new orders, nine new families, more than 75 new genera, and 300 new species. This extraordinary degree of novelty qualifies anchialine habitats as uniquely important. Because anchialine species commonly have a highly restricted distribution, often being found only in a single cave system on one island, pollution or destruction of the caves will result in their extinction.

Stygobitic anchialine fauna often have highly disjunct biogeographic distributions, inhabiting caves in isolated locations on opposite sides of the Atlantic and Pacific Oceans, as well as in the Mediterranean, and are considered Tethyan relicts. Various hypotheses have been proposed to explain the origin of anchialine fauna. In general, these theories invoke either vicariance (geological) or dispersal (biological) processes. Recently initiated molecular genetic comparisons of cave populations from distant locations may help provide data for determining the age and dispersal sequence of anchialine stygobites (Zakšek et al., 2007; Hunter et al., 2008).

#### *Lifestyle Adaptations*

The extreme environmental conditions in anchialine caves, such as the absence of light, hypoxia, and limited food reserves, present a unique set of challenges for the organisms that reside there. The lack of light precludes photosynthetic (primary) production of oxygen and food. Without light, organisms receive no visual information for orientation or communication and must function without diurnal timing mechanisms.

Adaptations to anchialine and marine caves can be morphological, behavioral, and physiological (Iliffe and Bishop, 2007). As a result of both food scarcity and hypoxia, there is a high selective advantage for economy of energy observed in many taxa, with possible adaptations including enhanced chemo-mechano-receptors for improved food finding capability, starvation resistance, and reduction in energy demand via reduced metabolism.

#### METHODS

##### *Diving Investigations*

Because anchialine stygobites are commonly found only at significant depths or distances from the water surface, cave diving is an essential component of the collection and study of anchialine fauna (Iliffe and Bowen, 2001). Cave diving requires specialized training, equipment, and techniques because a direct ascent to the surface is not possible and divers may be hundreds of meters from outside access. In case of equipment failure or loss of air supply, cave divers must have readily available backups. Special techniques for cave diving may include the use of side-mounted, instead of back-mounted, scuba tanks to allow divers to pass through low bedding plane passages. Closed circuit rebreathers, which recycle the diver's exhaled gases, reduce the amount of percolation, that is, of silt dislodged from cave ceiling or walls by the exhaust bubbles produced in conventional open circuit scuba, and lessen contamination of the cave waters, which are low in dissolved oxygen (Figure 1). Rebreathers allow for much longer dives and generally less decompression time. Deep dives, depths below 40 m, require the use of special breathing gas mixtures that replace part or all of the nitrogen with helium to reduce the effect of nitrogen narcosis. As many cave dives are for longer durations and/or to deeper depths, they frequently involve long decompression.

##### *Sampling and Fixation*

The exceptionally clear waters of anchialine caves facilitate visual observation and collection of stygobitic



**FIGURE 1.** A diver uses a Megalodon closed-circuit rebreather with full face mask to collect a small shrimp, *Typhlatya* sp., from a cave in Yucatan. Rebreathers recycle expired gas so that no bubbles are produced.

species. Collectors generally lead the dive to have undisturbed water in front of them. As they slowly sweep their dive lights back and forth in an arc, observing the water column illuminated by the light beam, animals as small as a few millimeters can be distinguished as white pinpoints, sharply contrasting with the black background of the cave. Specimens recognized in this manner can be collected either individually in clear glass vials or plastic tubes or in larger numbers using a type of suction device known as the “Sket bottle” (Chevaldonné et al., 2008). Plankton nets, of 93  $\mu\text{m}$  mesh with a 30 cm mouth diameter and 1 m length, can be used to collect smaller animals, such as copepods, from the water column. When collecting animals from the surface layer of sediments, divers can gently fan up the sediments with a hand and then sweep the plankton net through the disturbed water. This agitation should be done with care so as not to obscure overall visibility, which could cause the dive team to lose sight of their guideline leading back to the surface. Larger amounts of sediment can be collected in sealable plastic bags for later sorting in the laboratory. Finally, minnow traps or similar funnel-shaped traps made from plastic bottles (Manning, 1986) can be baited with a small amount of fish, crab, or other attractant and left within the cave for 6 to 24 h. If the trap

is carefully placed inside a sealed plastic bag when it is recovered, even small invertebrates can be collected.

If temperatures are kept close to cave temperature after collecting, specimens will remain alive for up to 24 h. Photographic documentation of color pattern and natural body position in live specimens is highly desirable. Smaller animals can be photographed using a phototube attachment on a dissecting microscope and larger specimens with the macro setting found on many digital cameras. If animals are too active to be photographed easily, they can be chilled in a small dish placed in a refrigerator or an ice bath until they stop moving. Digital video segments showing swimming and other behaviors can be made in the same manner. Specimens are sorted under a dissecting microscope using small pipettes to transfer them to individual dishes for each taxon. Depending upon the type of animal and its intended use, various fixatives can be used. Most animals are best preserved in 70% to 95% pharmaceutical grade ethanol, which allows them to be used for either morphological or molecular investigations. Specimens for confocal laser scanning microscopy can be fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) buffer (1:1 in seawater), while those intended for scanning or transmission electron microscopy are fixed in 2% glutaraldehyde in seawater.

## **GEOLOGICAL ORIGINS, AGE, AND DISTRIBUTION OF ANCHIALINE HABITATS**

Anchialine caves occur in both volcanic bedrock and karstic limestone. Lava tube caves form during volcanic eruptions of basaltic lava. They typically occur close to the earth’s surface and are thus relatively short lived (thousands to a few tens of thousands of years). Anchialine lava tubes may originate on land and extend out under the coastline and beneath the seafloor or can form from submarine eruptions. Anchialine lava tube caves are known from the Canary Islands, Galapagos Islands, Hawaii, and Western Samoa. The longest of these is the Jameos del Agua (Atlantida Tunnel) on Lanzarote in the Canary Islands, the submerged portion of which extends 1.6 km beyond the coastline, reaching a depth of 50 m (Iliffe et al., 2000).

The most extensive of known anchialine habitats are solutionally developed limestone caves that typically contain both freshwater and marine waters. Such caves are sometimes referred to as flank margin caves and were formed by mixing dissolution in a fresh groundwater lens (Mylroie and Carew, 1990). The largest anchialine cave is Sistema Ox Bel Ha located on the Caribbean coast of the Yucatan Peninsula in Mexico; it contains 180 km of

surveyed underwater passages interconnecting 130 cenote entrances. Extensive anchialine limestone caves are also known from the Bahamas, Bermuda, Belize, Dominican Republic, and Bonaire in the Caribbean, plus the Balearic Islands and Sardinia in the Mediterranean. Smaller anchialine caves are present on many islands in the Indo-South Pacific and in Western Australia.

Limestone caves last much longer than lava tubes and can be hundreds of thousands to many millions of years old. Commonly, massive stalactites and stalagmites occur underwater to depths in excess of 50 m in coastal limestone caves. Because speleothems form very slowly and only in air, these caves must have been dry and filled with air for long periods of time when glacial sea levels were as much as 130 m lower than today. The last low stand of Ice Age sea level occurred only 18,000 years ago.

Coastal tectonic faults that extend below sea level constitute another form of anchialine habitat. On Santa Cruz in the Galapagos Islands, vertical faults in coastal volcanic rock are locally called "grietas" (Iliffe, 1991). Wedged breakdown blocks have partially roofed over submerged portions of grietas so that they are in total darkness. Similar faults are present in Iceland. Fault caves also occur in uplifted reef limestone on the island of Niue in the Central Pacific, producing deep chasms containing anchialine pools. The Ras Muhammad Crack in the Sinai Peninsula consists of a water-filled crack in an elevated fossil reef formed by a 1968 earthquake (Por and Tsurumal, 1973). Many of the offshore ocean blue holes of the Bahamas consist of submarine faults running parallel to the platform edge. Ocean blue holes typically exhibit exceptionally strong, reversing tidal currents created by an imbalance between tides on opposite sides of the islands.

## ANCHIALINE CAVE ECOLOGY

### PHYSICAL AND CHEMICAL CHARACTERISTICS

The water column in most anchialine caves is highly stratified (Iliffe, 2000). The largest changes in chemical and physical parameters typically occur at the halocline where freshwater or brackish water is separated from underlying fully marine waters (Figure 2). It is not uncommon for caves to possess multiple haloclines. On larger islands and in continental regions such as Yucatan and Western Australia, freshwater occurs in the shape of a lens with thickness increasing in a direct relationship with distance inland from the coast. In Yucatan, the depth of the halocline and corresponding thickness of the freshwater lens increases from 10 m at 2 km distance inland to 20 m at 10 km inland.

Water temperature in Yucatan caves generally increases with depth, although in the Bahamas the inverse occurs and water below the halocline is generally cooler than surface water. Warmer waters below the halocline could be caused by geothermal heating at depth or evaporative cooling at the surface.

In the lightless interior of caves, there are no plants and hence no photosynthetic oxygen production; stable and stratified water masses also restrict vertical mixing and exchange of oxygen with surface waters. Thus, cave waters are typically hypoxic to anoxic. Where deeper, water-filled vertical shafts extend to the surface, such as in many cenotes and inland blue holes, input of leaves and other organic detritus has caused the total depletion of dissolved oxygen with resulting anoxia and hydrogen sulfide production. A cloud-like layer of hydrogen sulfide several meters thick occurs just below the halocline and may reduce underwater visibility to near zero, but water clarity improves considerably below the H<sub>2</sub>S layer. In some caves, dissolved oxygen levels can recover to 1 mg/L or less and populations of stygobitic animals occur.

A pH minimum generally occurs at the halocline, possibly arising from microbial oxidation of organic matter suspended at the density interface and resulting CO<sub>2</sub> production. Increased acidity at the halocline may explain the dissolution of limestone and the resulting development of cave passages at this depth.

### TROPHIC RELATIONSHIPS

Determination of stable carbon and nitrogen isotopes values from animals, sediments, and other sources of organic matter in Yucatan caves has been used to examine the trophic ecology of these systems (Pohlman et al., 1997, 2000). Four potential sources of organic matter were identified in Yucatan caves: the soil from the surrounding jungle, algae from the cenote pool, chemoautotrophic bacteria, and, to a lesser extent, organic matter originating from marine waters. Stable nitrogen isotope data determined that the food web comprised 2 to 2.5 trophic levels.

The paucity of food in anchialine caves drives organisms toward a generalist diet. Mysids and isopods tend toward omnivory, while ostracods and thermosbaenaceans occupy the roles of detritivores. The thermosbaenacean *Tulumella* and atyid shrimp *Typhlatya* have modified appendages that allow them to filter out even the tiniest particles. Remipedes, fishes, and some amphipods, operating either as top-level predators or as scavengers, feed on ostracods, thermosbaenaceans, copepods, isopods, amphipods, and shrimps.

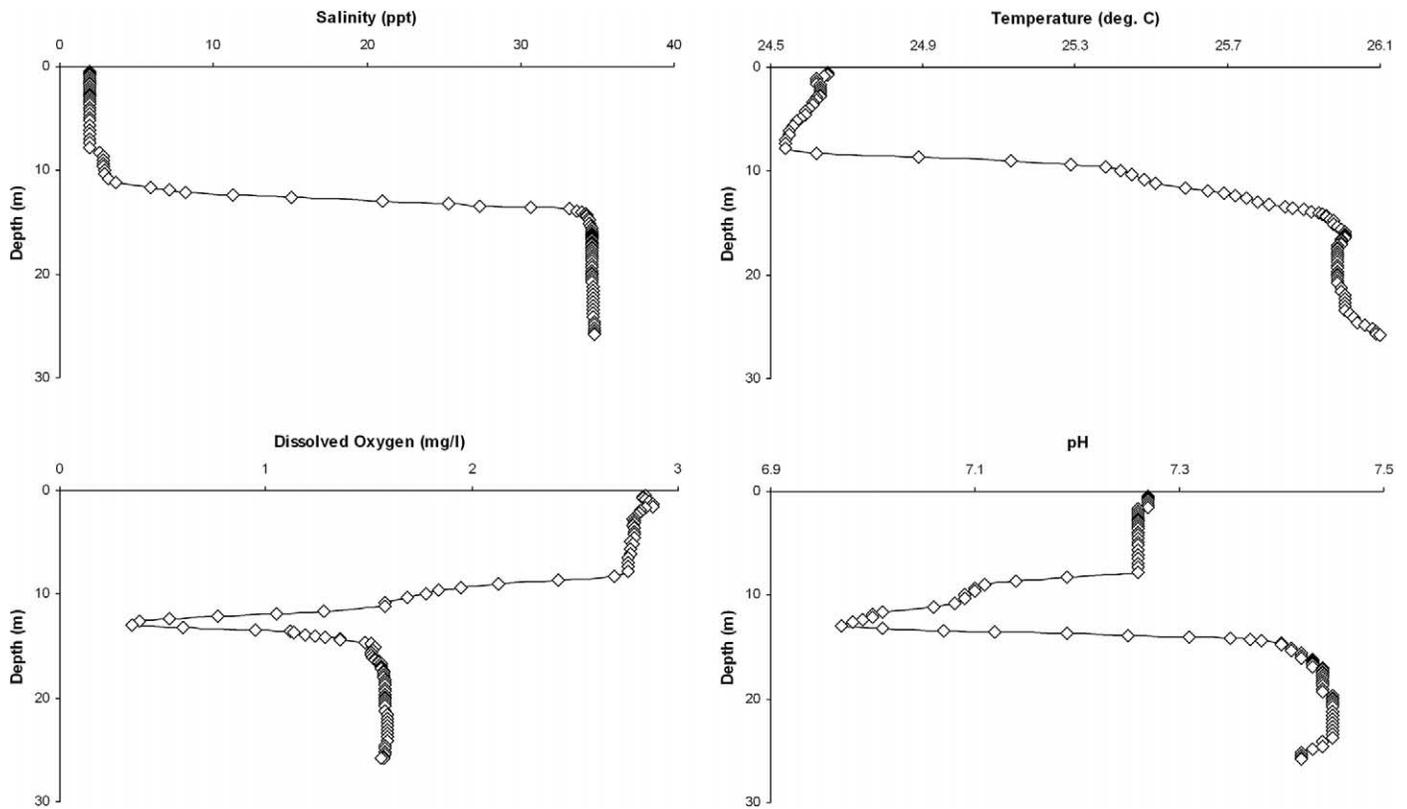


FIGURE 2. Depth profiles of salinity, temperature, dissolved oxygen, and pH from an anchialine cave, Cenote 27 Steps, Akumal, Mexico, 7 December 2003, recorded with a YSI 600 XLM multiparameter water quality monitor. Individual measurements (diamond symbols) were taken at 4 s intervals between the surface and 26 m water depth.

## BIODIVERSITY

### FISHES

Stygobitic anchialine fishes (Figure 3a) are represented in the families Bythidae (eight species in two genera from the Bahamas, Cuba, Yucatan, and Galapagos Islands), Eleotridae (one species from Northwestern Australia), Gobiidae (three species in two genera from the Philippines and Japan), and Synbranchidae (two species in one genus from Northwestern Australia and Yucatan) (Romero, 2001).

### NON-CRUSTACEAN INVERTEBRATES

Although most stygobitic anchialine invertebrates are crustaceans, a variety of non-crustacean invertebrate stygofaunal species have been described. Anchialine species include four sponges, one turbellarian, five gastropods, ten annelids, four chaetognaths, one tantulocarid, and three water mites. Although some of these species are questionable stygobites, several are clearly cave adapted. The poly-

chaetes *Gesiella jameensis* from the Canary Islands and *Pelagomacellicephala iliffei* from the Caicos Islands and Bahamas (Figure 3b) conserve energy by slowly swimming in the cave water column, while the chaetognath *Paraspadella anops* from the Bahamas lacks eyes and pigment.

### CRUSTACEANS

Crustaceans are the most abundant and diverse group present in both freshwater and anchialine cave habitats. Among the anchialine Crustacea, the largest numbers of species are represented by amphipods, copepods, decapods, ostracods, isopods, mysids, and thermosbaenaceans, approximately in that order.

### Remipedia

Remipedes are a class of Crustacea originally described from Bahamian caves by Yager (1981). Although their multi-segmented trunk and paired swimming appendages

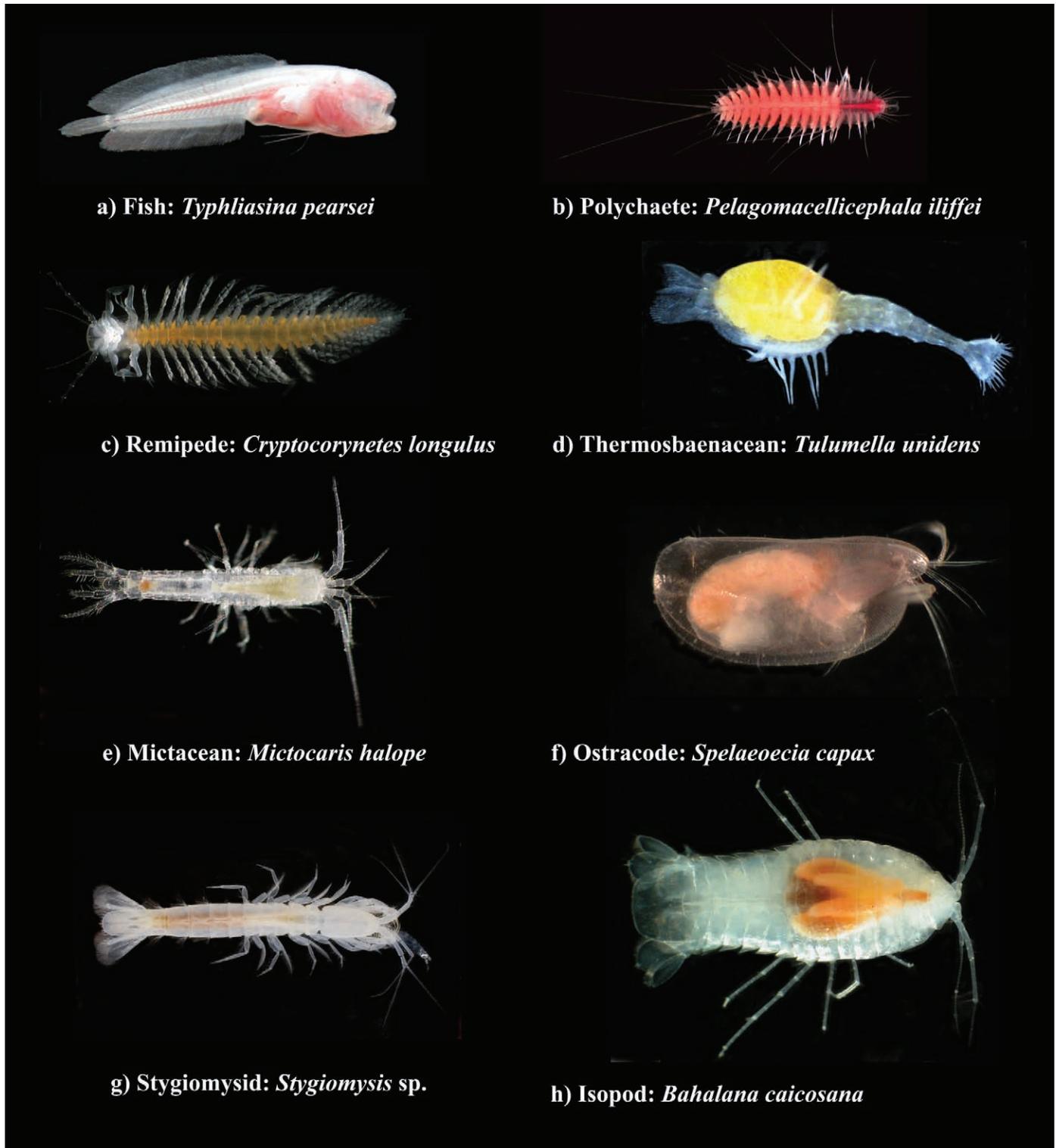


FIGURE 3. Characteristic anchialine cave animals include the (a) Yucatan cave fish *Typhliasina pearsei*; (b) polynoid polychaete worm *Pelagomacellicephala iliffei* from the Bahamas; (c) remipede *Cryptocorynetes longulus* from the Bahamas; (d) thermosbaenacean *Tulumella unidens* from Yucatan; (e) mictacean *Mictocaris halope* from Bermuda; (f) halocyprid ostracod *Spelaeoecia capax* from the Bahamas; (g) stygiomysid *Stygiomysis* sp. from Yucatan; and (h) cirrolanid isopod *Bahalana caicosana* from the Caicos Islands.

appear primitive, their head and mouth parts are highly specialized (Figure 3c). Remipedes have paired hollow fangs for capturing prey and are among the top predators in anchialine habitats. They are up to 4.5 cm in length, usually colorless and blind, with elongate, centipede-like bodies. Twenty species of remipedes inhabit fully marine, oxygen-deficient waters in caves from the Bahamas, Caicos Islands, Cuba, Yucatan Peninsula, Dominican Republic, Canary Islands, and Western Australia (Koenemann et al., 2008b; Daenekas et al., 2009). The recent discovery of free-living, nonfeeding remipede larvae promises to yield information on the reproduction and development as well as the evolutionary affinities of this enigmatic group (Koenemann et al., 2007, 2009).

#### *Thermosbaenacea*

Thermosbaenaceans (Figure 3d) are small (5 mm or less), eyeless or eye-reduced, anchialine and freshwater peracarid crustaceans with a dorsal brood pouch in females (Wagner, 1994; Jaume, 2008). They include at least 34 species with a wide distribution in caves and thermal springs around the Mediterranean and Caribbean, as well as in Australia and Cambodia.

#### *Mictacea*

Mictaceans (Figure 3e) are small (3–3.5 mm), eyeless and depigmented, nonpredatory crustaceans. This peracarid order is represented by only a single species that inhabits anchialine caves in Bermuda (Bowman and Iliffe, 1985).

#### *Bochusacea*

Bochusaceans are very small (1.2–1.6 mm), semi-transparent, and eyeless peracarid crustaceans that include two anchialine species from the Bahamas and Cayman Islands, plus two deep-sea species (Gutu and Iliffe, 1998; Jaume et al., 2006).

#### *Copepoda*

Platycopeid, misophrioid, cyclopid, harpacticoid, and calanoid (especially epacteriscid and ridgewayiid) copepods inhabit anchialine caves in tropical regions around the globe. They are small (typically 1–2 mm long) and have a short, cylindrical body with head and thorax fused into a cephalothorax. Most are planktonic filter feeders, but some, such as the harpacticoids and cycloids, are benthic, while epacteriscids are predators on other copepods.

#### *Ostracoda*

Halocyprid ostracods (Figure 3f) include anchialine species with a distribution and co-occurrence similar to that of remipedes (Kornicker et al., 2007). *Danielopolina* is the most widely distributed stygobitic genus with species on opposite sides of both the Atlantic and Pacific, inhabiting caves in the Bahamas, Cuba, Yucatan, Jamaica, Canary Islands, Galapagos, Western Australia, and Christmas Island. More than 300 species of podocopid ostracods have been found in springs, caves, and anchialine habitats.

#### *Mysidacea*

Stygobitic mysids are found in freshwater and anchialine habitats in Africa, the Caribbean, Mediterranean, and India. Their distribution suggests that they were stranded in caves by lowering of the sea level in the Tethys and Mediterranean. Recent molecular phylogenies of the mysids suggest that a new order is justified for the stygiomysids (Figure 3g), which inhabit caves in the Caribbean and Italy (Meland and Willassen, 2007).

#### *Isopoda*

Stygobitic isopods (Figure 3h) range from several millimeters to several centimeters in length. Anthurid isopods occur in anchialine and freshwater caves in the Canary Islands, Caribbean and Indian Ocean islands, Mexico, and South America. Asellot isopods inhabit anchialine and freshwater caves in the Caribbean, Europe, Galapagos, India, Indonesia, Japan, Malaysia, North and Central America, and Polynesia. Cirolanid isopods have been found in freshwater and anchialine caves clustered in Mexico and the Caribbean (Iliffe and Botosaneanu, 2006), as well as in Europe and the Mediterranean.

#### *Amphipoda*

Amphipods occur in freshwater and marine cave habitats. Stygobitic representatives are present in the bogidiellid, crangonyctid, hadziid, and niphargid families of the amphipod suborder Gammaridea. They are very widely dispersed, with large numbers of species inhabiting caves in Central and Southern Europe, the Mediterranean, eastern and southern North America, and the Caribbean.

#### *Decapoda*

The anomuran galatheid crab *Munidopsis polymorpha* inhabits an anchialine lava tube in the Canary Islands

(Wilkens et al., 1990). Brachyuran crabs are widely distributed in caves of the tropics and subtropics. Anchialine stygobitic shrimp include representatives from the caridean families Agostocarididae, Alpheidae, Atyidae, Hippolytidae, Palaemonidae, and Procarididae; the stenopodid family Macromaxillocarididae; and the thalassinid family Laomediidae.

#### *Other Crustacean Stygofauna*

One tantulocarid, an exceptionally tiny ectoparasite on anchialine harpacticoid copepods, occurs in the Canary Islands (Boxshall and Huys, 1989). A species of stygobitic nebalicean inhabits anchialine caves in the Bahamas and Caicos Islands (Bowman et al., 1985). Several species of cumaceans and tanaidaceans have been collected from anchialine caves in Bermuda and the Bahamas, but it is not clear whether they belong to the stygofauna.

### **BIOGEOGRAPHY**

Upon examining the biogeography of anchialine fauna, some extraordinary patterns are evident. A number of anchialine genera, including the remipede *Lasionectes*, ostracod *Danielopolina*, thermosbaenacean *Halosbaena*, and misophrioid *Speleophria*, inhabit caves on opposite sides of the Earth and are believed to be relicts whose ancestors inhabited the Tethys Sea during the Mesozoic (Humphreys, 2000). Some anchialine taxa are represented in the Mediterranean, but others, notably remipedes and *Halosbaena*, are absent. The presence of anchialine taxa at all in the Mediterranean is remarkable considering that this basin was completely dry for long periods of time during the Miocene. The aytid shrimp *Typhlatya* shows an especially interesting distribution with 17 known species inhabiting freshwater and anchialine caves in the Mediterranean region, Bermuda, Ascension Island, Caribbean locations including Cuba and Yucatan, and the Galapagos Islands (Alvarez et al., 2005). The shrimp family Procaridae contains one genus with species in the mid-Atlantic and Caribbean, as well as Hawaii.

Based on numbers of stygobitic species, the Bahamian archipelago appears to have been a possible center of origin for anchialine fauna. Among the Remipedia, 15 of 20 described species inhabit caves in the Bahamas (Koenemann et al., 2008b; Daenekas et al., 2009), whereas among anchialine halocyprid ostracods, Bahamian species account for 4 of 11 in the genus *Danielopolina*, 6 of 11 in *Spelaeoecia*, and all 8 species of *Deeveya* (Kornicker et al., 2007).

The Bahamas archipelago consists of a series of broad, shallow-water, highly karstified, carbonate platforms rising abruptly from the deep sea. The islands and cays consist of Pleistocene limestone covered by a thin veneer of Holocene carbonate reefs and sediments. Underlying these younger limestones is a continuous section of Tertiary and Cretaceous limestones and dolomites exceeding 11 km in thickness. If the position of the tectonic plates before the development of the Atlantic Ocean is reconstructed, virtually all the Bahamas overlap the African continent and its continental shelf. This finding suggests that the Bahama platform developed over oceanic crust during the earliest phase of the creation of the Atlantic. The extended shallow-water history of the Bahamas, coupled with the cavernous nature of the limestone, may help to explain its rich and diverse anchialine fauna.

### **ORIGINS OF ANCHIALINE BIOTA**

A number of theories have been proposed to explain the trans-oceanic distribution of many anchialine taxa. The *vicariance model* suggests that plate tectonics served as a mechanism for the dispersal of anchialine fauna (Rosen, 1976; Wiley, 1988). This model mainly describes the Tethyan track of ancient taxa that were rafted on the drifting continents to their present positions (Stock, 1993; Jaume et al., 2001). However, the existence of anchialine fauna on mid-ocean islands such as Bermuda, Ascension, and Hawaii that have never been part of or closer to a continent cannot be explained by this mechanism (Iliffe, 2000).

The *regression model* suggests that sea-level regressions, caused by tectonic uplift or eustatic glacial lowering of sea levels, stranded crevicular or interstitial marine littoral species that subsequently adapted to brackish or freshwater conditions (Stock, 1980). This model is supported by the observed correlation between the distribution patterns of numerous, marine-derived cave organisms and the position of shorelines during the Late Mesozoic or Tertiary seas. Nevertheless, the presence of anchialine fauna in caves that were completely dry and air filled (as evidenced by their now-submarine speleothems) less than 10,000 years ago indicates that these animals can migrate vertically with raising postglacial sea levels (Iliffe, 2000). Also, small islands such as Bermuda offer little chance for marine species to be stranded.

A *deep-sea origin* has been proposed for some anchialine species having close relatives that inhabit bathyal depths (Hart et al., 1985). Both caves and the deep sea are old, climatically stable, lightless, and nonrigorous environments. Anchialine habitats on islands and continental

margins could be connected via a continuum of crevicular corridors extending from shallow depths to the deep sea (Iliffe, 1990). However, evidence against a deep-sea origin of cave faunas includes the questionable ability of deep-sea species to cross the oceanic thermocline, the relatively recent nature of deep-sea species (resulting from the lack of oxygen in Atlantic bathyal waters during the late Oligocene), and phylogenetic analyses of morphological characters supporting independent colonization of deep-sea and anchialine habitats (Stock, 1986; Danielopol, 1990).

The *active migration model* involves the inland dispersal and colonization of subterranean habitats by expansionistic marine species with a high degree of salinity tolerance (Rouch and Danielopol, 1987). This process is independent of climatological and geological variations.

*Passive oceanic dispersal* of larval or postlarval stages of anchialine species by currents could explain the wide distribution of some anchialine shrimp species within the Indo-Pacific. Rafting on floating objects, such as wood, algae, kelp, and coconuts, or on mobile and migratory animals, for instance, sea turtles, fishes, and larger arthropods, could disperse anchialine species, even those without a free larval stage. However, oceanic dispersal is unlikely for many anchialine groups that produce few offspring or have narrow physiological tolerances.

## ADAPTATION TO LIFE IN ANCHIALINE CAVES

### BEHAVIORAL ADAPTATIONS

Behavioral adaptations are the most immediate adaptations for survival and colonization in cave systems. Cave organisms, in particular amblyopsid cave fishes, use a glide-and-rest technique to conserve energy in their search for food. Remipede locomotion is also designed for the economy of movement. Remipedes swim slowly, using less energy for the same distance than if they swam at higher speeds (Koenemann et al., 2008a). The power stroke produces drag by individual legs, but the recovery stroke is completed with the legs folded with other legs to reduce water resistance.

The stygobitic galatheid crab *Munidopsis polymorpha*, inhabiting an anchialine lava tube in the Canary Islands, has a number of specialized behaviors (Parzefall, 1992, 2000). These small crabs are most abundant in a dimly illuminated pool where they hide in rock crevices during the day but come out at night to feed on diatoms. Because of the large numbers of individuals in this pool, they spread in an almost regular pattern determined by the length of the second antennae.

*Munidopsis* crabs remain aggressive throughout the year. They detect intruders from water movements and attack with extended chelipeds. Male crabs are attracted by a molting hormone released by females. To prevent the females from fleeing, males rhythmically move their chelipeds as they approach, until the female responds by vibrating one of her chelipeds. The male then seems to turn the female over on her back to initiate insemination.

### MORPHOLOGICAL ADAPTATIONS

#### *Regressive Features*

The loss of features that in cave environments no longer have a function, such as eyes and pigmentation, is regarded as regressive evolution. There are two main theories explaining the driving force for regressive evolution. In an environment with a depauperate food supply, natural selection should favor reallocating energy from developing unused features, such as eyes and pigment, to growth and survival. A second explanation is that regressive evolution may be the result of nonselective processes such as neutral mutation and genetic drift. Features such as eyes and pigment that abruptly lose their biological function when animals enter caves are free to be turned off by now non-lethal mutations.

Unfortunately, the theory of energy economy by character reduction in stygobites is not well tested, especially with anchialine stygobites, yet the anchialine environment is dominated by blind, depigmented organisms.

#### *Constructive Features*

In the case of constructive features, priority is given to life history, metabolism, development, and starvation resistance, with sensory development such as mechano- and chemoreceptors being subordinate. For troglomorphy to occur, two factors must be present: (1) selective pressure in favor of the development and (2) genetic, physiological, or behavioral ability of the organism to respond to the selective pressure. A prerequisite for constructive traits is their genetic availability in epigeal forms: if traits are not present in epigeal ancestors, they will not be present in hypogeal descendants.

There are several areas of the body where constructive features occur. In crustaceans, appendages may be elongated, in particular, the antennae, and in fish, the head may become enlarged or flattened. Corresponding to the morphological changes, there is an increased sensitivity to chemical and mechanical stimulants. As a result of compensatory enhancement of extraocular senses, the signal-processing structures in the brain are altered.

## PHYSIOLOGICAL ADAPTATIONS

### *Adaptations to a Food-Poor Environment*

Food in the stygobitic environment may be in general scarce or at best patchy; therefore, the stygofauna need to cope with temporal periodicity of food availability and potentially tolerate long periods of starvation. This adaptation occurs through lipid accumulation or energy economy. In comparison with pelagic crustaceans, anchialine crustaceans sacrifice protein mass for increased lipid stores (Ilfie and Bishop, 2007). Lipids provide neutral buoyancy without energy expenditure, while also serving as an energy reserve when food is limiting. Anchialine stygobites also tend to be smaller than their epigeal counterparts. Their small size is a mechanism for energy economy.

### *Adaptation to Hypoxia and Anoxia*

As mentioned previously, the anchialine environment, especially at or below the halocline, is commonly hypoxic or even anoxic. As a result, hypogean organisms tend to have substantially lower oxygen consumption rates than their epigeal relatives (Bishop et al., 2004). Many organisms are capable of obtaining energy when faced with a reduction or absence of oxygen, but few are able to survive indefinitely without a return to oxygen. When the oxygen supply becomes inadequate, organisms switch to anaerobiosis to compensate for adenosine triphosphate (ATP) demand.

During periods of anaerobiosis, organisms conserve their energy stores by a loss in physiological functions such as motility, ingestion, and digestion, combined with a dramatic depression of their energy (ATP) demand. When oxygen is temporarily unavailable, many organisms switch to anaerobic glycolysis. Anaerobic glycolysis is, however, a fundamentally inefficient metabolic strategy and thus not an attractive solution for anchialine organisms.

By examining the activities of enzymes critical to metabolism and energy conversion, it is possible to determine the rate at which food is converted to cellular energy (Bishop et al., 2004). Citrate synthase (CS) is an indicator of an organism's maximum aerobic potential, or how fast an organism can aerobically convert glucose to energy. Malate dehydrogenase (MDH) functions in the presence as well as absence of oxygen, whereas lactate dehydrogenase (LDH) contributes to both aerobic and anaerobic metabolic pathways and serves as an indicator of glycolytic potential.

Anchialine organisms are anaerobically poised with both LDH:CS and MDH:LDH ratios tending to be greater

than one. The higher the MDH:LDH ratio, the greater is the tolerance to hypoxia. Such high ratios indicate an evolutionary adaptation to the anaerobic anchialine environment.

## CONSERVATION

Over the past 25 years, more than 400 new species of anchialine stygobites have been discovered and described. A high percentage of these species are known only from a single cave or cave system. Even within caves, species are characteristically found only at specific depths or locations as defined by a narrow range of environmental parameters. In many parts of the world, tourism development, limestone quarries, and groundwater pollution are either destroying or grossly polluting numerous caves, resulting in extinction of untold numbers of species.

Anchialine species qualify for inclusion on endangered species lists for reasons of their limited distribution and the declining environmental quality of their habitat. In Bermuda, 25 cave species are on the IUCN (International Union for Conservation of Nature) Red List of endangered species. Other cave species from the Yucatan Peninsula are on the official Mexican list of threatened and endangered species.

Maintaining groundwater quality is essential to the environmental health of the subterranean environment. For example, the small oceanic island of Bermuda is the third most densely populated country in the world and has the largest number of private cesspits per capita. Disposal of sewage and other wastewater into cesspits or by pumping down boreholes is contaminating the groundwater and cave water with nitrates, detergents, toxic metals, and pharmaceuticals; depleting the very limited amounts of dissolved oxygen in cave water; and generating toxic levels of hydrogen sulfide.

Some ocean caves such as the Blue Holes of the Bahamas have strong tidal currents sweeping through them for very considerable distances. In one such cave, plastic bottles and other trash have been observed littering the floor of the cave nearly a mile back into previously unexplored passages. Far too many caves and sinkholes are viewed as preferred locations for the dumping of garbage and other waste products.

Another serious environmental problem concerns the destruction of caves by limestone quarries or construction activities. Half a dozen or more Bermuda caves have been totally destroyed by two limestone quarries that produce crushed aggregate for construction purposes. Untold other

caves have been lost to enormous limestone quarries in the Yucatan Peninsula. Many caves have been filled in and built over by golf courses, hotels, and housing developments in Bermuda. Recently, a series of luxury town homes was built directly on top of the largest cave lake in Bermuda.

Sometimes even seemingly innocent activities can threaten caves and cave animals. Along the Caribbean coast of the Yucatan Peninsula, many open water cenote pools are inhabited by the freshwater fish *Astyanax fasciatus*. Some of these fish frequently follow divers into caves, moving in front of the dive team and voraciously darting in to consume any cave fish or crustaceans that are illuminated by the beam of a dive light. Considering the many thousands of cave divers who use these systems each year, it is not surprising that the caves most heavily visited by tourist divers are now essentially devoid of life.

Even the gas exhaled by divers may have adverse effects on cave animals. Because anchialine cave waters typically contain extremely low levels of dissolved oxygen, exhaust bubbles from open circuit scuba could have profound effects on the cave ecosystem. Several anchialine caves in Western Australia with unique fauna are currently off limits to open circuit divers and may only be visited by those using rebreathers (Humphreys et al., 1999).

Some anchialine caves in Bermuda, the Canary Islands, and Mallorca have been developed into commercial tourist attractions. Unfortunately, many of the tourists visiting these sites have viewed the deep clear water cave pools as natural wishing wells in which to throw a coin or two. Copper coins tend to rapidly deteriorate and dissolve in saltwater, producing high levels of toxic copper ions in the cave waters. In one such cave in the Canary Islands, the endemic crab *Munidopsis polymorpha* has shown a marked decline in abundance during the past decade or longer, probably in response to high levels of copper in the cave water.

#### ACKNOWLEDGMENTS

Investigations of the anchialine cave fauna of the Bahamas were supported by awards DEB-9870219 and DEB-0315903 from the U.S. National Science Foundation's Biodiversity Surveys and Inventories Program and by grants from the NOAA Caribbean Marine Research Center to T. Iliffe. Collection of specimens was carried out under a permit from the Bahamas Department of Fisheries. This research would not have been possible without the generous collaboration of numerous scientists, graduate students, and cave divers from around the world. Renee Bishop (Penn State University at Worthington Scranton) generously provided much useful information on physiological

adaptations of anchialine stygobites. This is contribution number 845 of the Caribbean Coral Reef Ecosystems Program (CCRE), Smithsonian Institution, supported in part by the Hunterdon Oceanographic Research Fund.

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