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OBSERVATIONS ON THE BIOLOGY AND BEHAVIOR OF *AMPHICUTIS STYGOBITA*, A RARE CAVE BRITTLE STAR (ECHINODERMATA: OPHIUROIDEA) FROM BERNIER CAVE, SAN SALVADOR ISLAND, BAHAMAS

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ABSTRACT

Amphicutis stygobita is the world's only known brittle star species that appears to be a cave endemic. Pigment is absent in arms and disk. This species was discovered by John Winter in 2009 and described by Pomory, Carpenter, and Winter in 2011. The few individuals available for the current study were collected in January 2011, January 2013, and June 2013.

Goals of the current study are to: (1) compare Bernier Cave to nearby Lighthouse Cave to possibly determine why *A. stygobita* are found only in Bernier Cave, (2) observe interactions between *A. stygobita* and two potential predators: cave isopods (*Bahalana geracei*) and mangrove rivulus fish (*Kryptolebias marmoratus*), (3) compare some biological and behavioral traits of *A. stygobita* (especially troglomorphisms) to those of other brittle stars and to those of cave dwelling species in other taxa, and (4) determine culture techniques to keep *A. stygobita* alive and healthy.

Amphicutis stygobita are difficult to find and collect because they are: found only in the dark, tiny (disk diameter 3-4 mm), and similar in appearance to the detritus on which they are found. They are challenging to maintain in the laboratory because they are susceptible to light, salinity changes, and elevated temperatures, and they don't accept food normally eaten by brittle stars.

Bernier Cave has a lower salinity range (~14-28 ppt) than Lighthouse Cave (~20-36 ppt); it is rare for an echinoderm to be endemic to such a low salinity environment. Tidal fluctuations and tidal flow are less in Bernier Cave, allowing for a substrate of detritus and soft mud, and its entrance room has larger openings over the water to provide more detritus. Cave isopods and mangrove rivulus fish do not appear to be significant predators of *A. stygobita*.

The statement that *A. stygobita* is a cave endemic is supported by a surprising array of troglomorphisms including: no body pigment, muted alarm response to light, reduced body size, elongated arm segments, reduced aggregation, slow movement (1-2 cm/min primarily with tube feet, rather than by swinging arms forward), and extremely slow regeneration rate of 0.03 mm/wk. Bernier Cave and its rare cave brittle star deserve much more study.

INTRODUCTION

The brittle star *Amphicutis stygobita* was found by John Winter in 2009 in a cave discovered by Don Bernier on San Salvador Island, Bahamas. John Winter and I collected additional specimens in January 2011; Pomory, Carpenter, and Winter (2011) described the brittle star as a new genus and new species. Distinctive physical traits include: no pigment in arms or disk, translucent layer of skin raised above surface of arms, small size (disk diameter 3-4 mm), and short arms (to 9 mm) consisting of relatively few segments that are elongated (Fig. 1).

In March 2014, Susan Hottenrott Spark kindly sent me unpublished information regarding a single brittle star specimen collected from Lighthouse Cave by a George Washington University class in 1992. She still has the specimen, which she studied extensively, and her detailed drawings and description confirm that it is clearly an *A. stygobita*. Many subsequent searches by
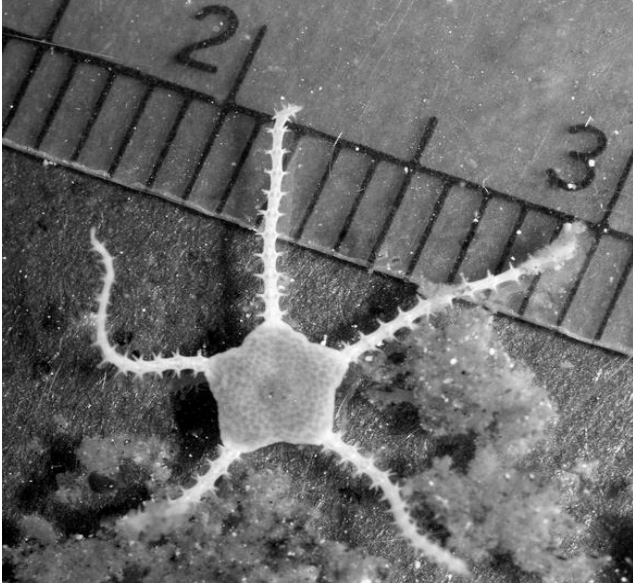
Figure. 1. Amphicutis stygobita with metric ruler and detritus.

George Washington University classes, as well as by my own classes from Northern Kentucky University, have never found another brittle star in Lighthouse Cave. This may indicate that this species has been extirpated from the cave, possibly as a result of disturbance from numerous classes that visit the cave every year. It is also possible that the microhabitats in Lighthouse Cave are simply not as favorable for *A. stygobita* as those in Bernier Cave.

The discovery of this species is especially noteworthy because it is the only brittle star that appears to be a cave endemic (Pomory et al. 2011). There are only rare examples of ocean dwelling species (e.g., *Ophioderma longicauda* and *Ophionereis sp.*) finding their way into caves (Pomory et al., 2011). According to Stöhr and O'Hara (2013) there are over 2,100 species and subspecies of ophiuroids (brittle stars and basket stars). Since they live in a large variety of habitats and are generally photonegative, it is surprising that more brittle stars have not been found in caves. In fact, it seems likely that more brittle stars do live in marine caves, but perhaps cave divers have not seen or collected these small benthic animals because divers tend to stay off the bottom to avoid clouding the water.

Limestone caves that are close to oceans often contain salt water or brackish water. Those caves without a surface connection to the ocean are called anchialine (Stock et al., 1986). Typical anchialine caves are stratified with a surface freshwater layer from rain water lying over a saltwater layer from the sea, and separated by a halocline layer of mixed fresh water/salt water. Cave divers often swim through several meters of fresh water and halocline to reach the salt water.

The anchialine caves on San Salvador Island do not have this stratification. Instead, they have salt water or brackish water all the way to the surface. This allows researchers to collect and observe a unique variety of marine cave animals without SCUBA diving. Examples include several new species described from Lighthouse Cave such as the cirolanid isopod crustacean *Bahalana geracei* (Carpenter, 1981), the asselote isopod *Neostenetroides stocki* (Carpenter and Magniez, 1982), and three species of sponges (van Soest and Sass, 1981). Another interesting example from San Salvador Island is the remipede crustacean *Speleonectes epilimnius* (Yager and Carpenter, 1999), described from Major's Cave; this is the only remipede species known to occur at the saltwater surface of a cave (hence the name "epilimnius"), rather than several meters deep in stratified cave waters.

Some intriguing questions arose with the discovery of the world's first cave brittle star. For instance, does Bernier Cave have unusual qualities that allow *A. stygobita* to live there? And why have we never found *A. stygobita* in nearby Lighthouse Cave, which my students, colleagues, and I have studied nearly every year since 1978? Thus, one of the goals of the current study was to compare Bernier Cave to Lighthouse Cave to possibly determine why *A. stygobita* are now found only in Bernier Cave.

One of my early observations in Bernier Cave was that cave isopods, *Bahalana geracei*, are rare there, while mangrove rivulus fish, *Kryptolebias marmoratus* (Poey), are fairly common. From years of personal observation of Lighthouse Cave organisms, I found that both these species prey on a variety of animals. Thus, a second goal of the current study was to observe interactions between these two potential predators and *A. stygobita.*

Several traits are often associated with cave dwellers, especially with obligate cave organisms known as troglobites (or stygobites, if aquatic). These special traits are called troglomorphisms (Christiansen, 1962; Romero, 2009). Familiar examples include loss of eyes and body pigment; Romero (2009) lists 20 others. It is tempting to think of troglomorphisms as adaptations for living in caves, but in many cases we don't know how (or if) the characters are actually advantageous in this habitat (Romero, 2009), and documentation of adaptation in extremely troglomorphic cave animals is difficult (Culver et al., 1995). Nevertheless, it is interesting to examine what troglomorphisms are found in this first cave brittle star and possibly support the statement that this is a cave endemic species. Thus, a third goal of the current study was to compare some biological and behavioral traits of *A. stygobita* (especially possible troglomorphisms) to those of non-cave dwelling brittle stars and to those of cave dwelling species in other taxa. In order to accomplish this third goal it was necessary to pursue a forth goal—to determine culture techniques to keep *A. stygobita* alive and healthy long enough to study some of these traits.

Amphicutis stygobita is one of the rarest brittle star species in the world (Pomory et al., 2011). On several collecting trips to Bernier Cave no specimens were seen or collected. Since it has been impossible to collect *A. stygobita* in large numbers, and this species is challenging to keep alive for extended periods, sample sizes have been inadequate for traditional quantitative experiments. Instead, I am herein describing my observations on what might be thought of as early trials in long-term research projects. I plan to conduct additional trials and experiments if and when more specimens become available. Nonetheless, it seems appropriate to report my findings now because of the rareness of the species and the uniqueness of cave endemism for a brittle star.

After the "Materials and Methods" section, this paper is divided into several parts. Each part describes one or more sets of experiments and/or observations, results, and my interpretation of observations.

MATERIALS AND METHODS

Animal Sampling

All *A. stygobita* specimens were collected from dark areas of Bernier Cave (24° 05' 37" N, 7° 27' 15" W) in shallow water 10-40 cm deep. The few individuals available for this study were collected on three trips: 6 January 2011 (15 specimens collected, but 7 preserved for type series and microscopic study); 9-11 January 2013 (n = 10); and 17 June 2013 (n = 4). *Kryptolebias marmoratus* fish were collected from Bernier Cave for fecal studies and predation experiments; collection dates and quantities were: 9-11 January 2013 (n = 5) and 17 June 2013 (n = 5). We also collected detritus and water samples. Cave isopods (*B. geracei*) were collected from Lighthouse Cave for predation studies; collection dates and quantities were: 8 January 2013 (n = 6) and 18 June 2013 (n = 7). More information about Bernier Cave and Lighthouse Cave will be covered in the section comparing these two caves.

Flashlights were used to find and collect specimens in dark areas of the caves. In January 2011 aquarium nets were used to collect *A. stygobita* from the substrate, but this stirred up detritus making it difficult to find other specimens, and arm spines became entangled in the nets. On later trips, fingers or spatulas were used to scoot specimens into 35 mm film canisters or small clear jars. If more than one specimen was placed in a container it was more likely for oxygen to be depleted and arms to be entangled and damaged, so we learned to transport specimens in separate containers. *Kryptolebias marmoratus* were collected with aquarium nets and transported in individual jars. *Bahalana geracei* were collected in Lighthouse Cave with aquarium nets and transported in individual jars to avoid cannibalism. Salinities were determined with hydrometers and/or refractometers. Temperatures were measured using a Weston metal probe thermometer and/or a Suunto Core wristwatch thermometer.

Soon after specimens were collected they were taken to the Gerace Research Centre for short-term observation. Later some were transported in small jars to Kentucky for long-term ob-

servation and experimentation. Some *A. stygobita* were placed in Petri plates or other shallow containers for brief observation with an Olympus dissecting microscope. Most *A. stygobita* specimens were photographed using a Nikon D80 camera with a 60 mm Micro Nikkor macro lens.

Culture Methods

Since I have successfully kept individual specimens of several other cave species (e.g., isopods, sponges, and freshwater planarians) alive for long periods (*B. geracei* for up to eight years), I used the same general methods for *A. stygobita*. However, I learned through trial and error that this species is more sensitive to light, salinity changes, and higher temperatures so the following methods were more successfully used for *A. stygobita*. Each specimen was kept in a small jar with ~30 ml of brackish water (20-28 ppt), similar to that of Bernier Cave. Depth in each jar was kept shallow (1-2 cm) for a high surface area to volume ratio to keep oxygen levels high. Jars had tight-fitting lids (rather than loose lids as with Petri plates) to limit evaporation that increases salinity. Salinities were checked weekly with a refractometer. If a salinity increased, I reduced it by adding 1-2 ml of water at a slightly lower salinity; drops were added slowly and away from specimens to avoid salinity shock. A thin layer of detritus from Bernier Cave was kept on the bottom of each jar to provide food and to help stabilize levels of oxygen and salinity. An additional 8-15 drops of new Bernier Cave detritus were added every 4-7 days. Animals ignored or rejected other foods offered including small pieces of Tetra Min© fish flakes, shrimp, boiled egg, and boiled lettuce. Each specimen jar was labeled so I could keep individual records of maintenance, experiments, and general observations. Animals were kept in darkness except during observations and maintenance. Observations and maintenance were done at night or in a room without windows to avoid even weak sunlight, and jars were shaded from direct overhead lights and microscope lights.

Photographs of some *A. stygobita* individuals were shot approximately weekly to record feeding activities and regeneration progress. The camera's built-in flash was dimmed by setting it on half power and using a diffuser to reduce possible disturbance or damage to specimens.

Specimens were kept near Bernier Cave temperatures of 23-25°C (73-77°F). I used either a 10-gallon tank with a heating pad below, or a water bath with an aquarium heater. Jars of Bernier Cave detritus were also kept in darkness at cave temperatures and salinities to try to keep them viable and to avoid temperature or salinity shock when detritus was added to specimen jars.

COMPARING BERNIER CAVE TO LIGHTHOUSE CAVE

Since *A. stygobita* has been found several times in Bernier Cave and only once (in 1992) in the extensively studied Lighthouse Cave only ~300 m away, a comparison of the two caves might help explain why. The geology and hydrology of Lighthouse Cave have been described by Mylroie (1980) and Davis and Johnson (1988) respectively, while many pertinent features of Bernier Cave were described by Pomory et al., (2011). The hydrology of Bernier Cave is currently being studied by Devin McGinty (a graduate student at University of New Haven) using data loggers to determine long-term tidal fluctuations and salinities (pers. comm.). The following comparisons are based mostly on my personal observations of these two caves.

Both caves are in the northeastern part of San Salvador Island, ~1-1.5 km from the ocean. They are reached by taking different paths from Dixon Hill Lighthouse (southerly for Lighthouse Cave and southwesterly for Bernier Cave). Tidal fluctuations in the caves occur through complex conduit systems; tides are delayed (compared to ocean tides) by ~45 minutes in Lighthouse Cave and ~2 hours in Bernier Cave, presumably because Bernier Cave is further from the ocean. Tidal fluctuations (changes in water depth between low and high tide) are much less and flow rates are much weaker in Bernier Cave, which help provide a substrate of detritus and soft mud; detritus appears to be the main source of food for *A. stygobita*. In Lighthouse Cave the substrate is

bare rock where tidal water moves rapidly, and it is silty in protected quiet water; also, the water and substrate are frequently disturbed by touring field classes and vacationers.

Both caves are entered through openings in the roof by climbing down ladders ~3-4 m to dry breakdown platforms below. Both caves have additional roof openings that permit entrance of sunlight, rain, leaf litter, and fauna including insects, birds, and bats. However, the ceiling openings in Bernier Cave are larger and directly above or near the water, so more detritus enters Bernier Cave water. The entrance room also has much more light so that mangrove rivulus fish can more easily see and attack prey such as insects, copepods, and cave isopods. This appears to have a major effect on populations of cave isopods, which are common in Lighthouse Cave and rare in Bernier Cave (only two have been seen in >60 hours of observation by researchers).

There is a significant difference in salinity ranges of the two caves. Lighthouse Cave is usually near full ocean salinity (35-36 ppt), but sometimes it drops to ~20 ppt or lower after heavy rains (my samples on 18 June 2013 varied from 20-32 ppt depending on location in the cave). Salinity readings in Bernier Cave range from ~14-28 ppt; these wide variations in readings are due mostly to strength of recent rains, but they also vary with depth (my samples on 19 June 2013 ranged from 15 ppt at the surface to 21 ppt at 20 cm depth near the substrate where brittle stars were found).

It is surprising that *A. stygobita* can live in such a low salinity, since echinoderms are generally marine because they lack excretory-osmoregulatory structures (Brusca and Brusca 1990). In fact, until the discovery of *A. stygobita* in brackish Bernier Cave, the estuarine brittle star *Ophiophragmus filograneus* appeared to be the only endemic brackish-water echinoderm (Turner and Meyer, 1980). Since *O. filograneus* and *A. stygobita* have the unusual ability to tolerate brackish environments, they may benefit from decreased competition from other echinoderms and avoidance of predators that cannot tolerate low salinities throughout their life cycles.

I believe the differences in these two caves that are most likely to explain why *A. stygobita* are found in Bernier Cave and not in Lighthouse Cave are: salinity range is lower in Bernier Cave, and slower flow rates and larger openings over the water result in abundant detritus on the substrate for *A. stygobita* to eat.

POTENTIAL PREDATORS OF CAVE BRITTLE STARS

Cave Isopods, *Bahalana geracei*

One other possible explanation for why *A. stygobita* are currently found in Bernier Cave and not in Lighthouse Cave is related to food chains. In previous studies my marine biology students and I observed predators from Lighthouse Cave, and we found that mangrove rivulus fish *K. marmoratus* readily eat *B. geracei* isopods, which eat a variety of other cave animals. Bernier Cave's well-lit entrance should make it easy for rivulus fish to prey on *B. geracei*, which may explain the rareness of the isopods in this cave. If the larger population of *B. geracei* in Lighthouse Cave preyed on *A. stygobita* they could have eliminated them from that cave, while the reduced population of this predator in Bernier Cave could allow *A. stygobita* to survive there. The big question is, "Will *B. geracei* prey on *A. stygobita*?" On five different occasions I put either a healthy or an injured *A. stygobita* with a *B. geracei* for several hours or days, and the isopods never showed any interest in the brittle stars. To make sure the isopods did not reject the brittle stars simply because they were not hungry, I offered them pieces of shrimp which they ate immediately. On several other occasions I collected another species of small brittle star (*Ophiocomella sexradiata*) from an aquarium and placed them with *B. geracei* for several days; again the isopods showed no interest in eating them. Since some other marine isopods such as the giant *Glyptonotus antarcticus* eat brittle stars (Dearborn, 1967; Janecki and Rakusa-Suszczewski, 2006), it is surprising that *B. geracei* appear to not like the taste or texture of them.

Mangrove Rivulus, *Kryptolebias marmoratus*

Since brittle stars are often eaten by fish (Emson and Wilkie, 1980), I wanted to see if mangrove rivulus fish from Bernier Cave showed any interest in *A. stygobita*. On 10 January 2013 (one day after they were collected) I added one *A. stygobita* to an 8 cm diameter jar with one rivulus, which soon started attacking tips of the brittle star's arms. I removed the rivulus from the arena jar to protect the brittle star and examine it for damage (one arm was now missing the terminal segment). The next day I returned the rivulus to the arena jar with the brittle star, and this time the rivulus watched the brittle star for ~12 hours but did not attack it (Fig. 2). I added a second rivulus to the arena, and both continued to watch but not attack for another 12 hours. The arena was moderately well lit for the fish to see, and they had plenty of opportunity to attack, so I concluded that *K. marmoratus* may not like the taste or texture of *A. stygobita*, and probably they are not major predators of these brittle stars, especially in dark areas of Bernier Cave. This was supported by microscopic examinations of fecal samples of ten *K. marmoratus*, which showed no remains of brittle stars.
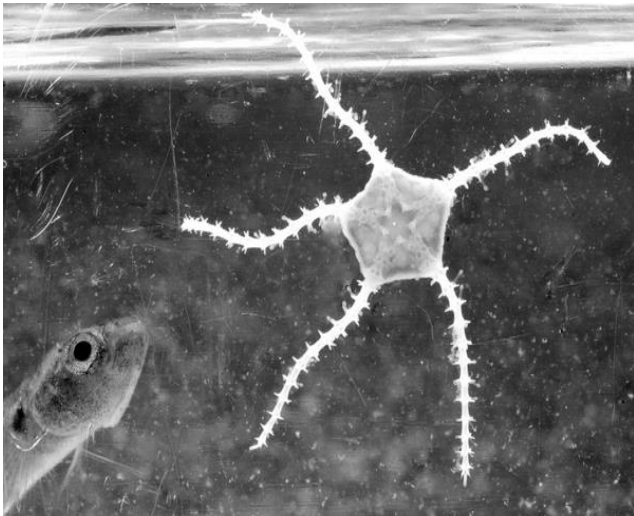

Figure 2. Mangrove rivulus (K. marmoratus) watching Amphicutis stygobita on side of jar.

Natural Arm Damage

Regeneration is so common in brittle stars that individuals observed in nature usually have regenerating arms at many different growth stages (Chinn, 2006). For most species, sub-lethal predation appears to be the major cause of arm damage, so the frequency of regenerating arms is often used as an estimate of predation pressure (Clark et al., 2007; Sköld and Rosenberg, 1996).

Based on photographs and recorded observations of 23 specimens of *A. stygobita* soon after collection, it appears that the incidence of natural arm damage is very low compared to that of other species. Only 8 of 23 specimens (= 35%) showed signs of arm damage or regeneration, and in each case only 1 arm showed damage so only 8 of 115 arms (= 7%) were damaged.

In comparison, Lawrence and Vasques (1996) summarized numerous sub-lethal predation studies on 30 species of brittle stars. Some studies only described the cause of damage (usually fish predation). Other studies reported the incidence of regeneration in three different ways: (1) 10 studies of 8 different species reported 43-99% of individuals in populations were regenerating; (2) 13 studies of 11 species reported the percentage of regenerating arms (25-85%); and (3) 6 studies of 5 species simply reported "% of arm regeneration," without specifying whether % of individuals or % of arms (range <10% to 30-85%). Thus, in comparison to these studies of at least 21 other species, the incidence of damage is very low in *A. stygobita*. This indicates that sub-lethal predation is low and probably has little influence on population density.

TROGLOMORPHISMS

The term "troglomorphism" was proposed by Christiansen (1962) to reflect the convergent morphologies of obligate cave animals (Culver and Pipan, 2009). Some troglomorphisms such as loss of eyes and body pigment seem to be a response to darkness (Romero, 2009). Others, such as slower metabolisms and increases in chemo- and mechanoreceptors to find food are apparent

responses to the lack of primary production and the resulting low food availability (Romero, 2009). Culver et al., (1995) listed 16 "major characteristics of cave organism," while Romero (2009) listed 22 troglomorphisms. Of course, "troglomorphy is not universal among subterranean organisms" (Culver et al., 1995), and some troglomorphic characters are specific for certain taxonomic groups.

One of my goals in the current study was to examine possible troglomorphisms of *A. stygobita*, which I assembled into Table 1. I omitted those listed by Culver et al., (1995) and Romero (2009) that are not applicable to ophiuroids, including reductions in: visual brain centers and pineal organs (absent in ophiuroids), cuticles (present in terrestrial arthropods), and scales and swimbladders (present in fish). I also omitted several that may apply to *A. stygobita*, but supporting information is not yet available, including: increased egg volume, longer life span, reduced circadian rhythms, and reduced aggression. I included the following troglomorphic traits of *A. stygobita* that I observed, even if only in anecdotally small numbers; these are discussed in the following sections.

Table 1. Troglomorphisms relevant to A. stygobita.

Eyes and body pigment reduced or absent
Responses to light
Reduction in body size
Elongated appendages
Enhanced chemoreceptors
Aggregation and fecundity reduced
Slow metabolism: movement and regeneration

Eyes and Body Pigment Reduced or Absent

These two traits are probably the most often recognized troglomorphisms. Although *A. stygobita* have no eyes, in this case it should not be considered as troglomorphic since eyes are absent in virtually all ophiuroids. However, lack of pigment in the arms and disk or *A. stygobita* is significant, since "other shallow-water Caribbean

ophiuroids have a wide range of coloration" (Pomory, 2007; Pomory et al., 2011).

Responses to Light

Although ophiuroids are eyeless, they do have the ability to detect light and to respond to it. Brittle stars show a strong tendency to avoid light by being nocturnal, which reduces predation by diurnal fish (Randall, 1967). In fact, light often acts as an alarm for brittle stars. Reef dwelling brittle stars (e.g., *Ophiocoma echinata* and *Ophionereis reticulata*) have a strong and immediate negative response when a microscope light is shined on specimens resting in the dark (personal observation). In contrast, many times I have shined a microscope light on *A. stygobita* to observe them, and they generally show little or no response, sometimes for >15 minutes.

Although *A. stygobita* have relatively little short-term response to light, the overall effect of light exposure can be severely damaging to brittle stars as demonstrated by Johnsen and Kier (1998). In their experiment, after four days of exposure to sunlight, 12 out of 13 *Opioderma brevispinum* were dead, and the 13th was damaged; control animals were protected by a UV-opaque filter and suffered almost no damage (Johnsen and Kier, 1998). Some cave animals are especially susceptible to light; Romero (2009) reported that Buchanan (1936) exposed the depigmented planarian, *Sphalloplana percaeca*, to direct sunlight and found that they did not move to shaded areas when given the chance, but they disintegrated within 12 hours of exposure. On 30 January 2011, I exposed seven *A. stygobita* to indirect sunlight for 100 minutes to see if they would move away from the light; the data indicated that there was virtually no directional movement. However, six of the seven specimens died within three days. This was not a controlled study for effects of UV light, but the dramatic results convinced me to protect *A. stygobita* from light. It seems likely that the translucent layer of raised skin and lack of pigment in *A. stygobita* make them more susceptible to UV light.

Reduction in Body Size

Many cave animals have a reduction in body size, perhaps as a response to a diminished food supply or quality. But Romero (2009) pointed out that reduction in body size should also be advantageous in an ecosystem with an abundance of crevices that could be used for hiding from predators, feeding, or reproduction. *Amphicutis stygobita* is one of the smallest brittle stars known, with a disk diameter of only 3-4 mm. Pomory (2007) provided a key and approximate disk size for 85 species of ophiuroids from the Gulf of Mexico and Caribbean Sea; the majority have disk diameters ~10-45 mm, and only two species have disks <5 mm (*Ophiactis algicola* ~2 mm, and *Ophiactis savignyi* ~4 mm.). Thus, it appears that reduction in body size is a troglomorphic trait of *A. stygobita*.

Elongated Appendages and Enhanced Chemoreceptors

Culver (1982) pointed out that "the most cherished tenet of biospeleology" is that "the fragile, delicate morphology of cave animals results from selection for increased sensory organs on appendages, which in turn results in lengthened appendages." But the arms of *A. stygobita* are relatively short at only 2-2.5X disk diameter because fewer (but much longer) arm segments are produced. If predation pressure is reduced in Bernier Cave, that would allow *A. stygobita* to conserve energy by moving slower and by producing fewer arm segments, fewer joints, and fewer spines which are located at the joints. This physical arrangement would result in "stiffer" arm movements, possibly making podial walking more efficient (see section below on "Slow Metabolism: Movement").

The greatly lengthened arm segments may also provide better surface areas for chemoreception. A distinctive trait of *A. stygobita* is its translucent raised layer of skin above the skeletal plates (see Fig. 4 in section below on "Slow Metabolism: Regeneration"). Pomory et al., (2011) speculated that the raised skin might be used to absorb dissolved organic matter (DOM). According to Brusca and Brusca (1990) sensory neurons in the epidermis of echinoderms "respond to touch, dissolved chemicals, water currents and light." Thus, it seems likely that *A. stygobita* may also use its raised skin for enhanced chemoreception and other functions in finding food or mates.

Podia (tube feet) of ophiuroids could also be considered a type of "appendage," and Pomory et al., (2011) noted that the podia in *A. stygobita* are relatively large. According to Ruppert and Barnes (1994) tube feet are used for gas exchange. Perhaps the enlarged podia (and possibly the skin) aid respiration in this warm cave environment with decaying detritus and no primary production or turbulent water to add oxygen.

Aggregation and Fecundity Reduced

Some ophiuroid species such as *Ophiothrix fragilis* occur in dense populations of 1000-2000 per square meter (Ruppert and Barnes, 1994). In contrast, most individuals of *A. stygobita* were found by themselves, but occasionally we have seen two or three within a square meter. This low density is probably a reflection of low food quality and/or quantity. Tests have not yet been performed to determine if *A. stygobita* tend to aggregate in captivity.

Little is known about reproduction and fecundity in *A. stygobita*. However, since juveniles have not yet been observed in Bernier Cave, it appears that reproduction may be an infrequent event.

Slow Metabolism: Movement

Many studies have found that subterranean species have lower metabolic rates in response to food scarcity (Hüppop, 2000). One indication of a slow metabolism in *A. stygobita* is its pattern of slow movement. Many times I measured the movement of specimens as they slowly glided around the perimeter of their 5 cm diameter jars. This usually took 8-15 minutes, yielding a very slow rate of ~1-2 cm/min. The method of movement is also unusual. Pomory et al., (2011) pointed out that "most ophiuroids move by bending the arms, rather than podial walking as is common in

asteroids," but *A. stygobita* "mainly uses podial walking for locomotion." Pomory et al., (2011) speculated that "this may be a function of the short arms and the relatively large podia." It also seems logical that moving by podia (rather than swinging arms) may require less energy, and rapid escapes from predators are apparently needed less frequently in this cave habitat.

Slow Metabolism: Regeneration Rates

Most of my *A. stygobita* specimens did not lose any arms, or if they lost arms they did not survive long enough to have significant regeneration. However, one specimen (R #1) collected in January 2013 that lost parts of four arms had steady regeneration for 21 weeks; a second specimen (R #2) collected in June 2013 lost parts of all five arms, and it regenerated for 24 weeks. Both specimens probably lost arms as a result of reduced water quality from increased temperature and decreased oxygen during their long trips home. The two specimens were similar in size (disk diameter 4 mm), both started with arms ~5.5 mm long and with 14 segments/arm, and they had similar patterns and rates of regeneration. They fed regularly on detritus and appeared to be healthy.

The number of regenerating segments on each arm was determined from microscopic examination and photographs taken at intervals of 1-3 weeks; the number of regenerated segments ranged from only 0-3 for R #1 in 21 weeks, and only 1-4 for R #2 in 24 weeks (Figs. 3-4). At first regeneration rates were recorded simply as number of segments added, but that did not correlate well to published regeneration rates for other species, which were typically reported as mm/wk. So, I measured the small growth in each regenerating arm of the two specimens based on photographs. Results varied from no growth for two arms to a maximum of 1 mm in 24 weeks for two arms (= 0.04 mm/wk). The average regeneration rate for all regenerating arms was 0.03 mm/wk.
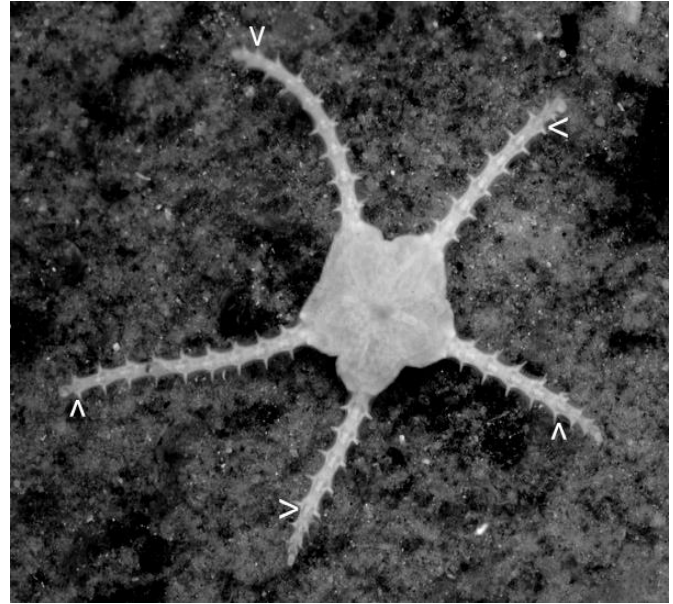

Figure 3. Amphicutis stygobita (specimen R #2) after 5 months of regeneration. Symbols indicate beginning of regenerated arm sections.
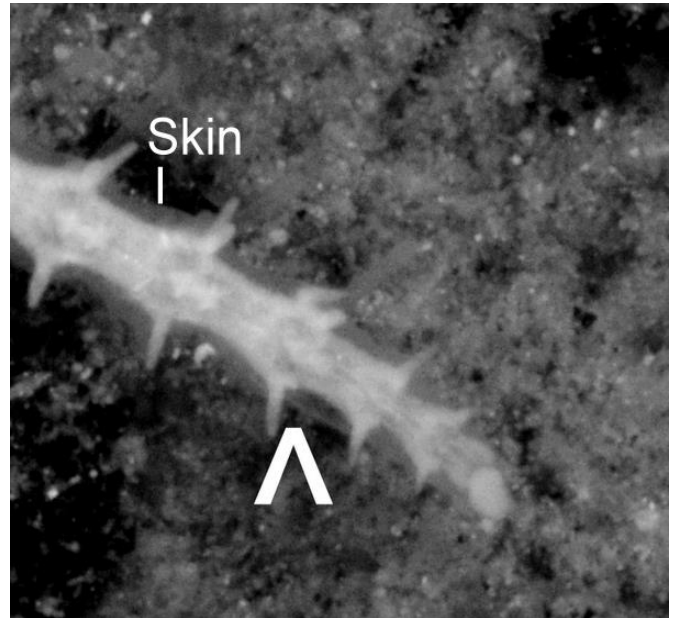

Figure 4. Amphicutis stygobita 3rd arm enlarged from Figure 3, showing skin and regenerated arm section (^).

Clark et al., (2007) presented a table of regeneration rates (RR = mm/wk) at various temperatures for 13 species of brittle stars as reported in 10 different studies. The fastest regeneration

rate was 6.25 mm/wk at 28°C for *Microphiopholis gracillima* (now known as *Amphipholis gracillima*), while the slowest regeneration rates were 0.25 mm/wk at 14°C for *Amphipholis squamata* and 0.22-0.68 mm/wk at 0.75°C for the Antarctic brittle star *Ophionotus victoriae*. So, the regeneration rate of *A. stygobita* of 0.03 mm/wk is much slower (by a factor of 10) than the slowest rates of the other species presented by Clark et al., (2007).

Since arms of *A. stygobita* are relatively short, 2-2.5X disk diameter (Pomory et al., 2011), theoretically the time required to regenerate an entire arm should also be fairly short, but at the slow regeneration rate of 1 mm in 5 months it would take 27.5 months (= 2.3 years) to re-grow a 5.5 mm arm, and 45 months (= 3.75 years) to grow a full-length 9 mm arm. According to Clark et al. (2007), "an Antarctic brittle star with an 80 mm arm could take over 3 yr to re-grow a complete arm." These re-growth times are extremely long compared to fast-growing species such as *Amphipholis gracillima*, which has a regeneration rate at 28°C that would "indicate complete replacement of lost tissue in 100 to 120d" (Stancyk et al., 1994).

One deficiency in the Clark table is that it does not address the concept that larger species would naturally add more mm/wk than smaller species. To better compare the regeneration rate in *A. stygobita* to that of other species, I collected disk diameters from various sources (e.g., Pomory, 2007). Then I modified the Clark table into my Table 2, which includes ten species, regeneration rates (RR = mm/wk), disk diameters (mm), relative regeneration rates (RRR = RR/disk mm), temperatures, and sources of information. Relative regeneration rates (RRR), which account for disk size, still show that *A. stygobita* has the slowest regeneration rate (<0.01 mm/wk/mm of disk), compared to the next slowest for *Ophionotus victoriae* (0.01-0.03 mm/wk/mm of disk). This difference is even more noteworthy because of the dramatically different temperatures (25°C for *A. stygobita* compared to 0.75°C for *O. victoriae*). According to the Q10 phenomenon, biological processes in ectotherms roughly double with each 10°C rise in temperature (Clark et al., 2007). By this measure the regeneration rate for *A. stygobita*

at 25°C should be at least four times faster than that for *O. victoriae*, instead of slower. Furthermore, the slow regeneration rate of *A. stygobita* is contrary to the "general phenomenon that smaller species of animals typically have higher per unit mass metabolic rates than larger species" (Pomory and Lawrence, 1999).

Table 2. Regeneration rates (RR) and relative regeneration rates (RRR) for ten brittle star species measured at different temperatures, adapted from Clark et al. (2007).

Species	RR (mm/wk)	Disk size (mm)	RRR (RR/ disk size)	Temp. (°C)	Source
Amphicutis stygobita	0.03	4	<0.01	25	This study
Amphipholis gracillima	6.25	8	0.78	28	D'Andrea et al. (1996)
Amphipholis squamata	0.25	5	0.05	14	Dupont et al. (2001)
Amphiura filiformis	0.8-1.5	10	0.11	7-12	Thorndyke et al. (2003)
Hemipholis elongata	2.1	10	0.21	20	McAlister and Stancyk (2003)
Macrophi- opholis longi- peda	6	20	0.30	26-29	Chinn (2006)
Ophiarthrum elegans	3	25	0.12	26-29	Chinn (2006)
Ophiocoma scolopendrina	3.6-4.2	20	0.20	26-29	Chinn (2006)
Ophionotus victoriae	0.22-0.68	20	0.02	0.75	Clark et al. (2007)
Ophiophrag- mus filogra- neus	2.94	10	0.29	23	Clements et al. (1994)

Several factors may influence the exceptionally slow growth rate in *A. stygobita*. First, cave animals often have inherently reduced metabolic rates, possibly as an adaptation to an energy poor environment (Poulson, 1963). A second factor—one associated with reduced metabolic rate—is that the detritus which *A. stygobita* eats would actually seem to be a relatively poor energy source compared to food given to regenerating brittle stars in some other studies (e.g., Chinn, 2006 used turkey meat, Clark et al., 2007 used white fish, and Pomory and Lawrence, 1999 used TetraMin© fish food).

A third factor to consider is salinity. Specimens of *A. stygobita* were maintained at Bernier

Cave salinities of 20-28 ppt. Donachy and Watanabe (1986) found that length of regenerated arms and number of ossicles formed in *Ophiothrix angulata* were significantly less at 23 ppt than at 28-38 ppt; they correlated this with reduced calcium concentrations at lower salinities.

Fourth, Clark et al., (2007) noted that in their study of the Antarctic brittle star, "the longer length of arm lost, the faster the regeneration rate." In the Clark study, "one arm was amputated from each individual approximately 10 segments from the disc;" this is relatively close to the disk in this long-armed species. In some other studies (e.g., Zeleny, 1903; Pomory and Lawrence, 1999) amputations were made at or near the disk. By comparison, the two *A. stygobita* specimens lost only ~28-64% of their total arm length, and regeneration rates appeared to slow as arms grew closer to full length. Thus, a short section of a damaged arm might be expected to grow slower than an almost completely removed arm.

A fifth factor is that several arms were regenerating simultaneously. As Chinn (2006) pointed out, "it is logical that the more arms a brittle star is regenerating, the slower the growth will be, due to increased energy expenditure." However, Zeleny (1903) in his study of *Ophioglypha lacertosa*, found that "the greater the number of removed arms (excepting the case where all are removed) the greater is the rate of regeneration of each arm." Thus, I suspect that the simultaneous regeneration of arms in *A. stygobita* did not contribute to the slow rate.

A sixth factor is the way lag phases were used in calculating regeneration rates. Clark et al., (2007) noted that *O. victoriae* had an unusually long "lag phase of up to 5 mo before reproducible amounts of new tissue are produced," so they only plotted regenerated arm lengths for months 5 to 12. The next longest lag phase found in the literature is "up to one month" for *Astrobrachion constrictum* at 10.5° to 14.5°C (Stewart, 1996). The lag phase for *A. stygobita* specimen R #2 was at least 26 days; the lag phase of specimen R #1 appeared to be less than 12 days. According to Clark et al., (2007) the lag phase in some species "can be minimal, often lasting 2-4 d." Thus, most regeneration studies (e.g., Chinn, 2006, Pomory

and Lawrence, 1999, and the current study) included lag phase days in calculations of regeneration rates. If Clark et al., (2007) had included lag phase days, the 0.44 mm/wk regeneration rate for *O. victoriae* would be reduced by 5/12 (= 43%) resulting in a rate of ~0.25 mm/wk; RRR (relative regeneration rate) would then be 0.25/20 mm disk size = 0.013 and nearly as low as the RRR calculated for *A. stygobita* at <0.01. Of course, another major factor in my calculations of RRR is disk size itself, and for this I used approximations from the literature rather than actual disk diameters because most studies (including Clark et al., 2007) did not state disk sizes of their regenerating specimens.

To summarize this section on regeneration, the regeneration rate of *A. stygobita* is one of the slowest ever observed. The most likely contributing factors include: (1) inherently slow metabolism related to cave endemism, (2) feeding on detritus as a poor quality food, (3) reduced salinity and calcium availability, and (4) relatively short sections of arms regenerating.

CONCLUSIONS

Bernier Cave's unusual features of brackish water, low tidal fluctuations, well-lit entrances open to the surface, and a substrate with abundant detritus provide a favorable environment for cave brittle stars and other organisms. Cave isopods and mangrove rivulus do not appear to be significant predators of *A. stygobita*. Special culture techniques are required for cave brittle stars because of restricted food requirements and susceptibility to light, salinity changes, and elevated temperatures. The statement that *A. stygobita* is a cave endemic is supported by its surprising array of troglomorphic traits including: no body pigment, muted alarm response to light, reduced body size, elongated arm segments, raised skin possibly for enhanced chemoreception and possibly for DOM absorption, reduced aggregation, slow movement, and extremely slow regeneration. Bernier Cave and its rare inhabitant, *Amphicutis stygobita*, deserve much more study by researchers. Hopefully, this special cave will be protected

from class tours and projects that could be detrimental to its unique cave brittle stars.

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