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EXTREME PHENOTYPIC VARIATION AMONG CRYPTIC CARIDEAN SHRIMP FROM SAN SALVADOR ISLAND, BAHAMAS.

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ABSTRACT

Extensive phenotypic variation is documented in specimens of cryptic cave shrimp collected on San Salvador Island, Bahamas in June of 2012. Sixty two of the 70 individuals exhibited such immense variation, including characters not previously described to *Barbouria cubensis*, *Barbouria yanezi*, *Parhippolyte sterreri*, or any member of the family Barbouriidae, that identification using a traditional morphological approach proved impossible. Examples of such characters include terminal eyestalk spines, gross spination of the telson and the presence of sensory dorsal organs on the carapace. The source and extent of morphological variation in these shrimp remains unclear, but may be due to environmental or genetic causes, or a combination of both. We note that rampant variation is observed in taxonomically important characters that were previously considered highly conserved and therefore were used to discriminate among species of shrimp. If these characters are highly variable then the taxonomic identity of shrimp in this group may need to be reconsidered.

INTRODUCTION

A common feature among many tropical islands in the Caribbean is the presence of seemingly disjointed tidal lakes and ponds. San Salvador (San Sal) is a small island in the Bahamian archipelago and nearly half of its land mass is covered by anchialine pools that range from brackish to hypersaline in nature (Mylroie and Carew, 1995). Subsurface tidal flows permit marine conditions to exist in many of the landlocked pools via seawater exchange through conduits created from

the dissolution of the underlying carbonate platform (Edwards, 1996; Mylroie and Carew, 1995). No study has successfully traced the lakes' connection to the surrounding ocean on San Sal. Our original objective was to compare species composition of cryptic caridean shrimp inhabiting the conduit mouths of anchialine pools and use this information to infer connections between lakes and the surrounding ocean.

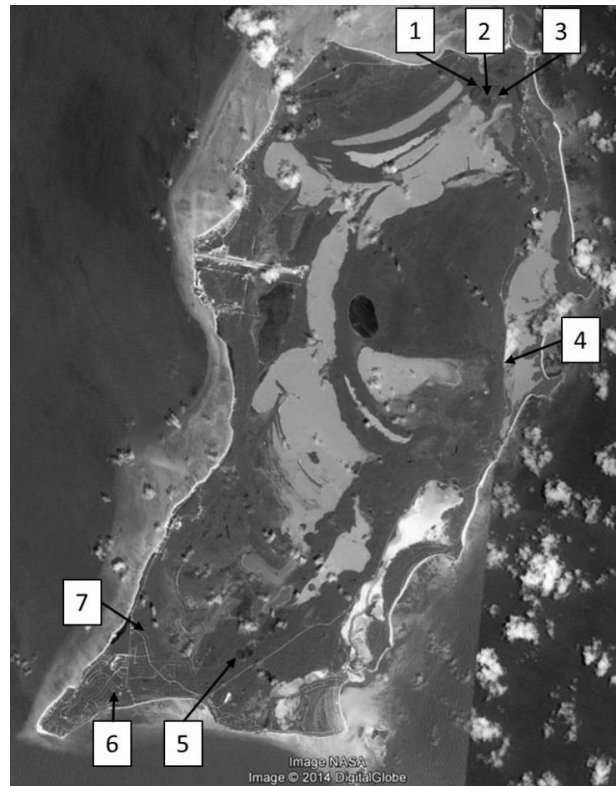


Figure 1. Map of San Salvador Island labeled with sites sampled in 2012; (1) Shrimp Holes, (2) Oyster Pond, (3) Wild Dilly Pond, (4) Fortune Hill Plantation Pond, (5) Mermaid Pond, (6) Watlings Blue Hole and (7) Blue Hole #5 (a.k.a. Double Decker Blue Hole).

It is not unusual for multiple species of cryptic carideans to be found inhabiting the same lake on other Caribbean islands (Wicksten, 1996; De Grave et al., 2009). Because these shrimp have planktonic larvae, inhabit conduits with tidal flow, and occur in multiple lakes, these shrimp are excellent candidates to examine possible interconnectivity among lakes (Manning and Hart, 1984; Bohonak, 1999).

In order to infer connections using anchialine shrimp, the species composition of shrimp on the island must first be identified. Only one species (*Barbouria cubensis*) has been previously recorded from San Sal (Hobbs, 1978). During 2011, another species (*Parhippolyte sterreri*) was collected from Mermaid Pond (Ditter and Erdman, personal observations). Both, *P. sterreri* and *B. cubensis* are listed as critically endangered on the IUCN Red List (Iliffe, 1996). A second species of *Barbouria* (*B. yanezi*) that is thought to be synonymous with *B. cubensis* (S. De Grave, R. Bauer and D. Felder, personal communication with Ditter) was described from Cozumel, Mexico in 2008 (Mejía et al., 2008) and is thought to occur only on the Yucatan Peninsula.

Typically, decapods can be identified based on morphology. We assumed we would be able to identify species using morphology and would require DNA to identify only unusual cases. However, we found such extensive phenotypic variation among specimens, species level identifications based on traditional methods proved impossible. Therefore, the objective of this study became documenting the extreme morphological variation ex-

hibited by specimens collected from anchialine pools on San Sal.

METHODS

Specimen Collection

During June 2012, 70 shrimp were collected from seven anchialine ponds and blue holes on San Sal (Figure 1). Samples were collected with hand nets or by baited minnow traps. Shrimp were transported to the Gerace Research Center (GRC) and maintained in running seawater until preservation. All specimens were preserved in 95-100% ETOH and transported to Florida Gulf Coast University (FGCU).

Morphological Identification

We examined 13 morphological characters (Figure 2) commonly used to discriminate between members of the family Barbouriidae (Chace and Manning, 1972; Hobbs, 1978; Hart and Manning, 1981; Manning and Hart, 1984; Wicksten, 1996; Mejía et al., 2008) using a Leica compound dissecting microscope equipped with a Lumenera INFINITY_x camera. INFINITY Analysis software was used to capture images and take measurements of morphological characters. Morphological characters that would require dissection were not examined to prevent damage to specimens (e.g. maxillipeds or the appendix masculina).

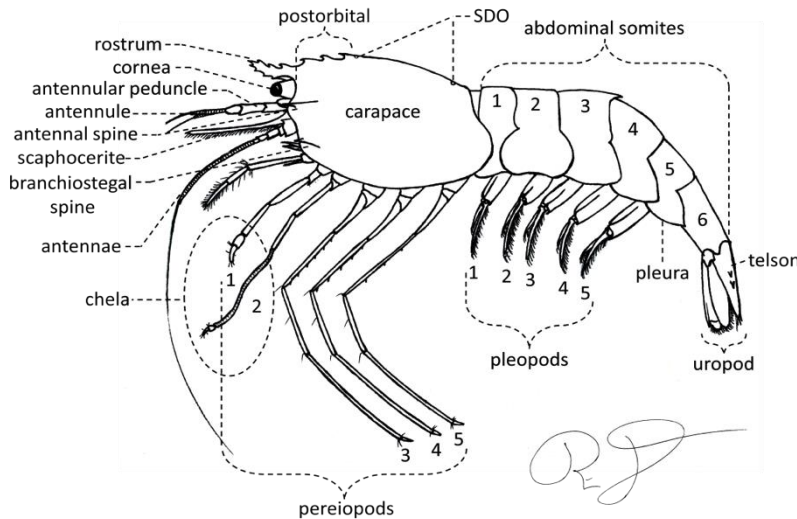


Figure 2. Morphological characters examined: (1) cornea width relative to eyestalk width; (2) rostrum length relative to antennular peduncle, (3) number of pre- and post-orbital dorsal and (4) ventral spines on rostrum; (5) terminal shape of telson, (6) number of dorsal and (7) terminal telson spines; (8) scaphocerite length to width ratio; (9) carapace spines, (10) carapace texture and sensory dorsal organs; (11) shape and (12) spines of pleura of the 4th & 5th pleomere; (13) 1st & 2nd pereopods chelate.

Table 1. Common morphological characters used to discriminate among *Barbouriidae* spp. separated by expected features for *B. cubensis* and *P. sterreri*, and the range of observed variation. Numbering in Character column corresponds to Figure 2 Legend.

Structure	Characters	Typical <i>Barbouria cubensis</i>	Typical <i>Parhippolyte sterreri</i>	Maximum Observed Range	Minimum Observed Range
Characters used to discriminate between Barbouriidae species					
Eyes	(1) cornea width relative to eyestalk width	cornea narrower than stalk	cornea wider than stalk	cornea width assymetrical (1 wider & 1 narrower)	cornea greatly reduced, terminal spine present
	(2) rostrum length relative to antennal peduncle	does not extend beyond 2 nd antennal segment	barely reaching 2 nd antennal segment	reaching 3 rd antennal segment	not reaching past eyestalk
	(3) number of dorsal rostrum spines (postorbital)	5-6 (3-4)	3-4 (1-2)	13 (8)	1 (0)
	(4) number of ventral rostrum spines	1-7	4-5	8	0
Telson	(5) terminal shape of telson	rounded	pointed	terminal spine present	unassignable shape
	(6) number of dorsal spines on telson (pairs)	4 (2)	4 (2)	13 (3)	0 (0)
	(7) number of distal spines on telson(pairs)	6 (3)	6 (3)	15 (7)	2 (1)
Scaphocerite	(8) length vs. width	2.9x as long as wide	3x as long as wide	3.5x as long as wide	2.7x as long as wide
Characters common between members of the family Barbouriidae					
Carapace	(9) carapace texture, and presence of SDO	carapace smooth, no SDO mentioned	carapace smooth, no SDO mentioned	not smooth with 4 sensory dorsal organs present	smooth with only single anterior SDO present
	(10) only antennal & branchiostegal spines present	antennal & branchiostegal spines present	antennal & branchiostegal spines present	extra antennal & branchiostegal spine present	antennal & branchiostegal spines absent
Pleura	(11) relative shape of 4th and 5th pleura	4th & 5th acute angle	4th & 5th acute angle	4th & 5th rounded	4th & 5th low angle
	(12) armorment of 4th and 5th pleura	4th unarmed 5th armed	4th unarmed 5th armed	both armed	both unarmed or malformed
Pereiopods	(13) 1st and 2nd pereiopods chelate	chelae present	chelae present	chelae present	chelae present

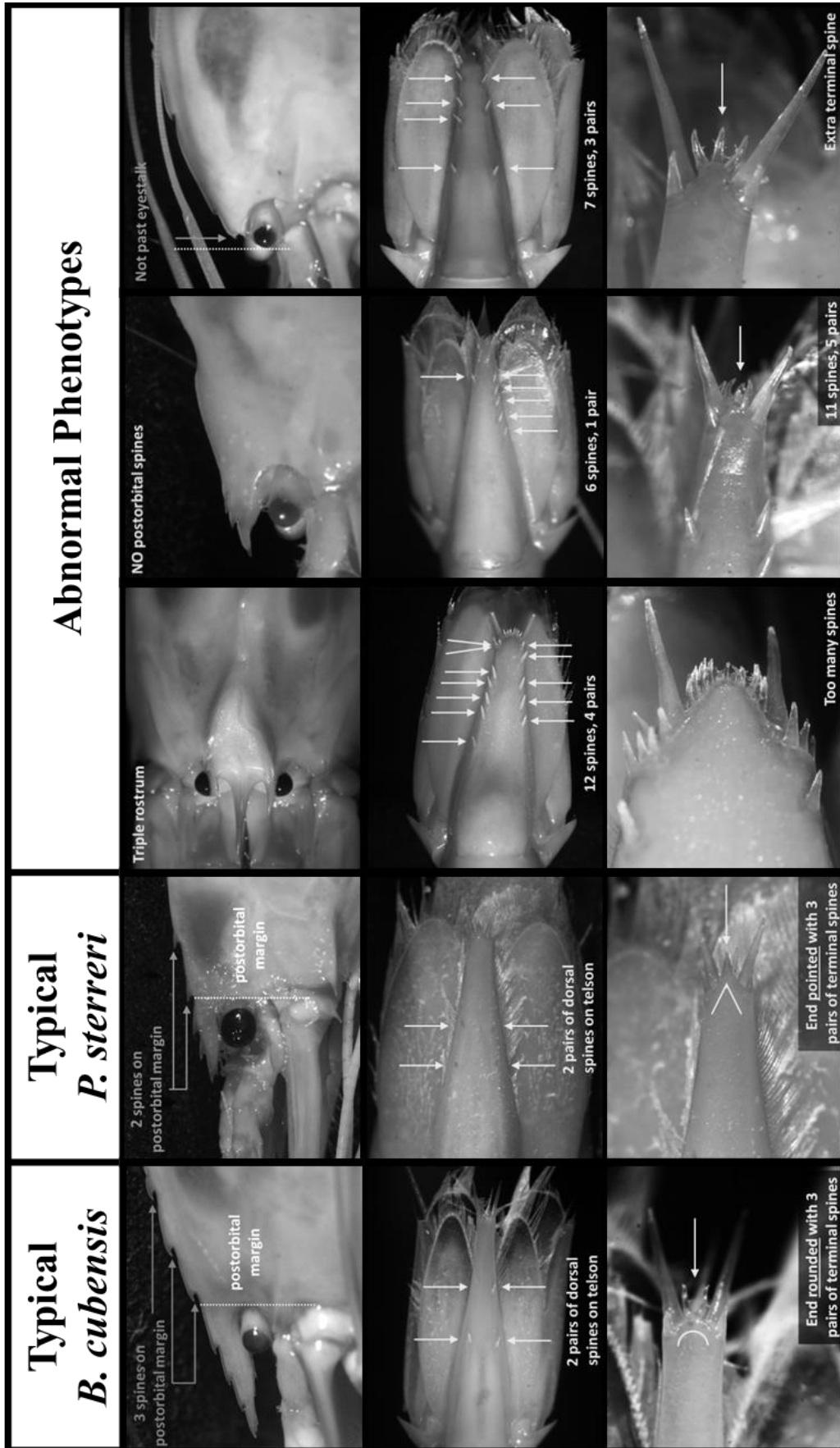


Figure 3. Expected phenotypes for *B. cubensis* and *P. sterreri* compared to examples of observed abnormal phenotypes; Top Row: spines on rostrum and rostrum length, Middle Row: number and pairing of dorsal spines on telson, Bottom Row: number of terminal spines and terminal shape of telson.

RESULTS

Based on 13 morphological characters (Table 1), only six shrimp were identified as *Barbouria cubensis* and two were identified as *Parhippolyte sterreri*. The species identity of the remaining 62 shrimp could not be positively identified based on morphological characters due to immense phenotypic variation (Figure 3). The variation exhibited by these shrimp often fell outside of characters prescribed to either genus (*Barbouria* or *Parhippolyte*). Extreme variation was common among all lakes with no discernable distribution pattern.

Eyes

Just over 34% of specimens had eyes that varied from the description of the eyes of *B. cubensis*. All specimens had some form of pigmented eyes. The cornea width of most individuals was typically narrower than the eyestalk, but 24 individuals had cornea that were equal to or larger than the width of the eyestalk, had malformed cornea, were asymmetrical in width or the cornea was greatly reduced and a single terminal spine was present on the eyestalk (Figure 4).

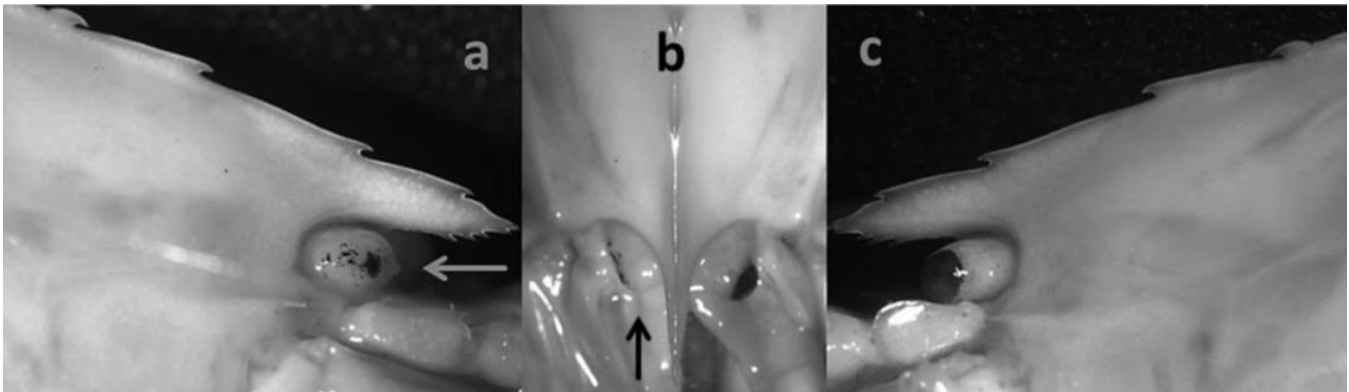


Figure 4. Example of asymmetry in right and left cornea and eyestalk morphology; a) right lateral cornea severely reduced and eyestalk with terminal spine present (indicated by arrows), b) dorsal view of eyes, c) left lateral cornea narrower than eyestalk width, eyestalk spine absent.

Rostrum

Nearly 74% of individuals had rostrums that did not match the prescribed character states for *B. cubensis* or *P. sterreri*. The number of rostral spines exhibited greater variation than has been previously recorded (Table 1). The rostrum did typically reach the second article of the antennular peduncle. Twenty one specimens had rostrums reaching the middle of the second article, while 22 specimens had rostrums reaching, or just reaching the second article and five specimens had rostrums reaching or nearly reaching the third article. Sixteen specimens had rostrums not reaching past the first article and the remaining six had rostrums that were either broken or not

nearly reaching the distal edge of the second penduncular segment.

Telson

Based on the prescribed characters, 67% of individuals could not be assigned as matching the telson of *B. cubensis* or *P. sterreri*. The shape of the posterior margin of the telson was highly variable; 24 specimens were described as having a rounded posterior margin, 24 were described as having a pointed posterior margin and the remaining 22 were intermediate in shape and could not be assigned. The number telson spines and terminal shape exhibited the greatest range of variation among morphological characters (Table 1).

Carapace

Only 13% of specimens had carapace spines that did not match that of *B. cubensis* and *P. sterreri*. Sixty one specimens had antennal and branchiostegal spines, while nine individuals were missing one or more spines that did not appear to be the result of breakage. The carapace of most specimens was smooth with the exception of small sensory dorsal organs (SDO) situated along the medial line of the cephalothorax (Figure 5). The anterior SDO was found associated with the distal postorbital rostral spine and posterior SDOs were found within the cardiac notch (Lerosey-Aubril and Meyer, 2013).

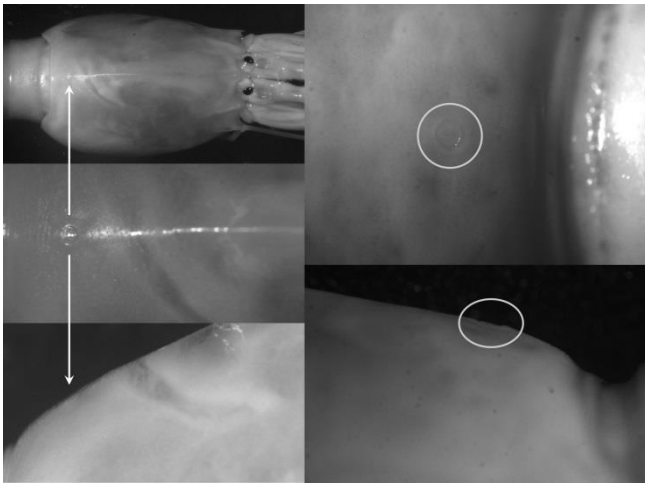


Figure 5. Representative examples of posterior sensory dorsal organ present on the post-dorsal margin of the cephalothorax from two specimens.

Pleura of the Abdominal Somites

In the majority of specimens the pleura of the third and fourth somites were unarmed (without posterolateral tooth), whereas the fifth and sixth were armed (with posterolateral tooth). In some instances only the sixth somite was armed or the presence of spines was asymmetrical. Additionally, the shape of the 4th and 5th pleura was highly variable and could most often be described as acute. However, it was not uncommon for the shape of the 4th pleura to be described as obtuse or round.

DISCUSSION

Many of the shrimp examined in this study exhibited characters synonymous with *P. sterreri*, *B. cubensis* or *B. yanezi*, and that are not described as being shared by the three species (i.e. terminal shape of telson or rostrum length). Additionally, some specimens exhibited morphological characters not present in any shrimp within the family Barbouriidae (Figures 3 and 5). This suggests that some characters which were considered to be highly conserved in taxonomic classification of caridean shrimp actually may not be conserved. If these characters are variable within and among multiple species then the classification of other species, based on these characters, should be reexamined.

Other characters found to be highly variable include; the number of spines on the appendix masculina of the 2nd pleopod, spination of the exopod of the uropod, carapace shape and the presence of SDOs on the dorsal margin of the telson. Documentation of variation in these characters is in progress. The spination of appendix masculina is the most important of the unreported characters as it may be the key to discriminating between species in the presence of so much other variation, and may lead to synonymizing *B. cubensis* and *B. yanezi* (Karge et al., 2013).

The source of variation in these shrimp is unclear and examples of phenotypic variation are abundant in the animal kingdom. This variation may be due to inherent phenotypic plasticity (DeWitt et al., 2000; Brian et al., 2006; Lardies and Bozinovic, 2008) although extensive phenotypic plasticity has not been previously reported in these shrimp. Other possible non-genetic based sources of extensive variation could be due to physical trauma during early development or during molting (Giménez, 2006, Vogt et al., 2008), damage due to parasitic infection (Goodman and Johnson, 2011), or environmental factors such as salinity and temperature (Ituarte et al., 2007; Reuschel and Schubart, 2007). If the source of variation has a non-genetic basis, then further study will be required to identify the source and implications for the conservation of these species in the Caribbean.

Alternately, the source of variation may have a genetic basis, such as severe inbreeding (Creasey et al., 2000; Fumagalli et al., 2002), the presence of hybrid swarms (Wolf and Mort, 1986; Perry et al., 2002), incipient speciation (Dawson, 2005; Malay and Pauley, 2009), cryptic speciation (Steinauer et al., 2007), or sympatric speciation (Turner et al., 2008). If the source of variation is due to severe inbreeding, then management efforts may be required to restore genetic diversity to these critically endangered populations. If the variation is due to factors associated with speciation, then it is likely a result of isolation or intense predation. If speciation is related to isolation, it may be possible to infer geologic timelines using genetic information. Furthermore, if speciation is a result of predation, it will be necessary to gain a better understanding of interactions among anchialine organisms.

In order to accurately identify these shrimp and identify the source of variation the use of molecular tools will be required. As these are critically endangered due to their unique cryptic habitats (Manning and Hart, 1984; Iliffe, 1996), a nondestructive tissue sampling technique has been developed using the common pink shrimp *Farfantepenaeus duorarum* as a model organism (Ditter et al., 2013).

If the extreme variation observed here has a genetic basis, then taxonomic revisions will be necessary. The phenotypic variation observed in these shrimp may be enough to support the reconsideration of the taxonomic classification of the genus *Barbouria* as being monophyletic. Additionally, these shrimp may represent previously unrecorded species. We anticipate that further molecular and morphological studies will identify the underlying cause of variation which may be due to a complex combination of genetic and non-genetic sources.

Although we had hoped to identify the species and their distribution among the supposedly connected lakes on San Sal, we were not able to do so with confidence in most cases. Instead we found abundant morphological variation. We plan to proceed with the two (possibly three) known species that could be identified with morphology, and concurrently use a combined molec-

ular and morphological approach to identify any new species and the role that isolation and gene flow through interconnected lakes may have played in their speciation.

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