Phenotypic hyper-variation, genetic species identification and population connectivity of enigmatic cave shrimp from San Salvador Island, Bahamas

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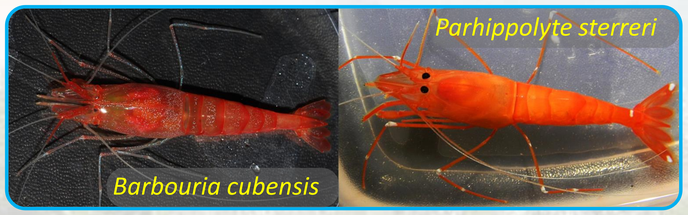
Introduction

San Salvador (San Sal) is a small karst island in the Bahamian Archipelago (Fig. 1). Nearly half of its land mass is covered by anchialine pools that range from brackish to hypersaline in nature[1]. These pools are believed to be isolated and act as ecological "islands within an island"[2,3]. Subterranean tidal flows greatly impact conditions in many of the pools via seawater exchange through conduits created by dissolution of the carbonate platform[1]. This landscape is rather unique to tropical islands, while many anchialine organisms are well documented, no study has successfully traced these subterranean aquatic connections on San Sal.

Among anchialine pool inhabitants is a group of enigmatic caridean shrimp that live in the caves and conduits[4,5]. Due to limited habitat availability, most anchialine species are found on the IUCN's Red List. Only *Barbouria cubensis* had been previously recorded from San Sal[6], but in 2011 *Parhippolyte sterreri* was discovered on the island[7] (Fig. 1). The occurrence of "red shrimp", with planktonic larvae, in many of the anchialine pools on San Sal makes them an excellent candidate to examine possible interconnectivity among anchialine pools[8,9,10].

Morphological examination of shrimp tentatively prescribed to *B. cubensis* reveals phenotypic hyper-variation (PhYV) among individuals, which confounds species identification. In many instances, individuals exhibit characters prescribed either to *P. sterreri* or not to the family Barbouriidae (*i.e.*, eyestalk spines). To clarify species identity and genetic connectivity among anchialine pools we used morphological and phylogenetic methods.



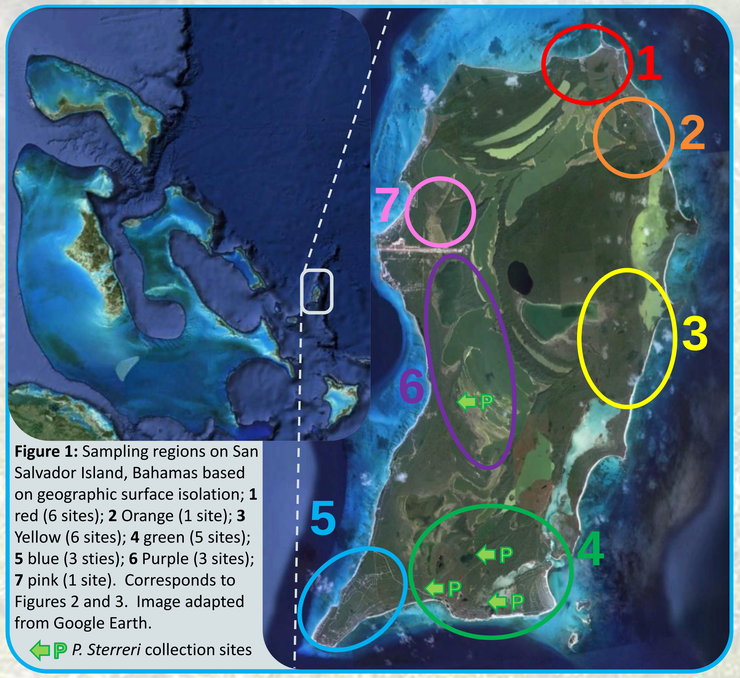


Figure 1: Sampling regions on San Salvador Island, Bahamas based on geographic surface isolation; **1** red (6 sites); **2** Orange (1 site); **3** Yellow (6 sites); **4** green (5 sites); **5** blue (3 sties); **6** Purple (3 sites); **7** pink (1 site). Corresponds to Figures 2 and 3. Image adapted from Google Earth.

P *P. Sterreri* collection sites

Objectives

1. Infer connectivity among anchialine pools based on phylogeny
2. Document phenotypic hyper-variation (PhYV) among shrimp across San Sal
3. Identify species using morphological and molecular tools
4. Examine population structure of *Barbouria cubensis* on San Sal

Methods/Results

Based on examinations of 226 shrimp collected between 2011-2014 from 25 anchialine pools on San Sal. It was found that ~90% of specimens could not be Identified to the species level, and ~10% could not be identified to the family level due to the presence of PhYV (Fig. 2). No evident pattern in PhYV was detected, as shrimp exhibiting PhYV are distributed among all regions sampled (Fig. 1).

Phylogenetic analysis of DNA sequences from two mitochondrial (16S & COI) and two nuclear (28S & PEPCK) gene regions amplified from 54 individuals (San Sal = 48; Mayaguana = 2, The Yucatan = 4) reveals that all of the shrimp examined are *B. cubensis* (Fig. 3), despite the presence of the PhYV. Regardless of the geographic barriers that occur, our analyses suggest that no population structure exists in these shrimp on San Sal. Additionally, our findings show a lack of genetic diversity within this population or between San Sal and Mayaguana and The Yucatan, suggesting gene flow is likely occurring among these populations.

Morphological Comparisons

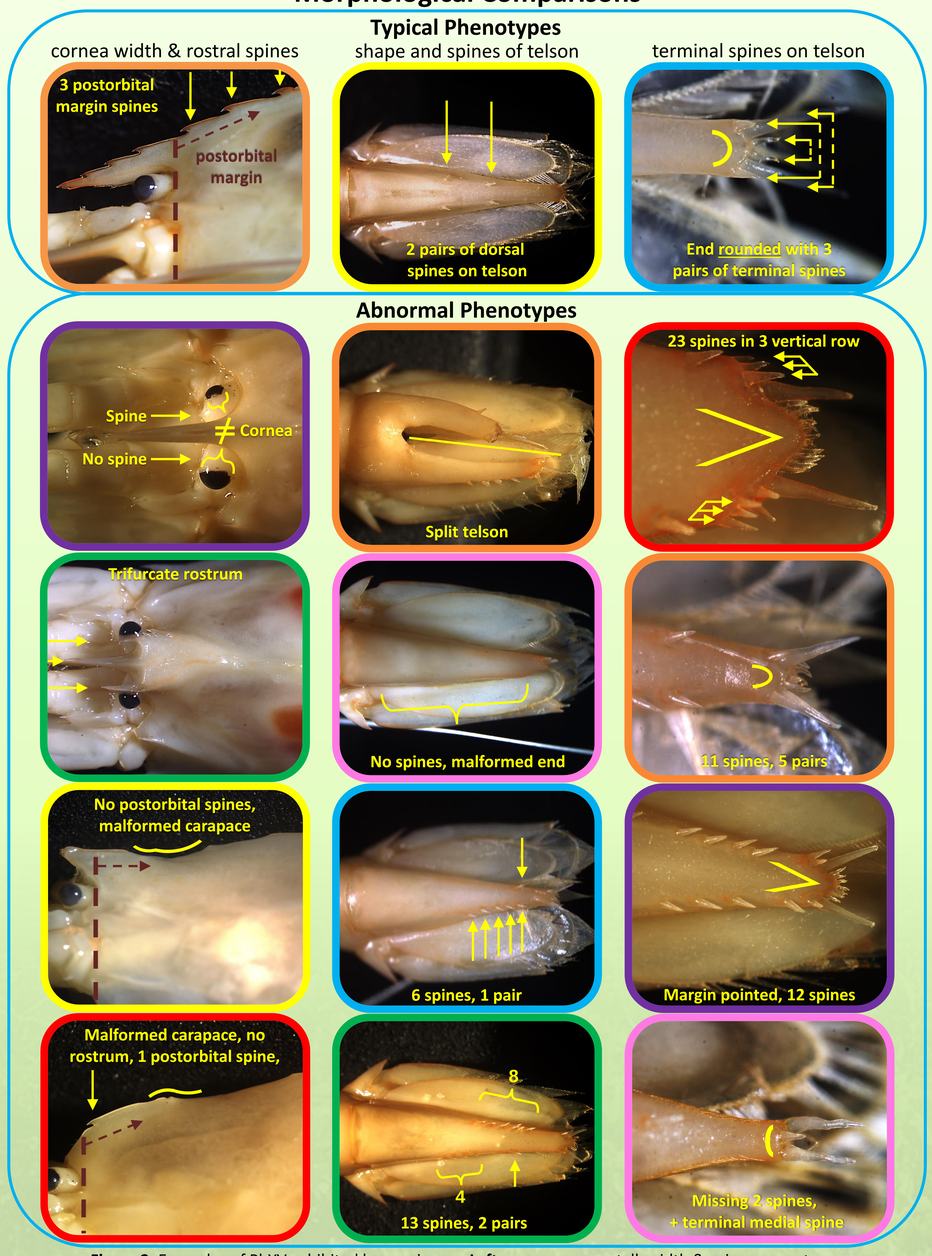


Figure 2: Examples of PhYV exhibited by specimens; **Left**: cornea vs. eyestalk width & spines on rostrum, **Center**: shape & spines of telson, **Right**: terminal spines on telson. Image outlines correspond to regions in Figure 1.

Discussion/Conclusion

When this study was conceived, it was assumed that species identification would be possible based on comparative morphology. However, due to the PhYV present among specimens collected, identifications based on traditional methods proved impossible (Fig. 2). Therefore, the use of molecular tools was employed to accurately identify the shrimp to the species level. Possible sources of the observed PhYV are; severe inbreeding, environmental toxins, damage from parasitic infection, hybridization, physical trauma during early development. This wide range of possibilities lends support for the use of next-generation sequencing (NGS) to examine these enigmatic populations. It is also necessary to sample other western Atlantic populations to determine how widespread PhYV is.

Our findings rule out incipient speciation, but it important to note that *B. cubensis* from San Sal, Mayaguana and The Yucatan are genetically identical, suggesting that gene flow is occurring. Because *B. cubensis* is critically endangered it is important for us to examine these connections with greater resolution through the use of NGS (*i.e.*, RADseq). It is imperative for us to further sample *B. cubensis* throughout the western Atlantic, due to rapid human development and the introduction of exotics species in these areas, which may destroy entire populations and create novel barriers to gene flow.

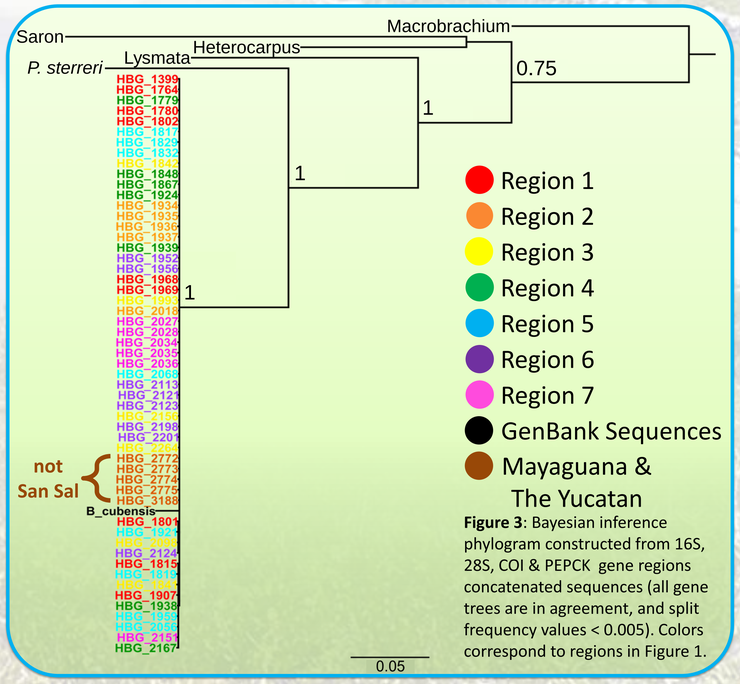


Figure 3: Bayesian inference phylogram constructed from 16S, 28S, COI & PEPCK gene regions concatenated sequences (all gene trees are in agreement, and split frequency values < 0.005). Colors correspond to regions in Figure 1.

Acknowledgements

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