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Widespread phenotypic hypervariation in the enigmatic anchialine shrimp *Barbouria cubensis* (Decapoda: Barbouriidae)

ROBERT E. DITTER[1,3], ROBERT B. ERDMAN[2],
ANNA M. GOEBEL[2] & HEATHER D. BRACKEN-GRISSOM[1]
[1]3000 NE 151st St., MSB 330, North Miami, FL 33181. E-mail: hbracken@fiu.edu
[2]10501 FGCU Blvd. South, WH 118 Fort Myers, FL 33965. E-mail: agoebel@fgcu.edu, rerdman@fgcu.edu
[3]Corresponding author. E-mail: rditt003@fiu.edu

Abstract

Classification and evolutionary relationships among anchialine shrimp from the family Barbouriidae Christoffersen, 1987, has long been a topic of debate amongst crustacean taxonomists. To date, no study has examined morphological or molecular variation among populations of these enigmatic shrimp. The present study documents and analyzes patterns of widespread morphological variation within populations of *Barbouria cubensis* von Martens, 1872, from anchialine pools on three Bahamian islands. Such extensive morphological variation confounds identification using classic taxonomical methods. Phenotypic variation is by no means a new topic, but studies of decapods are typically limited to isolated individuals or few morphological characters. Moreover, past studies of *B. cubensis* do not report extensive morphological variation, however we find that upwards of 90% of individuals are affected. Anomalous phenotypes are described in 54 morphological characters with no detectable pattern associated with geographic distribution. The term phenotypic hypervariation (PhyV) is used to describe morphological variation that greatly deviates from any previous taxonomic descriptions. Analysis of partial sequences of the 16S and COI mitochondrial genes confirm the identity of morphologically variable specimens as *B. cubensis* without population structure across the tropical western Atlantic. A test for cryptic diversity within *B. cubensis* suggests PhyV is not correlated with cryptic diversity. Morphological variation at this scale likely depends on recent changes either to their environment or genetic diversity.

Key words: molecular barcoding, taxonomy, cave shrimp, Bahamas, Atlantic, phylogeny, barbouriid species

Introduction

A common feature among many tropical islands in the tropical western Atlantic is the presence of seemingly disjointed anchialine pools, which are subterranean estuaries influenced by tidal flow (Stock, 1986; Bishop *et al*., 2015; Pérez-Moreno *et al*., 2016). Subsurface tidal flows can be strong enough to permit marine conditions in the landlocked pools via seawater exchange through conduits created by the dissolution of the underlying carbonate platform (Edwards, 1996; Mylroie & Carew, 2003). However, biological connectivity and diversity is largely restricted, which has led to the "ecological islands within islands" hypothesis (Edwards, 1996; Pérez-Moreno *et al*., 2016). Most physical connections between anchialine pools and the surrounding ocean remain unexplored and form extensive spatially complex subterranean networks (Becking *et al*., 2011; Mylroie & Mylroie, 2011; Bishop *et al*., 2015). The biota found within these pools is often rare and endemic, with crustaceans representing over 80% of animal biodiversity in these systems (Iliffe, 2000; Iliffe, 2005; Sket, 2005; Iliffe; 2009). Among anchialine pool inhabitants is a group of enigmatic caridean shrimp from the family Barbouriidae that live within the caves and pools (Manning & Hart, 1984; De Grave *et al*., 2009).

Barbouriid shrimps are described as having a tethyan distribution pattern along tropical latitudes, mostly confined to land locked pools (Manning & Hart, 1984). The family Barbouriidae Rafineque, 1815 is comprised of *Barbouria* Rathbun, 1912, *Parhippolyte* Borradaile, 1900, *Janicea* Manning & Hart, 1984 and *Calliasmata* Holthuis, 1973, which contain a total of 11 species. Until the recent reclassification of *Calliasmata* (De Grave *et al*., 2014), Barbouriidae was united by the presence of a unique subocular tooth on the carapace (Manning & Hart, 1984; Clark,

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1989; Chace, 1997). Additionally, these shrimps are described as having a mandible with a 3-jointed palp lacking incisor process, long slender pereiopods, the first two pairs chelate with a multiarticulated carpus and merus, and an appendix masculina shorter or subequal to the length of the appendix interna on the endopod of the 2nd pleopod in males (Chace, 1972; Manning & Hart 1984; Christoffersen, 1987). Characters used to discriminate among members of Barbouriidae include; width of the cornea relative to the eyestalk, ratio of the length versus the width of the scaphocerite, teeth of the rostrum, arrangement of arthrobranchs and podobranchs, subdivision of the articles of pereiopods three to five, ratio of the rostrum length versus height, number of setae of the appendix masculina, and shape of the terminal margin of the telson (Chace, 1972; Manning & Hart, 1984; Wicksten, 1996; Mejía *et al*., 2008).

Barbouria cubensis is a common species found within anchialine pools throughout the tropical western Atlantic ranging from Bermuda, the Bahamas, Turks and Caicos, Cayman Brac and the Yucatán Peninsula of Mexico (Mejía *et al*., 2008). Species within the genus *Barbouria* can most notably be identified by the presence of darkly pigmented cornea that are narrower than the eyestalk, and the lack of subdivision of the articles of pereiopods three to five (Fig. 1) (Chace, 1972; Manning & Hart 1984). Previous records have documented minor morphological variation for *B. cubensis* but are limited to the length and teeth of the rostrum, ratio of length versus width of the scaphocerite, number of subdivisions of the articles of the second pereiopod, length and spination of the appendix masculina and the body size (Rathbun, 1912; Holthuis, 1963; Chace, 1972; Hobbs *et al*., 1977; Hobbs, 1978; Hart & Manning, 1981; Manning & Hart, 1984; Mejía *et al*., 2008). However, recent examination of *B. cubensis* from anchialine pools on the Bahamian islands of Abaco, Eleuthra and San Salvador from 2012–2015 has revealed a wide range of morphological variation in these previously documented characters (Rathbun, 1912; Holthuis, 1963; Chace, 1972; Hobbs *et al*., 1977; Hobbs, 1978; Hart & Manning, 1981; Manning & Hart, 1984; Mejía *et al*., 2008), but also extends to an additional 50 morphological characters. For the purposes of this manuscript, we use the term phenotypic hypervariation (PhyV) to describe the morphological variation that deviates from previous taxonomic descriptions of *B. cubensis* (Rathbun, 1912; Holthuis, 1963; Chace, 1972; Hobbs *et al*., 1977; Hobbs, 1978; Hart & Manning, 1981; Manning & Hart, 1984; Mejía *et al*., 2008). To have a comparative framework for describing variation, we have defined "normal" to include all previous descriptions of morphological variation.

Molecular methods can be used to delimitate species, especially when the use of traditional taxonomic methods is not possible (Monaghan *et al*., 2005; Clare *et al*., 2007; Raupach *et al*., 2010). This can be particularly useful for *B. cubensis* as PhyV confounds traditional methods of identification. In recent years, several studies have shown the benefit of using molecular methods when cryptic species complexes exist with extensive phenotypic variation (Lefébure *et al*., 2007; Steinauer *et al.*, 2007; Trontelj *et al*., 2009; Neiber *et al*., 2012; Weese *et al*., 2012; Cornils & Held, 2014). With molecular barcoding it is possible to identify specimens exhibiting PhyV and determine if PhyV is the result of cryptic diversification.

The objective of this study is to document PhyV exhibited by *B. cubensis* collected from three Bahamian islands, and to determine if PhyV is associated with geographic distribution or cryptic genetic diversity. Timely documentation of this species is imperative, as rapid human development, pollution and continued natural disturbances (i.e. hurricanes) of tropical coastal areas is a formidable threat to these critically endangered animals. Fundamental studies of biodiversity are still needed to adequately protect endemic anchialine organisms.

Methods

Sample Collection. Between June of 2012 and July 2015, 363 shrimps were collected from 25 anchialine pools on San Salvador, Bahamas (Fig. 2a). Suitable habitats were identified from previous records, Google Earth and information provided by island residents. Samples from this island were collected with hand nets or by baited minnow trap. For sites where conduits could not be located from land, snorkeling was utilized to locate the conduits and place minnow traps. In addition to the collection on San Salvador, 41 specimens from Abaco were donated by Dr. Craig Layman from three anchialine pools to the south of Little Harbour (Fig. 2b). Ninety-five specimens from Eleuthera were donated by Dr. Jocelyn Curtis-Quick from six anchialine pools distributed across the entire island (Fig. 2c). Tissue samples from Mayaguana, Bahamas and Mexican specimens were donated by Dr. Darryl L. Felder and Dr. Tom Iliffe respectively. A maximum of 30 shrimp were retained from each site per sampling effort. A total of 529 shrimp were examined for this study, however 463 were included in the final morphological analysis. Data

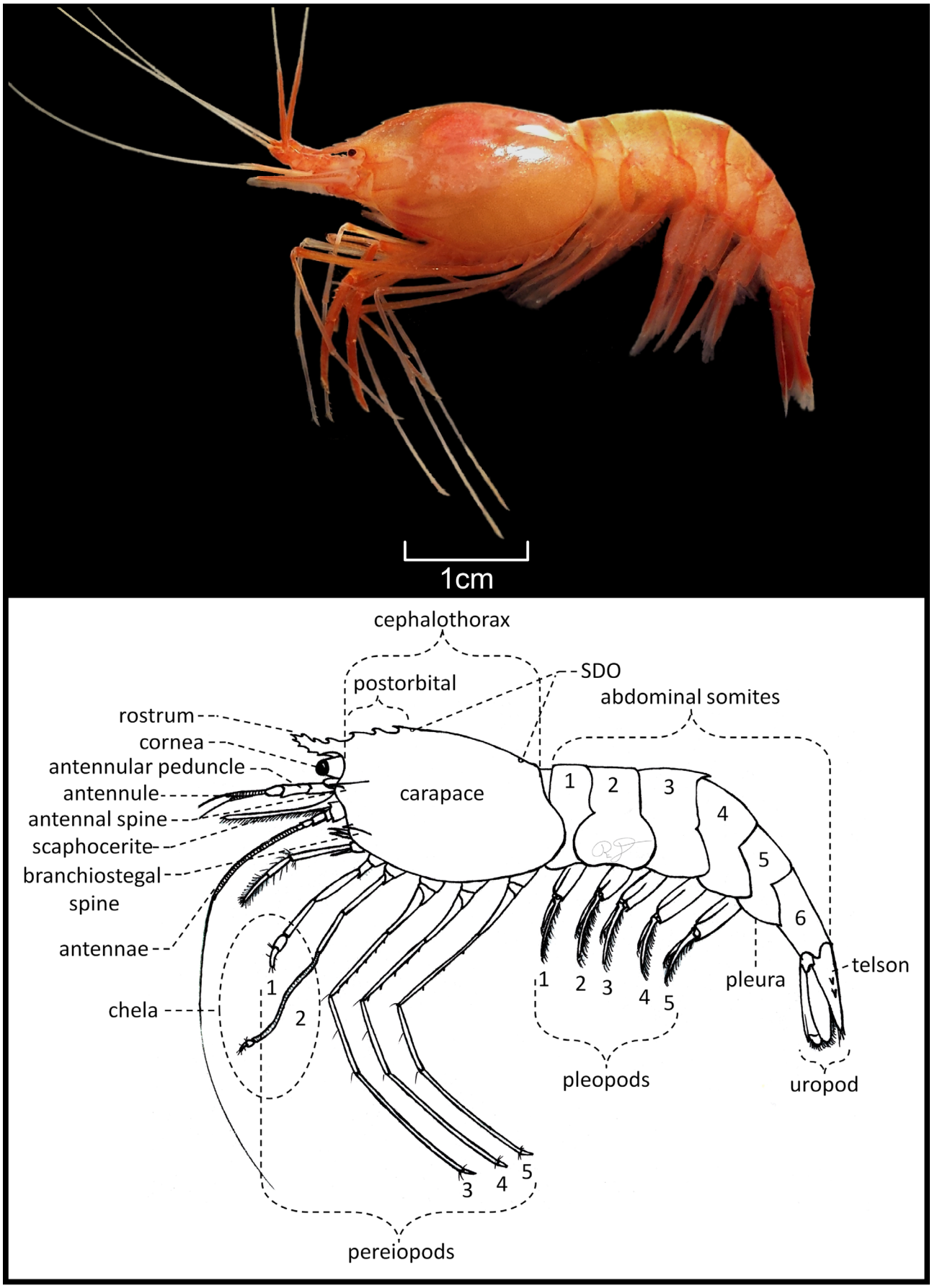

FIGURE 1. *Barbouria cubensis*, (**top**) left lateral view of live specimen (HBG1911), (**bottom**) schematic drawing by R.E. Ditter of general morphological characters within Caridea.

collected from 65 individuals determined to be juveniles, too heavily damaged or identified as *Parhippolyte sterreri* were excluded from analyses. Most individuals could not be identified to *Barbouria cubensis* based on taxonomic keys, however individuals were confirmed using DNA barcoding techniques. Specimens are deposited at the Oxford University Museum of Natural History Zoology Collection (OUMNH.ZC) and the Florida International Crustacean Collection (FICC). Specimens archived within the FICC are cataloged with the prefix HBG.

Morphological Data Collection. Fifty-four morphological characters were examined to document the extent of morphological variation in specimens collected from the Bahamas (see appendix, Tables AI–III). Initially, 14 morphological characters were chosen for examination because they represent common diagnostic characters in caridean taxonomy (Hobbs *et al*., 1977; Hobbs, 1978; Manning & Hart 1984; Chace, 1996; Bauer, 2004; De Grave *et al*., 2014). These include: three carapace characters, three rostral characters, two eye characters, three telson characters and three pereiopod characters (Fig. 2). The presence of gills and exopods of the maxillipeds and pereiopods were excluded in analyses because of inconsistency among previous records (Christoffersen, 1987; Wicksten, 1996). After PhyV was found during preliminary examinations of diagnostic characters, 13 additional characters believed to be conserved among carideans were included based on the advice of caridean taxonomic experts, Drs. Sammy de Grave and Raymond Bauer. These characters include five pereiopod characters, two pleopod characters and six mouthpart characters. Twenty-seven additional characters diagnostic to the family Barbouriidae *sensu lato* were examined to document the extent of family and species-specific morphological variations across individuals (Rathbun, 1912; Chace, 1972; Hart & Manning, 1981; Manning & Hart, 1984; de Grave *et al*., 2014). Lastly, the presence and position of sensory dorsal organs (SDO) were examined as they are believed to be present in some form in most crustaceans (Laverack *et al.*, 1996; Laverack & Macmillan, 1999; Lerosey-Aubril & Meyer, 2013). Although the ultrastructure and function of SDOs can only be examined with the use of scanning electron microscopy (SEM) and histology their presence and external aspects can easily be found and described (Brandt, 1988; Lerosey-Aubril & Meyer, 2013). This character was examined for its potential as a new diagnostic character for the family Barbouriidae due to the recent reclassification of the genus *Calliasmata*, which lacks characters considered as synapomorphies for barbouriid shrimps (de Grave *et al*., 2014).

Examinations were conducted using a Leica S8AP0 stereo microscope equipped with a Lumenera INFINITYx or a Leica MC170 HD camera (Leica Microsystems, Buffalo Grove, IL; Lumenera Corporation, Ottawa, Ontario, Canada). Over 4000 photographs were taken for 294 individuals of the 54 morphological characters as vouchers of expected phenotypes and characters exhibiting PhyV. INFINITY Analyze or Leica Acquire software packages were used to capture and analyze images (Lumenera Corporation, Ottawa, Ontario, Canada; Leica Microsystems, Buffalo Grove, IL;). Images of individuals and complex characters were rendered using Helicon Focus *ver*. 6.7.1Pro (Helicon Soft, Kharkiv, Ukraine).

Morphological Data Processing. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was conducted to determine if there is any pattern in the distribution of PhyV across Abaco, Eleuthera and San Salvador, Bahamas. For the purposes of this manuscript we define the expected phenotype of *B. cubensis* to include the combination of all previous morphological descriptions (Rathbun, 1912; Holthuis, 1963; Chace, 1972; Hobbs *et al*., 1977; Hobbs, 1978; Hart & Manning, 1981; Manning & Hart, 1984; Mejía *et al*., 2008). Qualitative characters (see appendix, Tables A1–3) were coded for analysis, as follows: character states matching previous descriptions of *B. cubensis* were coded as 1 and characters states not matching *B. cubensis* were coded as 2. These values were selected to prevent artificial bias. Subset #1 was generated to maximize the number of individuals included in analyses, using data for 25 characters that was collected for all 463 specimens (see appendix, Table A1). Subset #2 was generated to maximize the number of characters included in analyses, using data for 50 characters for 121 specimens (see appendix, Table A2). Subset #3 was generated to compare examine sexual dimorphism (see appendix, Table A3). All subsets included individuals from Abaco, Eleuthera and San Salvador, Bahamas. Data collected on the relative lengths of the segments of the antennule peduncle and pereiopods was excluded, because the diagnosed states are estimated measures. Data on the arrangement of the gill complement was deemed unreliable to establish the expected state for *B. cubensis* and was also excluded due to inconsistencies in previous records and the possibility of inaccuracy due to damage (Christoffersen, 1987; Wicksten, 1996).

Normality of data was tested using the Shapiro-Wilk statistics in R version 3.3.1 (R Core Team, 2016). Where necessary, values were normalized using log-transformation (Brian, *et al*., 2006). Values for each character were averaged by locality to avoid any bias resulting from the differences in samples size (Baltanás, *et al*., 2002). Individuals were grouped by Longitude. A UPGMA tree was constructed using Euclidean distance between rows with

1000 bootstraps in SPSS ver. 22 (IBM, Armonk, NY). Based on the results of the UPGMA, no further analyses were conducted.

Molecular Data Collection. To investigate the presence of cryptic speciation across the Bahamas, molecular data was collected from 69 specimens of *Barbouria cubensis*. Sampling localities on San Salvador Island were grouped into seven regions based on geographic distance and topographic features separating each region. Six individuals were randomly selected from each region, with the criteria individuals from each sampling site were included and that half of the individuals exhibit PhyV to ensure diversity. Two specimens were randomly selected from each sampling locality from Abaco and Eleuthera. Tissue was collected using non-destructive methods to preserve specimen integrity, and preserved in 95-100% EtOH and stored at -20°C. All tissue samples from Mayaguana, Bahamas and the Yucatán Peninsula of Mexico were included. Additional sequences were acquired from GenBank.

Total genomic DNA was extracted from muscle tissue of the abdomen, antennule or the 3rd to 5th pleopod using DNeasy® Blood and Tissue Kits (Qiagen, Valencia, CA). For incomplete tissue digestions, 10µl of 10% DTT and 10µl Proteinase K was added, and samples were incubated until complete digestion was achieved. Total genomic DNA quality was visualized using 2% agarose gels and concentration was measured using the Qubit dsDNA HS Assay kit on the Qubit 2.0 Fluorometer (Invitrogen, Life Technologies, CA) according to manufacturer's instructions.

Two partial mitochondrial genes were selected for their utility in decapod studies of identification (DNA barcoding), genetic diversity, and phylogeny (Bracken *et al*., 2009; Beaza, 2013; De Grave *et al.,* 2014; Aznar *et al*., 2015). The mitochondrial genes included the 16S large ribosomal subunit (~550 basepairs (bps)) and protein-coding cytochrome oxidase I (~600 bps, COI). The large ribosomal subunit (16S) was amplified with primers 16S-1471/1472 (Palumbi *et al.,* 1991; Crandall & Fitzpatrick 1996), COI was amplified with primers F/10, or LCO1490/HCO2198 (Folmer *et al.,* 1994; Bracken-Grissom *et al.,* 2014). Amplification was performed in 25µl volume reactions containing 12.5µl GoTaq DNA Polymerase (Promega, Madison, WI), 1µl forward and reverse primer for each gene, 9.5µl sterile H_2O and 1µl template DNA. The thermal cycling profile conformed to the following parameters: Initial denaturation for 5 min at 95°C followed by 35 cycles of 30 secs at 94°C, 45 secs at 48-56°C, 45 secs at 72°C and a final extension of 5 min at 72°C. PCR products were sent to GENEWIZ for amplicon purification and subsequent sequencing (South Plainfield, NJ,). All sequencing data was visually inspected, quality trimmed, manually cleaned and assembled using Geneious 9.1.7 (Biomatters Ltd., Newark, NJ). Once assembled, sequences were aligned using MAFFT v7.308 (Katoh & Standley, 2013). To identify potential pseudogenes, we translated protein-coding sequences and checked for insertions and deletions, stop codons and identified the open reading frames, and compared sequences among conspecifics following the protocol of Song *et al*. (2008).

Phylogenetic Analyses. Individual gene trees for 16S and COI were constructed alongside a concatenated dataset of 16S and COI sequences to investigate cryptic diversity in *Barbouria cubensis* (Table 1). Missing data were designated as a "?" in our alignment. To improve resolution, both genes were concatenated into a single alignment and partitioned by gene (Ahyong & O'Meally, 2004; Porter *et al.*, 2005; Robles *et al.*, 2007; Page *et al*., 2008). We conducted a partition test of heterogeneity and incongruence length difference test to determine if the gene regions were appropriate to combine for analyses, as implemented in PartitionFinder 2.7.1 and PAUP* respectively (Swofford, 2002; Lanfear *et al*., 2016). The model of evolution that best fit the individual data genes was determined by Partitionfinder 2.7.1. Independent models of evolution and parameters were partitioned in the Bayesian concatenated analysis.

The Maximum Likelihood (ML) analyses were conducted using RAxML 7.2.8 (Randomized Accelerated Maximum Likelihood, Stamatakis, 2014) with computations performed on the high-performance computing cluster at Florida International University. Likelihood settings followed the General Time Reversible Model (GTR) with a gamma distribution and RAxML estimated all free parameters. Confidence in the resulting topologies was assessed using Rapid Bootstrapping and a search for the best-scoring tree with 1000 replicates (Felsenstein, 1996). Bayesian Inference (BI) analyses were performed using parameters selected by PartitionFinder 2.7.1 and conducted in MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001). A MCMC algorithm ran for 10,000,000 generations, sampling 1 tree every 1000 generations. Observation of likelihood scores allowed us to determine burn-ins and stationary distributions. Once split frequency in the Bayesian analysis reached < 0.01 a 50% majority-rule consensus tree was obtained from the remaining trees. Posterior probabilities for clades were compared for congruence between analyses, Bootstrap values >70 for RAxML and >0.90 for Bayesian are presented on the phylograms.

TABLE 1. Common morphological characters used to discriminate among *Barbouriidae spp.* separated by prescribed character states for *Barbouria cubensis*, *B. yanezi*, *Parhippolyte sterreri* and *Janicea antiguensis*, and the most common, extremes and percentage of individuals exhibiting PhyV. * denotes percentages for which the character was examined for ≥ 121 individuals.

	Barbouria cubensis	*Barbouria yanezi*	*Parhippolyte sterreri*	*Janicea antiguensis*	*Barbouria cubensis sp.* **Examined**		
Carapace					**Most Common**	**Extremes**	**% PhyV**
antennal tooth	present	present	present	present	present	absent / bifurcate	16.4%
branchiostegal tooth	present	present	present	present	present	absent / 4 spines	7.3%
Rostrum	simple	simple	simple	simple	simple	absent / trifid	7.1%
length vs antennular peduncle	past 1st article	end of 2nd article	scarcely past 1st article	end of 1st article	medial 2nd article	absent / end of 3rd article	25.9%
total dorsal teeth	4–7	3–7	3–4	3–4	5 ± 1.29	0 / 15	13.5%
postorbital dorsal teeth	3–4	2–4	1–2	1–2	3 ± 0.86	0 / 10	30.2%
ventral teeth	1–7	3–9	4–5	1	3 ± 1.61	0 / 8	9.1%
Eye							
eyestalk spine/tubercle	absent	absent	absent	absent	absent	absent / present	18.5%
cornea pigmentation	darkly pigmented	darkly pigmented	darkly pigmented	darkly pigmented	darkly pigmented	unpigmented	6.9%
cornea width vs eyestalk	narrower	narrower	broader	broader	narrower	absent/broader	16.6%
Scaphocerite							
length vs width	2.9X	2.47X	3X	4X	3.04X ± 0.28	2.6X / 4.73X	87.0%*
Abdominal Pleura							
1st to 4th	rounded	rounded	rounded	rounded	rounded	acute / square	12.7%
5th & 6th	posteroventrally armed	posteroventrally armed	posteroventrally armed	posteroventrally armed	posteroventrally armed	rounded & unarmed	13.0%
Telson							
total dorsal spines	4	4	4	4	4 ± 1.23	0 / 13	20.8%
total posterior spines	6	6	6	6	6 ± 1.49	0 / 23	41.7%
terminal margin shape	broadly rounded	rounded	pointed	pointed	rounded	flat / pointed	13.0%
2nd Pereiopod							
ischium subdivisions	0	4	0–4	0	3–4 ± 1.39	0 / 8	95.5%*
merus subdivisions	11–17	11	10	11–14	13 ± 3.21	8 / 20	20.1%*
carpus subdivisions	21–32	23–34	25–27	26–31	30 ± 4.44	20 / 40	40.3%*
Pleopod (male)							
appendix masculina vs interna (~ length)	shorter (~1/2–3/4)	shorter (~2/3)	subequal	longer	shorter (~2/3)	shorter (~1/3) / longer	10.0%*
appendix masculina terminal setae	5–7	11	14	8	10 ± 2.37	5 / 16	93.0%*

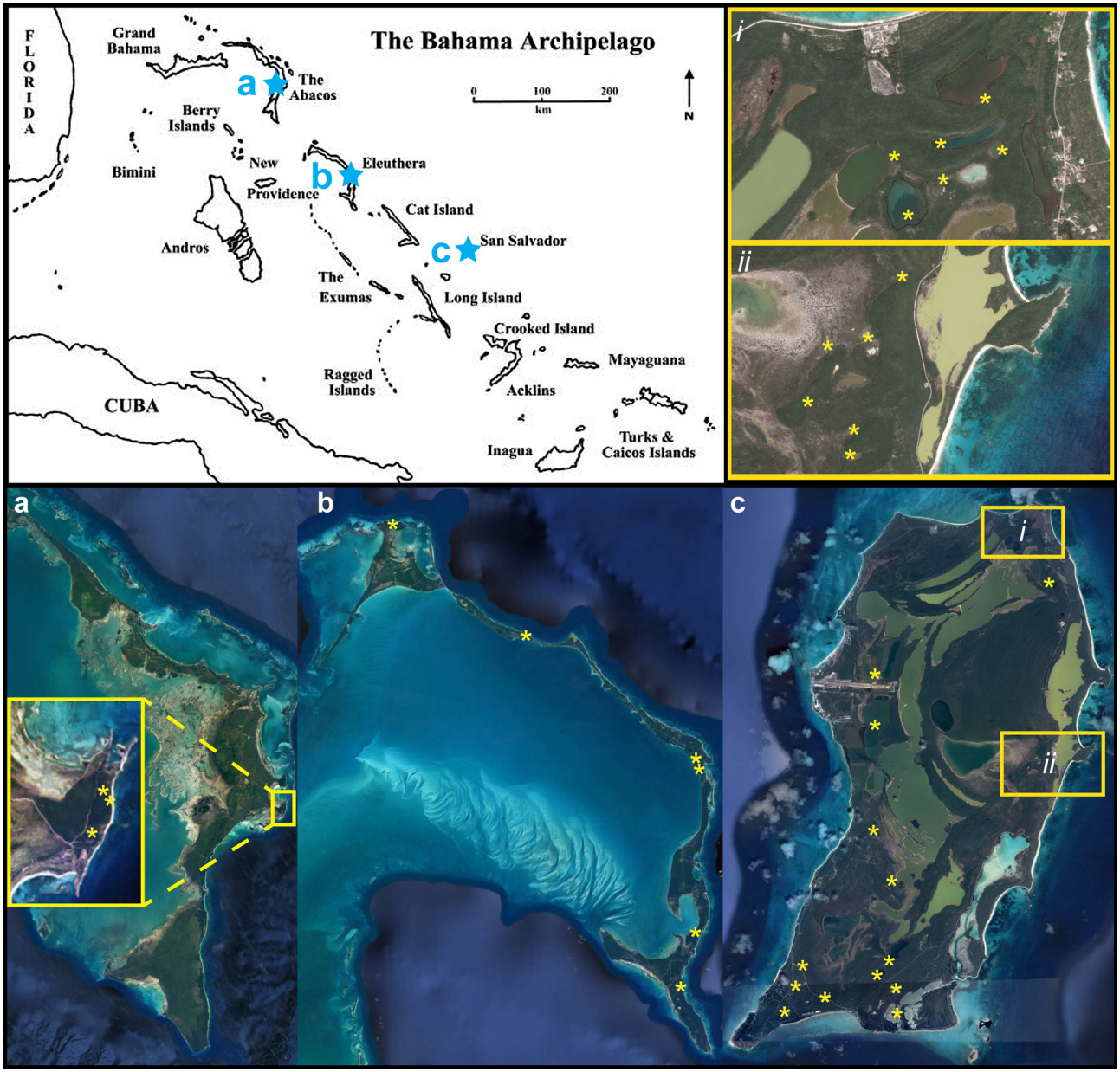

FIGURE 2. Map of Bahamian Islands with sites sampled from 2012–2016 (**a**) Abaco site codes and names labeled from North to South: ADS, Dripping Stones; ALP, Lora's Pond; ARS, Runge's Sinkhole (**b**) Eleuthera site codes and names labeled from North to South: EPC, Preacher's Cave Blue Hole; EDM, Dump Pond; ETD, Too Deep Pond; ESS, Savannah Sound; ESP, Shrimp Pond; EMN, Mackery Nixon Pond (**c**) San Salvador site codes and names labeled from northern most moving clockwise: LHC, Light House Cave; SSL, South Stout's Lake; MDP, Mermaid Pond; MNP, Merman Pond; PCC, Pigeon Creek Conduit; DCP, Dunk City Pond; WBH, Watling's Blue Hole; BH2, Blue Hole #2; BH5, Blue Hole #5; RED, Redrum Pond; WLL, William's Pond; LIL, Little Lake; MJC, Major's Cave. ***i***; site codes and names labeled from northern most moving clockwise: RHP, Reckley Hill Pond; PNP, Pain Pond; WDP, Wild Dilly Pond; OYP, Oyster Pond; SHP, Shrimp Holes. ***ii***; site codes and names labeled from North to South: PTN, Plantation Pond; SPX, Small Pox Pond; RBH, Rolle's Blue Hole; LRP, Tilde Pond; BRP, Big Rob Pond; BDP, Big Drink Pond.

Results

Over 90% of the 463 *B. cubensis* examined exhibit morphological variation beyond previous descriptions in one or more characters. PhyV exhibited in these shrimps often falls outside of characters prescribed to any genus of Barbouriidae or members of superfamily Alpheoidea (Table 1, Fig. 3: a–x). PhyV is present in individuals from all 34 localities sampled across Abaco, Eleuthera and San Salvador, Bahamas.

Carapace. Carapace is smooth; in some cases, a strong protuberance laterally or lateral grooves originating from the cardiac region are present (Fig. 3: f, g & l). While in most individuals the dorsal margin of the carapace forms a gradual rounded slope from the postorbital margin to the posterior margin, in some individuals the dorsal margin forms a distinct hump (see appendix, Fig. A1: a–c). Antennal and branchiostegal teeth are present in most specimens, but either could be absent. Some specimens lacking teeth the carina is present and multiple teeth originate from the carapace margin anterior to the carina (see appendix, Fig. A1: d–e). Approximately 18% (n=463) of individuals do not match previous descriptions of *B. cubensis* for these characters. Additionally, all specimens have at least two sensory dorsal organs (SDO) situated dorsally along the carapace (Lerosey-Aubril & Meyer, 2013). The first is allied posteriorly with the epigastric tooth and is present even when the epigastric tooth is absent. The second is medially situated along the dorsal margin of the carapace within the cardiac region and may have a smaller pair juxtapose and slightly posterior to the larger SDO (see appendix, Fig. A1: g–i).

Rostrum. Rostrum slender, typically reaching the middle of the second article of the antenullar peduncle. However, it was common for the rostrum to be absent (Fig. 3: b, c & f), not reaching beyond the first article of the antenullar peduncle or the eyestalk (Fig. 3: g) or reaching past the distal end of the second article (Fig. 3: d). The rostrum bears 4–7 total dorsal teeth, 3–4 along the postorbital margin, and 1–7 ventral teeth. However, the rostrum bears 0–15 dorsal (0–10 postorbital) and 0–8 ventral teeth. Additionally, in some individuals the rostrum can have bifurcated spines, or is bifid or trifid (Fig. 3: c, g & h). Over 51% (n=463) of individuals exhibit character states inconsistent with the diagnosis of *B. cubensis*

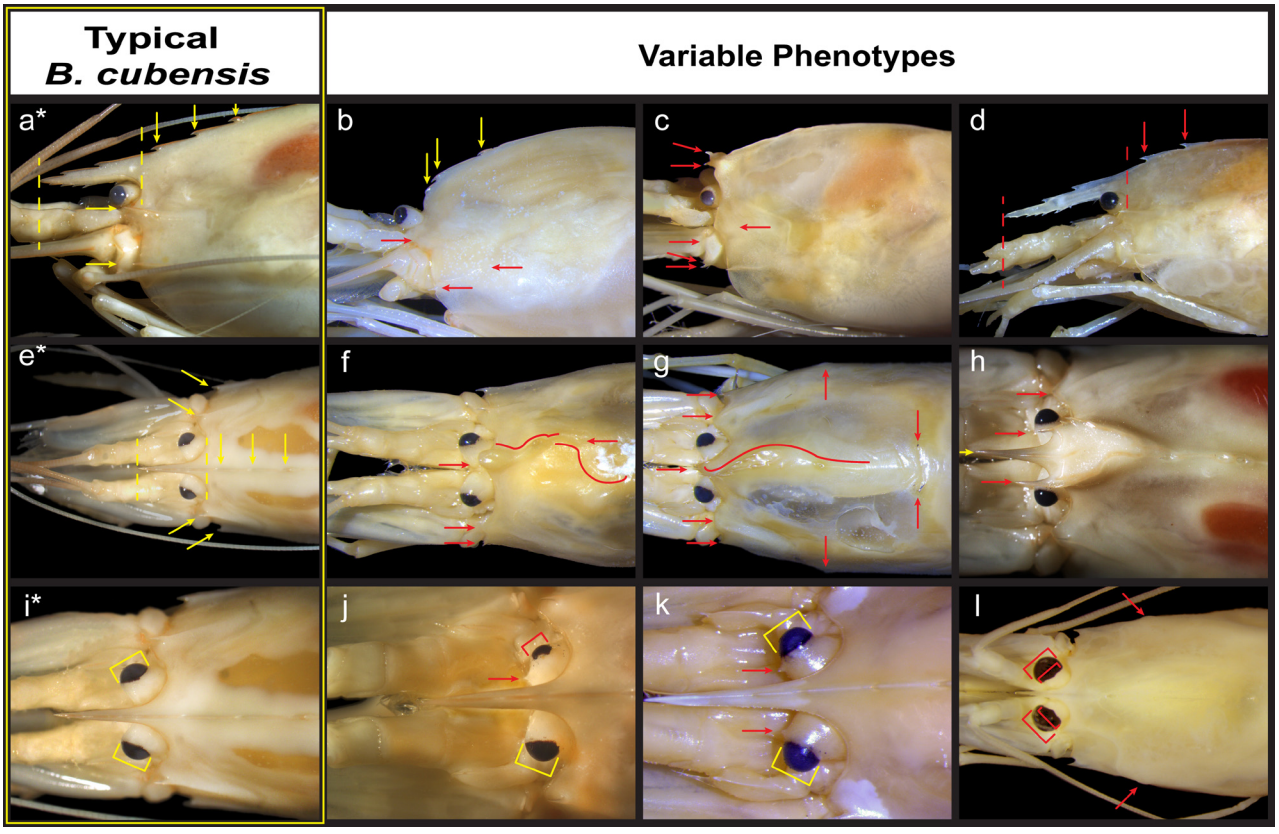

FIGURE 3. Expected phenotypes for *B. cubensis* (left column) compared to examples of observed variable phenotypes; (**a–d**) teeth on rostrum and rostrum length, and the presence of antennal and branchiostegal spines (**e–h**) rostrum length, teeth and shape, antennal and branchiostegal teeth and abnormalities of carapace (**i–l**) eyes, cornea width versus eyestalk width, and the presence of spines on eyestalk. Images correspond to the following catalog numbers for specimen vouchers housed in the Florida International Crustacean Collection: a*, HBG1843; b, HBG2162; c, HBG2011; d, HBG5644; e*, HBG1843; f, HBG2139; g, HBG2095; h, HBG1935; i*, HBG1843; j, HBG 1952; k, HBG1776; l, HBG2217.

Eyes. The eyes often have well-developed cornea that are narrower than the eyestalk (Manning & Hart, 1984). However, eyes with greatly reduced or absent pigmentation are present in some individuals (Fig. 3: j), or in some

instances the cornea was subequal to or broader than the eyestalk (Fig. 3: l). Frequently a medial spine or tubercle is present along the interior margin of the eyestalk (Fig. 3: j & k). Just over 26% (n=463) of specimens exhibiting character states that are not prescribed to any species within Barbouriidae (see appendix, Fig. A1: j–l).

Scaphocerite. The scaphocerite on average is approximately 3.1 times as long as wide. Some individuals exhibit lateral compressions in the anterior third of the scaphocerite (refer to appendix, Fig. A2: a), or exhibit asymmetry between the left and right scale. The length versus width ratio of approximately 66% (n=121) of individuals is greater than previously reported for species of *Barbouria.*

Mouth Parts. Variation was present in the maxilla and maxillipeds, predominantly in the shape of the caridean lobe. Epipods are present on the third maxillipeds. The distal margin of the epipods terminated in a single hook, or between two and four juxtaposed hooks. In some specimens a reduced incisor appeared to be present, but it could not be determined if the structure was a true incisor process. Variation was also observed in the gill complements. However, this variation is not reported here due to inconsistent results and the possibility of inaccuracy due to damage.

Pereiopods. Pereiopods 1 and 2 chelate, but the finger may be shorter or longer than, or equal to the length of the palm. Additionally, the interior margins of the chelae may be straight or curved, and either strongly serrated or smooth. Pereiopod 2 exhibits a wide range of articulation from the ischium with 0–8 subdivisions, merus with 8–20 subdivisions and carpus with 20–40 subdivisions (refer to appendix, Fig. A2: b & c). The posterior 3 pairs of pereiopods are undivided but exhibit a wide range of spines running medially along the posterior margin. Spines may be absent or reduced to well-developed occurring either as pairs or alternating along the length of the segment. Epipods were present on the anterior four pereiopods, bearing between 1–4 hooks in varying positions similar to those on the third maxillipeds. Of the individuals in which pereiopods were examined, nearly all exhibit character states not assigned to *B. cubensis*.

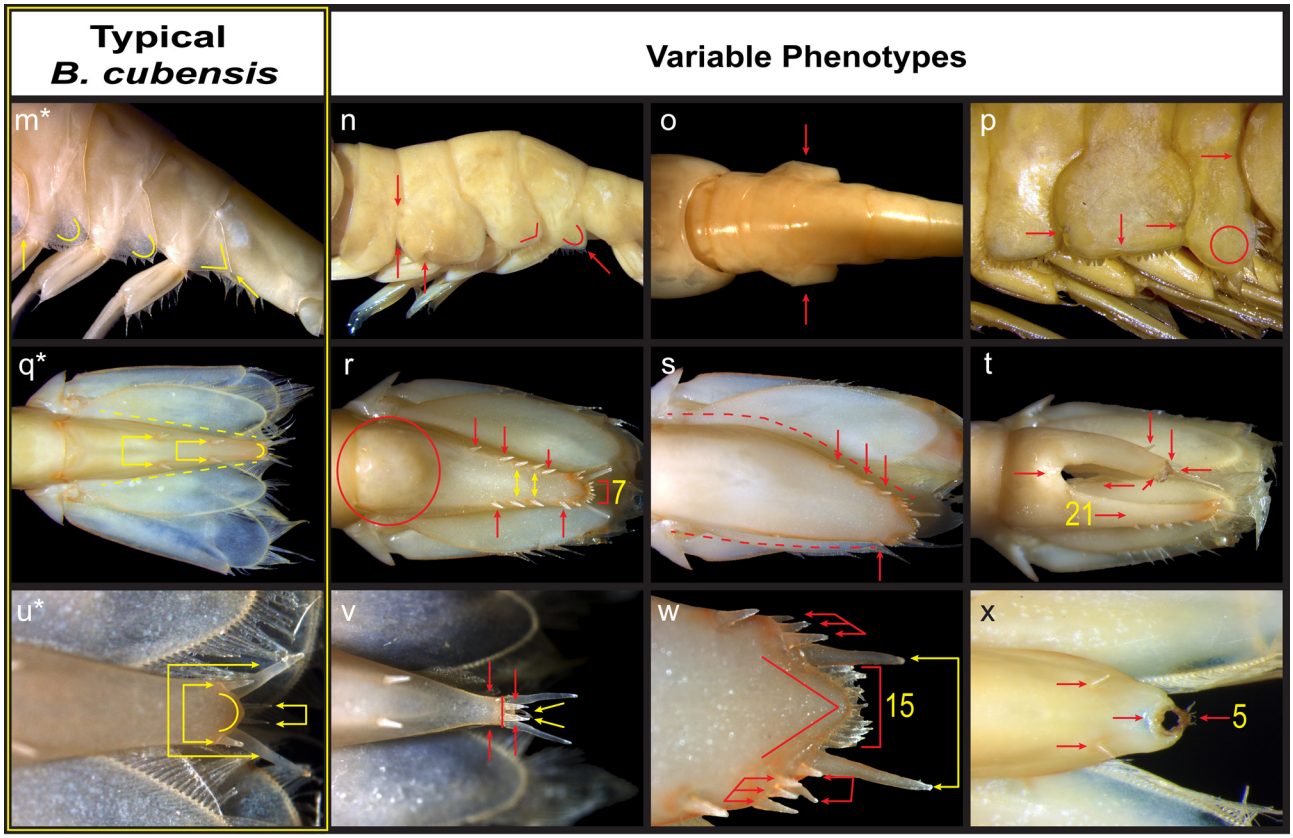

FIGURE 3 con't. (**m–p**) shape and armament of pleura of the abdominal somites (**q–t**) telson shape, dorsal spines and terminal spines (**u–x**) shape and spines of terminal margin of telson. Yellow lines and arrows indicate expected characters, red lines and arrows indicate variable characters. * denotes examples of expected phenotypes for *B. cubensis*. Images correspond to the following catalog numbers for specimen vouchers housed in the Florida International Crustacean Collection: m*, HBG1843; n, HBG1932; o, HBG1934; p, HBG1398; q*, HBG1843; r, HBG1936; s, HBG1937; t, HBG1970; u*, HBG1843; v, HBG1777; w, HBG1937; x, HBG2098.

Abdominal Pleura. The abdominal pleura of the somites typically match the previous descriptions (Manning & Hart 1984). It was found that for some specimens the first pleura may or may not overlap the carapace of the cephalothorax, and the first or third pleura may overlap the second (Fig. 3: n & p). The anterior three pleura may be square or have abnormal dentation along the margins (Fig. 3: n & p). In some instances, the pleura exhibit folding and can protrude laterally away from the abdomen (Fig. 3: o) similar to the findings of Fernandes *et al.* (2011). The fourth abdominal pleura ranged from obtusely to acutely round, or the posteroventral corner may be produced into a small spine or tooth. The fifth and sixth abdominal pleura could lack spines (Fig. 3: n) or be produced into multiple small spines or teeth. Nearly 29% (n=463) of specimens exhibiting abnormalities in the pleura of the abdominal somites not described for any species within Barbouriidae.

Pleopods. The endopod of the male exhibits an appendix masculina that may be shorter by two-thirds or longer than the appendix interna and bearing 5–16 terminal setae arranged in a ring. Of the 140 individuals in which endopods were examined, 66 individuals were identified as male. Approximately 93% of male endopods exhibit character states outside of variation prescribed to *B. cubensis*.

Telson. The telson ranged in shape from very slender and elongate, reaching well beyond the posterior margin of the uropods, to being short and stout not reaching beyond the posterior margin of the uropods (Fig. 3: r). Among many individuals with an elongate telson, the posterior third of the telson is laterally compressed (Fig 3B: v). Among individuals with an abnormally short telson, within the anterior third the telson becomes dorsoventrally compressed (Fig. 3: r). Alternatively, some individuals exhibit a telson with the medial third wider than the anterior and posterior third (Fig. 3: s) or could have a telson that is bifid (Fig. 3: t) or with a cavity anterior to the posterior margin (Fig. 3: x). The telson may bear 0–13 dorsal spines, and 0–23 terminal spines. Telson spines may be paired or unpaired. The terminal margin of the telson ranges in shape from being flat (Fig. 3: v) to pointed with an apical spine or tooth. The telson of 70% (n=463) of individuals could not be assigned as matching that of *B. cubensis*.

Uropods. Exopods of the uropods are armed with 0–2 posterolateral teeth. It was found that few specimens possess additional teeth medially along the lateral margin of the exopod, or that the lateral margin may be straight or exhibit multiple curves.

***Data analysis*.** Statistical analyses were conducted to test the hypothesis that the distribution of phenotypic hypervariation is correlated with geographic location. All characters exhibit a normal distribution based on Shapiro-Wilk test. PhyV is present in specimens from Abaco, Eleuthera and San Salvador, Bahamas. No clustering or discernable distribution of PhyV is detected under all parameters in which UPGMA was conducted (Fig. 4).

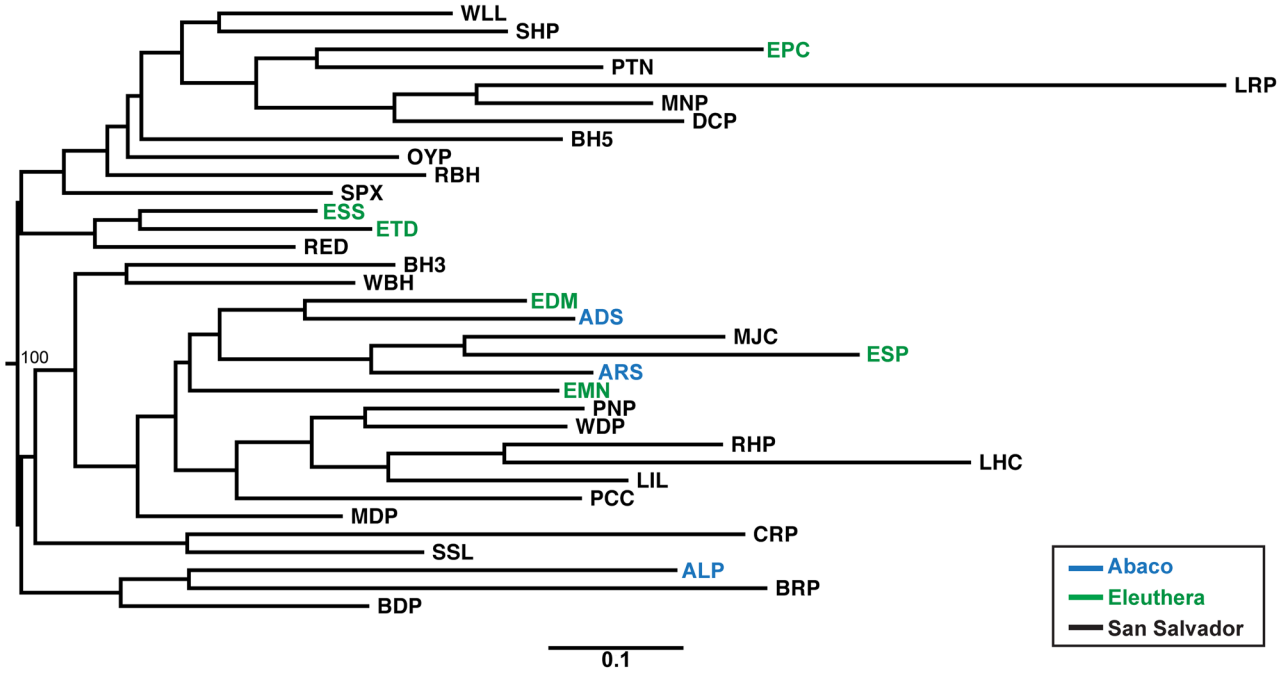

FIGURE 4. An unweighted pair-group method with arithmetic averages (UPGMA) dendrogram of morphological data in Subset #2 labeled with site codes corresponding to figure 2. Bootstrap values >90% are noted to the right of nodes.

Cryptic Diversity . The combined 16S and COI tree included 70 individuals of *B. cubensis* as the ingroup taxa (Fig. 5). The species, *Parhippolyte misticia*, *Parhippolyte sterreri*, *Parhippolyte uveae* and *Janicea antiguensis* from the family Barbouriidae and *Lysmata amboinensis* from the family Lysmatidae were included as the outgroup taxa. For this dataset, 144 new sequences were generated including 72 new sequences for 16S, and 72 new sequences for COI (Table 2). Individual gene trees were without conflicting topologies. Results provided no support for cryptic speciation among *B. cubensis* (Fig. 5). Individuals identified as *B*. cubensis from all localities form a single polytomy, and we find no evidence for population structure across localities. All individuals in this data set that grouped with *B. cubensis* included a wide range of PhyV, and no patterns relating to morphological variation were detected.

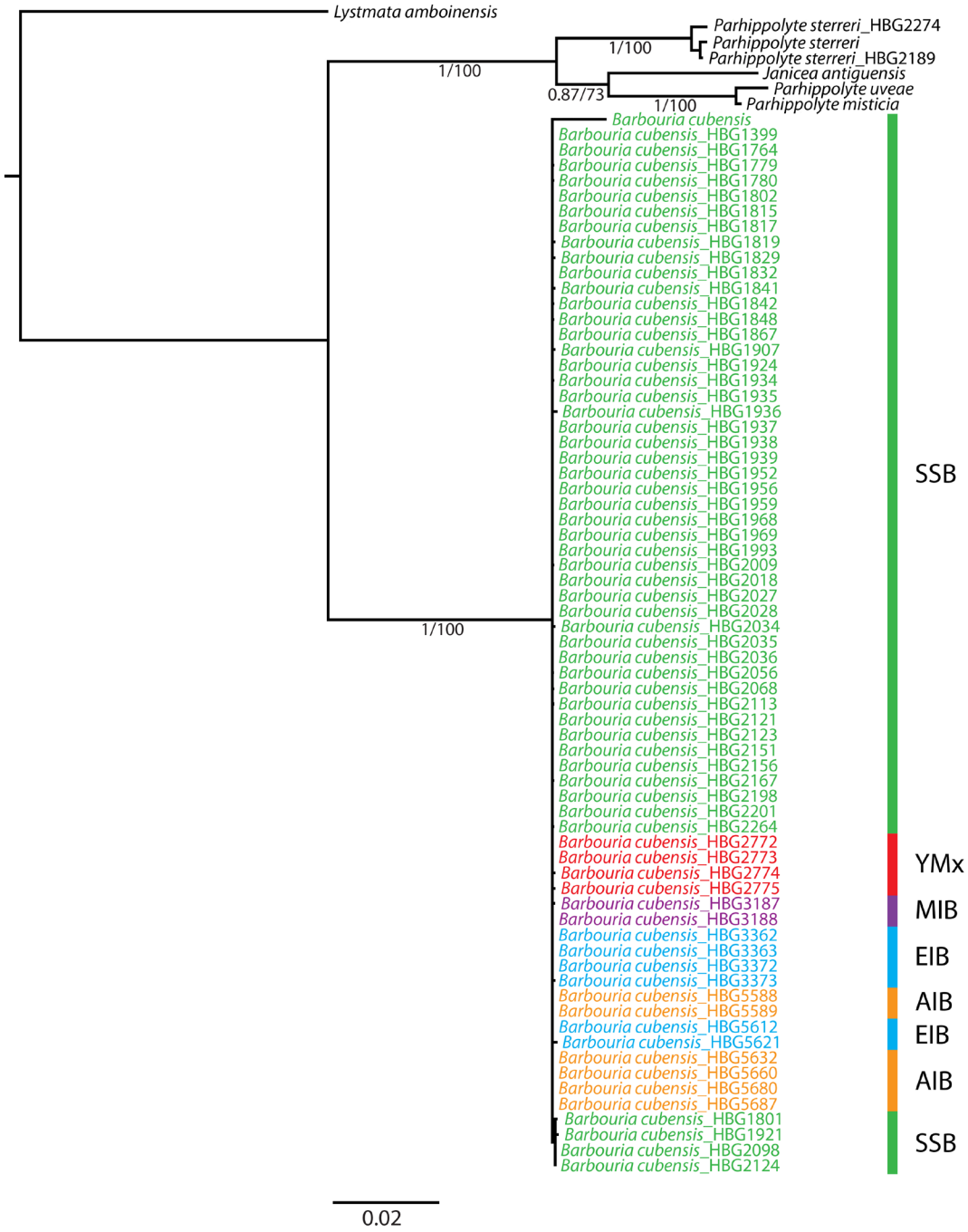

FIGURE 5. Bayesian (BI) phylogram for *Barbouria cubensis* (n = 70) based on a 16S & COI concatenated data set. BI posterior probabilities and Maximum Likelihood (ML) bootstrap values noted below branches. Values >0.7 for ML and >90% for BI are shown and represented by percentages. Vertical bars represent collection locality. AIB = Abaco, Bahamas, EIB = Eleuthera, Bahamas, MIB = Mayaguana, Bahamas, SSB = San Salvador, Bahamas, and YMx = Yucatán, Mexico. Catalog numbers represent tissue vouchers from the Florida International Crustacean Collection (FICC).

TABLE 2. Species used for phylogeny reconstruction for examining cryptic diversity within *Barbouria cubensis* (Dataset 1), showing taxon, collection locality, catalog number, and GenBank accession numbers for partial sequences of 16S, COI respectively [for museum abbreviations see Material and Methods].

Taxon	Collection Locality	**Catalog No.**	**16S**	**COI**
Barbouria cubensis	Abaco, Bahamas	HBG5588	MK501714	MK575421
von martens, 1872		HBG5589	MK501715	MK501722
		HBG5632	MK501718	MK501725
		HBG5660	MK501719	MK575426
		HBG5680	MK501720	MK575427
		HBG5687	MK501721	MK575428
	Eleuthera, Bahamas	HBG3362	MK501710	MK575417
		HBG3363	MK501711	MK575418
		HBG3372	MK501712	MK575419
		HBG3373	MK501713	MK575420
		HBG5612	MK501716	MK575423
		HBG5621	MK501717	MK575424
	Mayaguana, Bahamas	HBG3187	MK501708	MK575415
		HBG3188	MK501709	MK575416
	San Salvador, Bahamas	OUMNH.ZC.2010-05-003	KF023098	-
		HBG1399	MK501653	MK575359
		HBG1764	MK501654	MK575360
		HBG1779	MK501655	MK575361
		HBG1780	MK501656	MK575362
		HBG1802	MK501650	MK575363
		HBG1815	MK501657	MK575364
		HBG1817	MK501658	MK575365
		HBG1819	MK501659	MK575366
		HBG1829	MK501660	MK575367
		HBG1832	MK501661	MK575368
		HBG1841	MK501662	MK575369
		HBG1842	MK501663	MK575370
		HBG1848	MK501664	MK575371
		HBG1857	MK501665	MK575372
		HBG1907	MK501666	MK575373
		HBG1924	MK501667	MK575374
		HBG1934	MK501668	MK575375
		HBG1935	MK501669	MK575376
		HBG1936	MK501670	MK575377
		HBG1937	MK501671	MK575378
		HBG1938	MK501672	MK575379
		HBG1939	MK501673	MK575380
		HBG1952	MK501674	MK575381
		HBG1956	MK501675	MK575382
		HBG1959	MK501676	MK575383
		HBG1993	MK501677	MK575384
		HBG2009	MK501678	MK575385
		HBG2018	MK501679	MK575386
		HBG2027	MK501680	MK575387
		HBG2028	MK501681	MK575388

.....continued on the next page

TABLE 2. (Continued)

Taxon	Collection Locality	**Catalog No.**	**16S**	**COI**
		HBG2034	MK501682	MK575389
		HBG2035	MK501683	MK575390
		HBG2036	MK501684	MK575391
		HBG2056	MK501685	MK575392
		HBG2068	MK501686	MK575393
		HBG2113	MK501687	MK575394
		HBG2121	MK501688	MK575395
		HBG2123	MK501689	MK575396
		HBG2151	MK501690	MK575397
		HBG2167	MK501691	MK575398
		HBG2198	MK501692	MK575399
		HBG2201	MK501693	MK575400
		HBG2264	MK501694	MK575404
Barbouria cubensis	Yucatán Peninsula of	HBG2772	MK501704	MK575411
von Martens, 1872	Mexico	HBG2773	MK501705	MK575412
		HBG2774	MK501706	MK575413
		HBG2775	MK501707	MK575414
Outgroups				
Janicea antiguensis Chace, 1972	Cape Verde, Africa	OUMNH.ZC.2004-15-002	KF023112	-
Parhippolyte sterreri Hart & Manning, 1981	Iguana Cay, Bahamas	MNHN-IU-2012-1057	KP725619	KP759480
	San Salvador, Bahamas	HBG2189	MK501722	MK575429
		HBG2274	MK501723	MK575430
Parhippolyte uveae Borradaile, 1900		MNHN-IU-2012-1001	KP725621	-
Parhippolyte misticia Clark, 1989			HQ315560	-
Lysmata amboinensis De Man, 1888	Hong Kong/ Bise Point, Okinawa, Japan	*MSLH:CA23Lyamb*	KF023091	-
		UNML:32.9451	-	JF346249

Discussion

Extent of Phenotypic Variation in *Barbouria cubensis*. Examples of phenotypic variation are abundant in the animal kingdom (Allegue *et al*., 2017); however, the rate and extent of variation we report has never been documented in crustaceans (Agnalt *et al*., 2013). Examples of "abnormal" morphologies of decapods have been reported in crabs, crayfish, penaeid prawns, and *Palaemon* shrimp (Béguer *et al*., 2008; Duarte *et al*., 2008). Reported abnormalities include: duplication and asymmetry of chelae, bifurcation of rostrum and telson, rostrum size, curve and tooth number or absence of rostrum, reversal of asymmetry, abnormal sexual appendages, carapace spines, pereiopods, backwards folding and abnormal positioning of the abdominal epimera, and deformed telson and uropods (Aguirre & Hendrickx, 2005; Béguer *et al*., 2008; Duarte *et al*., 2008; Fernandes *et al*., 2011). These records are not comparable to the morphological variation we report in this study because they are limited to one or few individuals and are typically limited to a single morphological character (Aguirre & Hendrickx, 2005; Béguer *et al*., 2008; Fernandes *et al*., 2011).

The 463 *B. cubensis* examined in this study represent a unique example of phenotypic hypervariation (PhyV). We define PhyV as morphological variation beyond the combined variation described in previous records of *B.* cubensis (Rathbun, 1912; Holthuis, 1963; Chace, 1972; Hobbs *et al*., 1977; Hobbs, 1978; Hart & Manning, 1981;

Manning & Hart, 1984; Mejía *et al*., 2008). Less than 7.5% (n = 463) of individuals are without PhyV and PhyV was present in all 54 characters examined in at least one individual. For each morphological character the frequency of PhyV present is between 6.9% (n=463) to 95.5% (n=121) of individuals. The presence of PhyV among shrimp sampled from Abaco, Eleuthera and San Salvador without any discernable pattern suggests PhyV is not associated with locality and is likely present among all populations of *B. cubensis* across the western Atlantic (Fig. 4).

Previous records indicate abnormal phenotypes typically occur at low rates in crustaceans (Béguer *et al*., 2008) with one notable example. The highest rate of "deformities" is reported at 40% (n=1,578) and 58% (n=539) of individuals in *Palaemon longirostris*, which is far less than the observed 90% (n=463) in *B. cubensis* (Béguer *et al*., 2008; Béguer *et al*., 2010). Morphological "abnormalities" in *P. longirostris* is limited to four characters, which include cephalothorax anomalies, rostral "deformations" and pronounced bilateral dissymmetry of the scaphocerite and uropods (Béguer *et al*., 2008). The nature of morphological variations of *P. longirostris* appears similar to those of *B. cubensis*, however PhyV in *B. cubensis* extends to 54 morphological characters compared to the four of *P. longirostris*. As recently as 1984, extensive variation has not been reported for *B. cubensis* by carcinologists, which includes records of specimens collected from localities sampled in this study (Hobbs, 1978; Manning & Hart, 1984). Similar to *P. longirostris*, PhyV of *B. cubensis* has likely appeared within the past 30 to 40 years (Hart & Manning, 1981; Manning & Hart, 1984; Béguer *et al*., 2008). We do not believe that the appearance of PhyV is the result of increased sampling effort. Based on the numbers of specimens previously collected from sites included in this study if aberrant individuals were present at the time of those collections they would have been reported, especially considering the high rate at which we find PhyV is present in this study.

Many of the shrimp examined in this study exhibited characters synonymous with *Barbouria cubensis*, *Barbouria yanezi*, *Parhippolyte sterreri* or *Janicea antiguensis* and that are not described as being shared by the four species (i.e. terminal margin shape and spination of telson). Additionally, some specimens exhibited morphological characters not present in any shrimp within the family Barbouriidae (Fig. 3). It is important to consider revisiting and sampling other species of barbouriid shrimp in the western Atlantic to determine if PhyV is limited only to *B. cubensis* or if it is common among all species.

Potential Causes of Phenotypic Hypervariation. It is possible that phenotypic hypervariation may represent cryptic diversification as reported in atyid shrimp, if island populations are effectively isolated. *Caridina rubella* is reported to exhibit regional morphological variation in the length of the rostrum as either long or short (Weese et al., 2012). The two morphotypes of *C. rubella* correspond to two genetically divergent populations with two discrete COI haplotype networks as the result of cryptic speciation. We feel this is not comparable as the morphological variation of *C. rubella* occurs in a single character correlated to locality and resulting from speciation. In our phylogenetic analysis we included several individuals that exhibited severe PhyV along those with the individuals matching the normal *B. cubensis* phenotype. We found all individuals to be genetically identical with no population structure attributed to morphological variation. Our findings provide some insight into the source of PhyV, as we can conclude that PhyV is not related to cryptic diversity.

Alternatively, PhyV may be caused by low genetic diversity due to severe inbreeding or population bottleneck, which can lead to an accumulation of deleterious mutations, increased expression of abnormal phenotypes and ultimately extinction (Creasey *et al*., 2000; Fumagalli *et al*., 2002, O'Grady *et al*., 2006; Lampert *et al*., 2007; Duarte *et al*., 2008; Lacy & Alaks, 2012; Hedrick & Garcia-Dorado, 2016). Highly connected island populations are capable of inbreeding when species exhibit strong dispersal capabilities Kano & Kase, 2004; Santos, 2006; Russ *et al.*, 2010). Previous records of *B. cubensis* only include two ovigerous females, in which abundant very small oocytes were found in the gonads (Hobbs, 1978). It is likely that these shrimps have extended planktonic development with strong dispersal capabilities similar to other caridean species that produce numerous small oocytes (Bauer, 2005; Russ *et al.*, 2010; Weese *et al*., 2013). For anchialine organisms with planktotrophic larvae genetic connectivity occurring between populations has mostly been found to be limited to 600 km (Kano & Kase, 2004; Santos, 2006; Russ *et al.*, 2010; Weese *et al*., 2013; Gonzales *et al.*, 2017). The shortest direct route between the Yucatan and the Bahamas is >1200 km, and the closest known population of *B. cubensis* to the Yucatan occurs ~700 km away in the Cayman Islands. In this case we would expect highly connected populations within the Bahamas, distinct from the Yucatan population (Kano & Kase, 2004; Santos, 2006; Russ *et al.*, 2010; Weese *et al*., 2013; Gonzales *et al.*, 2017). Distinct isolated clades of *Typhlatya* are reported from the Yucatan and the Bahamian archipelago (Hunter *et al.*, 2008). However, our phylogenetic analysis finds no population structure of *B. cubensis* suggesting genetic connectivity is high across localities (Fig. 5). If a recent population bottleneck reduced genetic diversity, inbreeding

among highly connected populations could maintain low genetic diversity. It is possible the markers used in this study lack sufficient resolution to determine population level structure and patterns that exist between PhyV and genetic diversity. If the PhyV is due to low genetic diversity, then management efforts may be required to restore genetic diversity to these critically endangered populations.

Multiple species of Barbouriidae have been reported inhabiting the same localities (Hart & Manning, 1981) and PhyV may indicate the presence of hybrid swarms due to interbreeding between these species (Wolf & Mort, 1986; Perry *et al*., 2002; Cristescu *et al*., 2010; McInerney *et al*., 2014; Ribardière *et al*., 2017). Hybrid swarm is characterized by major morphological variation between individuals in populations with interbreeding hybrid individuals that back-cross with parent types (Cockayne & Allan, 1926). The presence of intermediate character states between *B. cubensis* and *P. sterreri*, such as the terminal margin of the telson being pointed or nearly pointed and produced into a spine, and the number of spines on the postorbital margin of the rostrum support hybridization. Future studies using next generation sequencing techniques and fine-scale markers may provide further insights into the relationship between the abnormally rampant morphological variation and genetic diversity within Barbouriidae.

Deviations from perfect symmetry in bilaterally paired structures, or fluctuating asymmetry, may be used to evaluate developmental instability due to environmental factors, geographic location or as an indicator of environmental stress (Duarte *et al*., 2008; Maia *et al*., 2009; Klingenberg, 2015; Nishizaki *et al*., 2015). It is noteworthy that the presence of PhyV was commonly asymmetrical, especially in the situation of extra spines along the dorsal margin of the telson. However, most characters utilized in this study are either not paired structures (i.e., characters associated with the rostrum) or the PhyV expressed in paired structures were symmetrical (i.e., the number of subdivisions of segments of the second pereiopod). Due to the lack of geographic pattern in the distribution of PhyV and insufficient symmetry measures we were unable to assess fluctuating asymmetry.

The anchialine pools in which *B. cubensis* are found are known to have strong turbulent flows through a complex series of subterranean passages in the karst landmass (Bishop *et al*., 2015). Physical trauma during early development or following ecdysis can lead to extensive variation, possibly resulting from abrasion against the conduit walls due to strong tidal flows (Moncada & Gomes, 1980; Bohonak, 1999; Giménez, 2006; Luppi & Spivak, 2007; Follesa *et al*., 2008; Vogt *et al*., 2008). Similarly, damage due to parasitic infection can lead to abnormal phenotypes (Goodman & Johnson, 2011).

Environmental factors such as salinity, pH, temperature and contamination have been identified as causes of extensive variation in crustaceans (Smith & Palmer, 1994; Stibor & Lüning, 1994; Trussel, 1996; Trussel & Smith, 2000; Agrawal, 2001; Kappes & Sinsch, 2002; Chown *et al*., 2007; Ituarte *et al*., 2007; Reuschel & Schubart, 2007; Béguer *et al*., 2008; Duarte *et al*., 2008; Béguer *et al*., 2010 Agnalt *et al*., 2013). Routine migration of *B. cubensis* in and out of anchialine pools (across the pycnocline) regularly exposes them to acute changes in temperature, pH, salinity and dissolved oxygen levels (Bishop & Iliffe, 2012). Because of this life style and no prior record of extensive variation for *B. cubensis*, it is unlikely PhyV is due to salinity, pH or temperature. However, contamination such as lead remains a viable source of PhyV. If the source of PhyV has a non-genetic basis, then further study will be required to identify the source and implications for the conservation of endemic anchialine species in the western Atlantic.

Conclusion

We found that PhyV is present in populations of *B. cubensis* from Abaco, Eleuthera and San Salvador, Bahamas with no detectable pattern of geographic distribution or cryptic diversification. Future studies will use population genomic methods to investigate potential drivers of PhyV across western Atlantic populations of *B. cubensis*. It is important for us to further investigate the low level of genetic diversity and high levels of population connectivity suggested by our results (Fig. 5) with greater resolution through the use of next generation sequencing, such as restriction site associated DNA sequencing (Miller *et al.*, 2007; Davey *et al*., 2011; Timm & Bracken-Grissom, 2015). We anticipate that further molecular and morphological studies with the addition of environmental data will identify the underlying cause sources of PhyV, which may be due to a complex combination of genetic and environmental factors.

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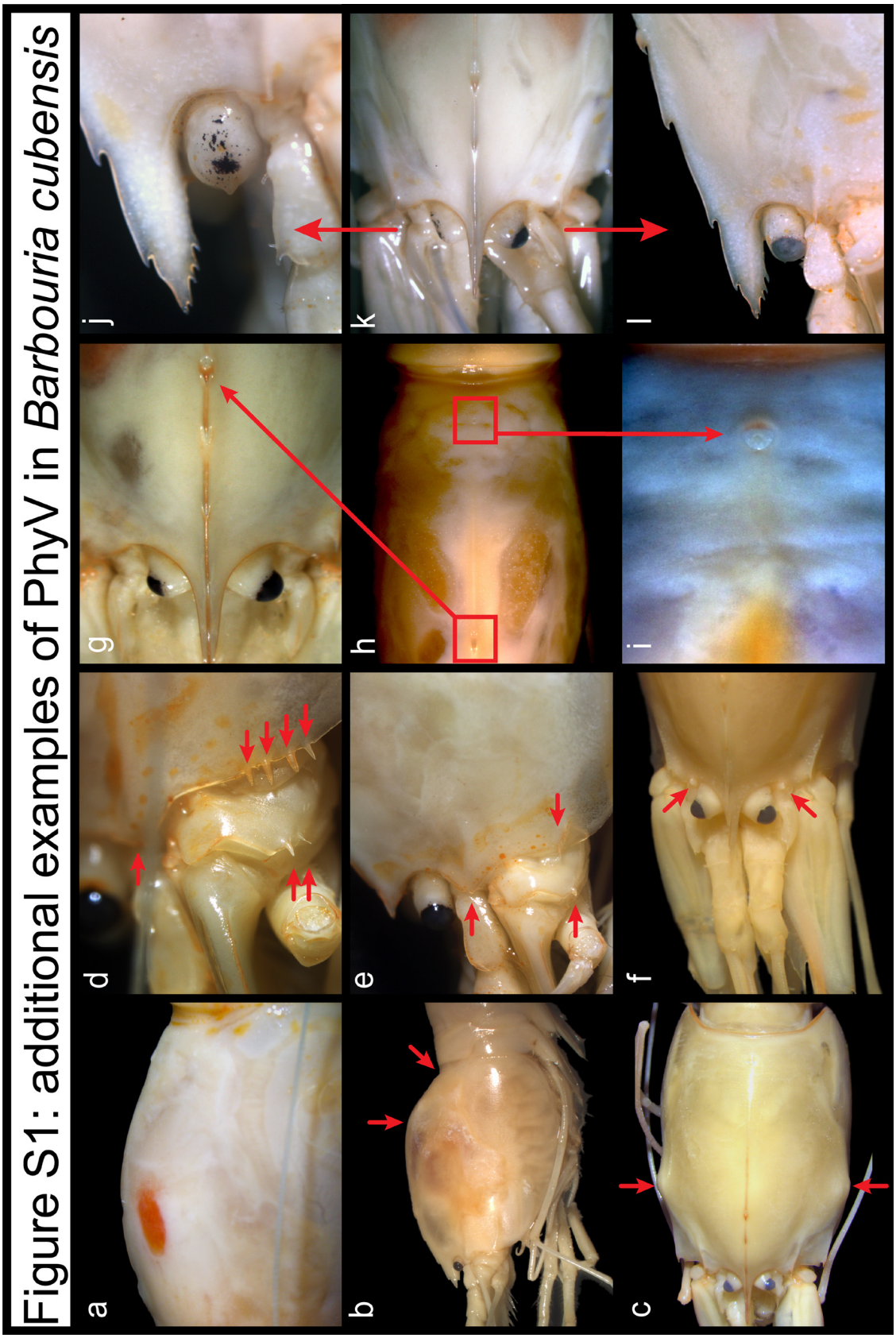

FIGURE A1. Phenotypic hypervariation exhibited by *B. cubensis*; (**a–c)** variations in the shape and surface of the carapace, (**d–f)** additional and missing teeth without carina, (**g–h**) position of sensory dorsal organ associated with epigastric tooth and within the cardiac region **(j–l**) asymmetry in the presence of cornea pigmentation and a terminal spine or tubercle on the eye-stalk. Images correspond to the following catalog numbers for specimen vouchers housed in the Florida International Crustacean Collection: a, HBG1793; b, HBG1918; c, HBG2215; d & e, HBG1808; f, HBG1909; g–i, 1801 HBG; j–l, HBG1849.

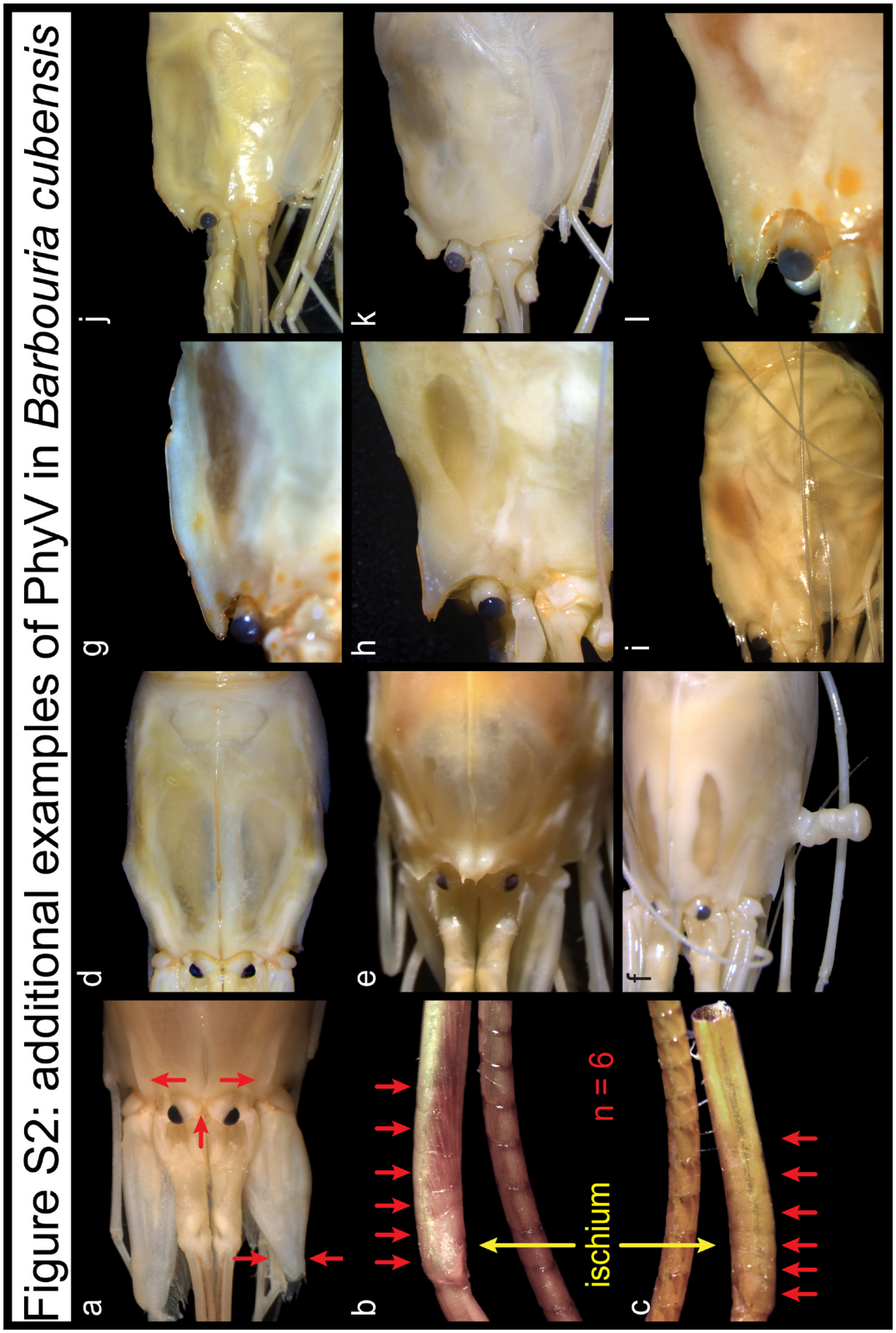

FIGURE A2. Phenotypic hypervariation exhibited by *B. cubensis*; (**a**) asymmetrical lateral compression of scaphocerite, and the absence of the rostrum and antennal tooth while the carina are present (**b & c**) dorsal and ventral view of the ischium of the 2nd pereiopod with six subdivisions (**d**) lateral extrusions along carapace, (**e**) bifid rostrum, antennal and branchiostegal spines absent and carapace not smooth (**f**) rhizocephalan parasite, (**g–l**) additional examples of PhyV in character located on the cephalothorax. Images correspond to the following catalog numbers for specimen vouchers housed in the Florida International Crustacean Collection: a, HBG1907; b & c, HBG1904; d, HBG2227; e, HBG2011; f, HBG2083; g, HBG1816; h, HBG1872; i, HBG1882; j, HBG2095; k, HBG1982; l, HBG1793.

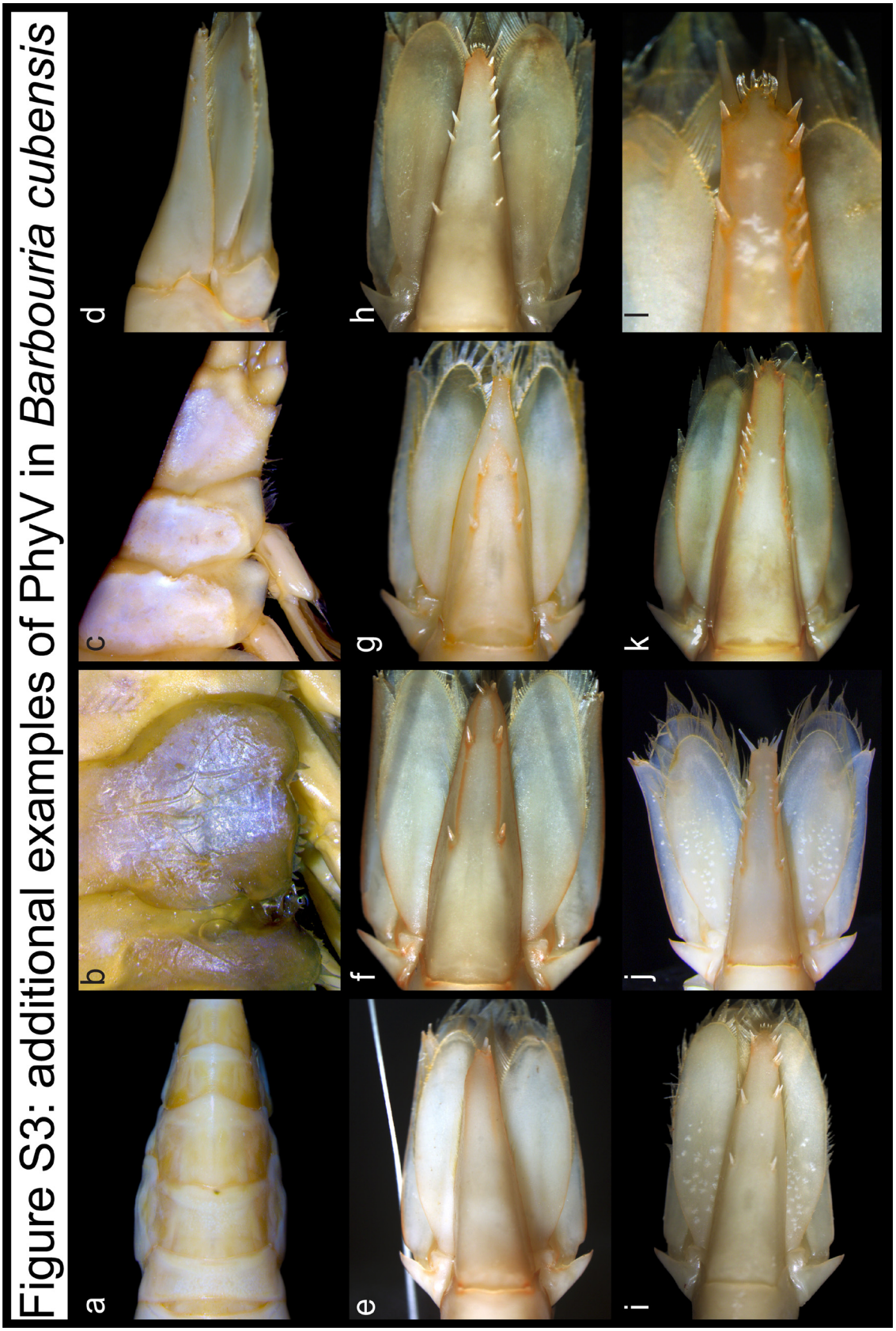

FIGURE A3. Phenotypic hypervariation exhibited by *B. cubensis*; (**a** & **b**) abnormal shape and armament of the pleura of the abdominal somites, (**d–l**) additional examples of PhyV in characters located on the telson and uropods. Images correspond to the following catalog numbers for specimen vouchers housed in the Florida International Crustacean Collection: a, HBG2093; b, HBG1398; c, HBG1399; d, HBG1793; e, HBG1802; f, HBG1791; g, HBG1777; h, HBG1395; i, HBG1954; j, HBG1982; k, HBG1855; l, HBG1853.

TABLE A1. Morphological characters included in data subset 1

Character *(# of characters used)*		prescribed state
Carapace *(2)*		Smooth
1	*suborbital tooth (antennal spine)*	Present
2	*branchiostegal tooth*	Present
Sensory Dorsal Organ *(2)*		
1	in cardiac notch	Undescribed
2	posterior to epigastric spine	Undescribed
Rostrum *(4)*		margins dentate
1	length vs. antennular peduncle	not past 2nd article
2	dorsal rostral teeth (total)	4 to 7
3	postorbital rostral tooth count	3 to 4
4	ventral rostral teeth (total)	1 to 7
Eye *(2)*		
1	*cornea pigmented*	pigmented cornea
	cornea width vs. stalk width	narrower than eyestalk
2	*eyestalk bearing spine/tubercle*	Absent
Abdomen *(4)*		
1	pleura 1 & 2	rounded laterally
	pleura of 2nd somite	overlapping 1st & 3rd
	pleura of 3rd somite	rounded/obtuse
	3rd posterolateral tooth	Absent
2	pleura of 4th somite	Rounded
	4th posterolateral tooth	Absent
3	angle of pleura of 5th somite	Acute
	5th posterolateral tooth	Present
	angle of pleura of 6th somite	short and acute
4	posterior ventral angle tooth	Present
	6th posterolateral tooth	Present
Telson *(4)*		
1	telson shape	elongate & slender
2	dorsal margin spines	2 pairs of spines
3	terminal margin shape	blunted apex
4	terminal margin spines	3 pairs
	length of terminal spines	middle pair longest
Uropod *(3)*		
1	length vs telson	slightly longer
2	outer margin shape	exopod straight
3	terminal tooth	Present
	inner movable spine	Present
Antennular Peduncle *(2)*		
1	basal segment spine/tubercle	Absent
2	basal segment length	Longest
	stylocerite terminal spine	Present
	2nd segment length vs. 1st	Shorter
	2nd segment length vs 3rd	Longer
	simple flagella	Present
Pereiopod *(2)*		
1	2nd articles subdivided	Present
2	3rd-5th articles not subdivided	Absent

TABLE A2. Additional morphological characters used in data subset 2

Character *(# of characters used)*		prescribed state
Scaphocerite *(1)*		
	ratio of length vs. width	2.9X
Pereiopod *(14)*		
	1st	
1	length vs scaphocerite	not past scaphocerite
2	chelae finger length vs palm	Longer
	chelae palm shape	Broad
3	upper dactylus, lower propodus	Convex
	cutting edge dactylus/propodus	Straight
4	carpus length vs chelae	slightly longer
	merus vs carpus length	Subequal
5	subdivided segments	None
	2nd	
6	merus+ischium vs carpus+chelae	Equal
7	merus posterior margin setae	stiff & curved
8	articulatlation (carpus)	21-32
9	articulatlation (merus)	11-17
10	articulatlation (Ischium)	0 (4 minor subdivisions)
11	3rd - 5th	about same shape
12	**3rd** see Hobbs *et al*., 1977	matches description
13	**4th** see Hobbs *et al*., 1977	matches description
14	**5th** see Hobbs *et al*., 1977	matches description
	Pleopod *(3)*	
	1st	
1	coupling hooks (male)	Absent
	2nd	
2	2nd appendix masculina (male)	Present
3	Gender	male or female
Mouth Part *(6)*		
	Mandible	
1	molar process	Stout
	incisor process	Absent
	3-jointed palp	Present
	1st & 2nd Maxillae	
2 & 3	see Hobbs *et al*., 1977	matches description
	1st-3rd Maxilliped	
4–6	see Hobbs *et al*., 1977	matches description

TABLE A3. morphological characters used to evaluate sexual dimorphism in place of characters used in data for 1st and 2nd pleopods

Character *(# of characters used)*		**prescribed state**
Pleopod *(2)*		
	2nd	
1	appendix masculina setae	5-9 or 5-7
2	appendix masculina vs interna	1/2 to 2/3