DNA Barcoding of echinopluteus larvae uncovers cryptic diversity in Neotropical echinoids

Rachel Collin[1*], Dagoberto E. Venera-Pontón[1], Amy C. Driskell[2], Kenneth S. Macdonald[2], Laura Geyer[1], Harilaos A. Lessios[1], Michael J. Boyle[3]

[1]Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Balboa Ancon, Panama.
[2]Laboratories of Analytical Biology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA
[3]Smithsonian Marine Station, Fort Pierce, Florida

[*]Corresponding author: e-mail: collinr@si.edu; (202) 633-4700 x28766. Address for correspondence: STRI, Unit 9100, Box 0948, DPO AA 34002, USA.

ORCID
Rachel Collin: https://orcid.org/0000-0001-5103-4460
Dagoberto E. Venera-Pontón: https://orcid.org/0000-0003-3320-3616
Amy C. Driskell: https://orcid.org/0000-0001-8401-7923
Kenneth S. Macdonald: https://orcid.org/0000-0002-0923-2460
Laura B. Geyer: 0000-0002-1532-197X
Harilaos A. Lessios: https://orcid.org/0000-0003-4726-1382
Michael J. Boyle: https://orcid.org/0000-0002-7287-1353

Journal: Invertebrate Biology
Keywords: Panama, Caribbean, sea urchin, meroplankton, Tropical East Pacific

April 2020

Abstract

Surveys of larval diversity consistently increase biodiversity estimates when applied to poorly-documented groups of marine invertebrates such as phoronids and hemichordates. However, it remains to be seen how helpful this approach is for detecting unsampled species in well-studied groups. Echinoids represent a large, robust, well-studied macrofauna, with low diversity and low incidence of cryptic species, making them an ideal test case for the efficacy of larval barcoding to discover diversity in such groups. We developed a reference dataset of DNA barcodes for the shallow-water adult echinoids from both coasts of Panama and compared them to DNA sequences obtained from larvae collected primarily on the Caribbean coast of Panama. We sequenced mitochondrial cytochrome *c* oxidase subunit I (COI) for 43 species of adult sea urchins to expand the number and coverage of sequences available in GenBank. Sequences were successfully obtained for COI and 16S ribosomal DNA from 272 larval and assigned to 17 Operational Taxonomic Units (OTUs): 4 from the Pacific coast of Panama, where larvae were not sampled as intensively, and 13 from the Caribbean coast. Of these 17 OTUs, 13 were identified from comparisons with our adult sequences and belonged to species well-documented in these regions. Another larva was identified from comparisons with unpublished sequences in the Barcode of Life Database (BOLD) as belonging to *Pseudoboletia*, a genus scarcely known in the Caribbean and previously unreported in Panama. Three OTUs remained unidentified. Based on larval morphology, at least two of these OTUs appear to be spatangoids, which are difficult to collect and whose presence often goes undetected in standard surveys of benthic diversity. Despite its ability to capture unanticipated diversity, larval sampling failed to collect some species that are locally common along the Caribbean coast of Panama, such as *Leodia sexiesperforata*, *Diadema antillarum*, and *Clypeaster rosaceus*.

Introduction

Surveys of adults are typically used for documenting the diversity of marine animals but they may be of limited value for taxa whose adults are difficult to collect or occur at depths not easily reached with SCUBA diving. In the few cases where larval diversity has been surveyed, it has resulted in the discovery of more or different species than those documented in adult surveys from the same location (Barber & Boyce 2006; Collin, Venera-Pontón, Driskell, Macdonald, & Boyle, 2019a;b; Collin et al. 2019; Mahon, Thornhill, Norenburg, & Halanych, 2010). This pattern may be due in part to the choice of study taxa, as these previous studies focused nemerteans, phoronids, and hemichordates, groups in which adult diversity is likely to be severely underestimated. Regardless of the reasons for the discrepancy, these results suggest that it is vital to include larvae in efforts to document the diversity of marine invertebrates.

Here we use sea urchins as a test case in this proof-of-concept study to determine how well larval diversity reflects local adult diversity in a well-documented, low-diversity (~1,000 extant species; Kroh & Mooi 2020) class of marine invertebrates. Echinoids have a long history of population genetic and taxonomic research, which should provide a strong base for genetic barcoding allowing the ready identification of larvae of common species. Regular echinoids are large and conspicuous in many marine habitats, where they are well-known eco-system engineers that play important roles as herbivores in coral reef, rocky reef, and seagrass meadows (Birkeland, 1989; Heck & Valentine, 1995; Kuempel & Altieri, 2017; Lessios, 2016; Ling *et al.*, 2015; Perkins, Hill, Foster, & Barrett, 2015; Valentine & Heck, 1991). Both regular and irregular echinoids can contribute significantly to erosion or bioturbation (Asgaard & Bromley 2008; Bak, 1994; Davidson & Grupe, 2015; Telford, Mooi, & Harold, 1987). Thus, diversity surveys targeting planktonic larvae may provide rapid discovery and documentation of these functionally important taxa.

The taxonomy and phylogeography of neotropical echinoids have been well-documented (Coppard & Lessios 2017; Coppard, Zigler, & Lessios, 2013; Hendler, Miller, Pawson, & Kier, 1995; Lessios, 2005; Lessios, Kessing, & Pearse, 2001; Lessios, Kane, & Robertson, 2003; Lessios et al. 2012; Zigler & Lessios 2004), and the shallow-water fauna is considered to be well known in the Caribbean. Despite being the subject of numerous genetic studies, global reference databases (e.g., BOLD and GenBank) contain few sequences from neotropical echinoid taxa for the "barcode" fragment of cytochrome *c* oxidase subunit I (COI) and the fragment of ribosomal

16S (16S) most commonly used for biodiversity studies. This is an important knowledge gap as assessments of biodiversity now focus on metabarcoding analyses of bulk mixed samples such as gut contents (Berry et al., 2015; Leray et al., 2013), settlement plates (Zaiko et al., 2016), plankton samples (Bucklin, Lindeque, Rodriguez-Ezpeleta, Albaina, & Lehtiniemi, 2016), or environmental DNA from sediment samples (Fonseca et al. 2010; Fonseca, Fontaneto, & Di Domenico, 2018), or water samples (Stat et al., 2017; Yamamoto et al., 2017). These samples are generally analyzed with markers that amplify across the most diverse set of taxa. The most commonly used markers for studies of species-level diversity are a fragment at the 5' end of the COI (Bucklin 2011), and a fragment of 16S for some taxa (Moura et al. 2008; Pruski & Miglietta, 2019) . When taxon coverage in the reference databases (e.g., GenBank and BOLD) is nearly complete, unknown samples can be identified to genus or species. However, with poor coverage, saturation in rapidly evolving markers means that they may fail entirely to provide useful information to aid in sample identification (Ekrem, Willassen, & Stur, 2007; Kvist 2013; Vences, Thomas, Bonett, & Vieites, 2005).

As part of a larger effort to document the diversity of Neotropical marine invertebrates by surveying the diversity of their planktonic larval stages on both coasts of Panama, we collected, photographed, and DNA-barcoded echinoid larvae (echinoplutei) from Bocas del Toro on the Caribbean coast and from the Bay of Panama on the Pacific coast. We compared the resultant barcode sequences to other sequence data generated from a tissue collection of adult Neotropical echinoids to answer the following questions: (1) Does larval diversity adequately capture the known local adult diversity? (2) Does larval diversity better capture soft-bottom and deep-water species that may be difficult to obtain as adults? Our structured sampling in the Caribbean also provides information on the density of echinoplutei in shallow waters.

Materials and Methods

Sample Collection and handling – Caribbean samples were collected from Bahia Almirante in Bocas del Toro Province on the Caribbean coast with a 0.5m diameter 125μm mesh plankton net towed behind a small boat that was moving slowly. In 2013, Caribbean larvae were collected incidentally as part of the Larval Invertebrate Diversity, Form and Function short-course at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station (BRS). Samples were collected over 2 weeks from various sites around Bahia Almirante in July, 2013 and sorted

by a team of 12 students. In 2015-2016, structured sampling involved a campaign of four Caribbean plankton surveys evenly spaced throughout a year (August 2015, November 2015, February-March 2016, and June 2016). Each survey consisted of three to five tows conducted on different days over a 9-day interval, between 7am and 9am. All tows were conducted at the same location in the channel between Isla Colon and Isla Cristobal (latitude: 09° 20' 8.9" N to 09° 20' 36.3" N; longitude: 82° 15' 41.0" W to 82° 15' 50.0" W). A flowmeter was attached to the mouth of the net to determine the volume of water sampled, and a depth meter confirmed that tows maintained a depth of 10-20m. Samples were sorted under a stereomicroscope, and echinoplutei were moved to dishes of filtered sea water. The 2015-2016 Caribbean samples were sorted exhaustively, providing data on larval density. Other sets of plankton samples were collected periodically during the same time span in the Bay Panama, on the Pacific coast, where echinoid larvae were rare, but those encountered were processed in the same way. Individual larvae were photographed alive, often moving, in a depression slide under a dissecting microscope prior to preservation for DNA sequencing. Notes were taken on the overall appearance, morphological details, and approximate size of each larva before they were preserved. When available, at least six larvae were preserved for each morphotype on each tow.

Tissue samples from adult specimens of 40 species, collected mainly from the Caribbean and Pacific coasts of Panama over the last 30 years by multiple collectors (see Acknowledgements), were preserved in high salt DMSO buffer or 95% ethanol. In a few cases, specimens came from localities other than Panama (See Table 1 for details). All shallow-water specimens were identified to species. Additionally, some adult echinoids were collected by trawling during a 2011 cruise of the RV "Miguel Oliver" from deeper waters off the Caribbean coast of Panama and Costa Rica. Tissues from these cruise samples were treated in the same way as the larvae (described below). Samples from the cruise were vouchered at the Smithsonian National Museum of Natural History. Due to the poor condition of the material and the difficulty identifying deeper water echinoids, two of the three could only be identified to genus. Detailed information about collecting localities are available in the BOLD and GenBank records of these specimens.

Larval DNA Extraction and Sequencing – Individual larvae were preserved in 150 µl of M2 extraction buffer (AutoGen), frozen and shipped to the Smithsonian's Laboratories of Analytical

Biology (LAB) for extraction and sequencing. Larval samples were extracted using an AutoGenprep 965 extraction robot after overnight digestion in the AutoGen buffer with proteinase-K. The resuspension volume of extracted DNA was 50 µl. The DNA barcode fragment of the cytochrome c oxidase subunit I (COI) was amplified using primarily the primer pair jgLCO1490/jgHCO2198 (Geller et al., 2013), although the pairs dgLCO1490/dgHCO2198 (Meyer, 2003) and COIceF/COIceR (Hoareau & Boissin, 2010) were also used. For amplification and sequencing of the 3' end of COI, the primer pair COIf/COIa (Palumbi & Benzie, 1991) were used. Primers for the amplification and sequencing of the 18S region were EukF/SR7 (Medlin, Elwood, Stickel, & Sogin, 1988). The 10 µl PCR cocktail for COI and the occasional 18S included 5 µl GoTaq Hot Start Mix (Promega), 0.1 µl BSA, and 0.3 µl of each 10 mM primer. For amplification and sequencing of 16S, the primer pairs 16S AR/16S BR (Palumbi et al., 1991) or 16SL2/16SH2 (Arnedo, Orom, & Ribera, 2001; Schubart, Cuesta, & Felder 2002) were used. The cocktail for 16S used Biolase Taq (Bioline) with the addition of 0.5 µL 50 mM $MgCl_2$. The annealing temperature for the 5' ends of COI, 16S, and 18S was 50° C; the annealing temperature for the 3' end of COI was 48°C.

Adult DNA Extraction and Sequencing – Total DNA was extracted by Proteinase K digestion from gonads as described in Lessios, Kessing, Wellington, & Graybeal (1996). In cases where this method did not recover adequate DNA for polymerase chain reaction (PCR), a subsequent extraction was obtained using the DNeasy Blood and Tissue Kit (Qiagen). The barcoding segment of COI was amplified from each specimen using either the primers dgLCO1490/dgHCO2198 (Meyer, 2003) or jgLCO149/jgHCO2198 (Geller, Meyer, Parker, & Hawk, 2013). Each sample was amplified using 0.04 units/µl of GoTaq® Flexi Polymerase (Promega) and with a final concentration of 1.6 mM $MgCl_2$. The PCR consisted of an initial denaturation incubation at 96°C for 5s, followed by 40 cycles of 94°C for 30s, 50°C for 45s, and 72°C for 1 minute, followed by a final extension step of 72°C for 5 minutes. Each PCR product was prepared for sequencing by incubation with 0.4 units of Exonuclease I and 0.075 units of Shrimp Alkaline Phosphatase per µL of PCR product suspension for 30 min at 37°C, followed by 15 min at 80°C. Each specimen was then cycle sequenced with Big Dye Terminator v3.1 (Applied Biosystems) using the amplification primers, according to the manufacturer's

recommendations. The sequenced samples were analyzed on a 3500xL Genetic Analyzer (Applied Biosystems).

DNA Sequence Analysis – Sequences were screened for quality and aligned to produce contigs of forward and reverse amplicons with Sequencher 5.4.6 (Gene Codes). Only sequences with more than 90% of the expected length and with a Phred quality score of at least 30 for more than 85% of the bases were combined into contigs and used for analysis. To check for potential contamination, sequences from our project were compared to each other within the BOLD project workbench (www.boldsystems.org) and to sequences available in GenBank using BLAST searches. Suspected products of contamination were removed from our datasets.

Neighbor joining trees with Kimura's two parameter model (K2P) distances were constructed using BIONJ (Gascuel, 1997) separately from our COI or 16S sequences with other COI or 16S sequences available in GenBank or BoLD for echinoid species known from Panama. COI alignments were inferred with the BoLD aligner [amino acid based Hidden Markov Model (Ratnasingham & Hebert, 2007)] and 16S was aligned using ClustalX (www.clustal.org); these automatic alignments were followed by manual editing. Operational Taxonomic Units (OTUs) were identified with the Automatic Barcode Gap Discovery method (ABGD; Puillandre, Lambert, Brouillet, & Achaz, 2012). The parameters for the ABGD analyses were: P_{min}: 0.001; P_{max}: 0.1, Steps: 10; X (relative gap width): 1.; Distance: Kimura (K80); TS/TV: 2. Most larvae could be easily identified on the trees as they clearly fell into monospecific clades that included known adults, had very small intra-clade divergences (typically <1%) for both the COI and the 16S markers and were separated from other such clades by larger inter-clade divergences (generally >5%). The only case where more than one named species fell into such a group is that of the *Lytechinus williamsi* Chesher, 1968 - *L. variegatus* (Lamarck, 1816) clade (Zigler & Lessios, 2004). We considered this entire clade and its sister clade, which is hardly divergent and is made up of the remaining *L. williamsi* samples, to be a single OTU. DNA sequences generated by this project have been deposited in GenBank (accession numbers: MN708558- MN708799 for COI from larvae; MN701199-MN701462 for 16S from larvae; MN683880-MN683991 for COI of adults), and BoLD (dataset DOI XXX for the adults and XXX for the larvae). Alignments are archived on FigShare at: 10.6084/m9.figshare.12141249

Results

Echinoid larvae were very abundant in Bocas del Toro, reaching densities of 39/m^3 in some tows (mean = 11.6; s.d. = 10.8; min = 3.8). Overall, we preserved 273 Caribbean echinoid larvae for DNA sequencing and obtained 228 COI and 249 16S sequences from them. In contrast, echinoplutei were uncommon in samples from the Bay of Panama and only 15 larvae were collected for sequencing.

Molecular Identification of the Larvae

Larval COI and 16S sequences fell into 17 OTUs, 13 from Bocas del Toro and four from the Bay of Panama (Figure 1). Most of these OTUs were > 5% divergent from each other in both COI and 16S fragments. The only exceptions were *Echinometra viridis* A. Agassiz, 1863 and *Echinometra lucunter* (Linnaeus, 1758), which had a pairwise distance of 3% in COI and ~2% in 16S. These both diverged from *Echinometra vanbrunti* A. Agassiz, 1863 by ~4.5% with 16S. Twelve of the larval OTUs contained a sequenced adult, with similarities >99% between the larval and adult sequences. We took this to indicate that they were conspecific. The larval OTUs from the Pacific were identified in this manner as belonging to *Toxopneustes roseus* (A. Agassiz, 1863), *Echinometra vanbrunti*, and *Agassizia scrobiculata* Valenciennes, 1846, while one (OTU P1), composed of 10 larvae, remained unidentified. The closest BLASTn matches to OTU P1 were *Abatus agassizii* Mortensen, 1910, *Schizaster doederleini* (Chesher, 1972) and *Brissopsis* sp., all of which showed 82-84% sequence similarity in COI, and the closest 16S match had 88% sequence similarity with *Schizaster doederleini*. This may indicate that OTU P1 is likely a spatangoid but the best matches are so distant that we cannot with confidence identify the genus to which it belongs. Reference sequences from adults collected in the Tropical Eastern Pacific which could be eliminated as potential matches for the unidentified OTU P1 included the following: *Tripneustes depressus* A. Agassiz, 1863, *Eucidaris thouarsi* (L. Agassiz & Desor, 1846), *Lovenia cordiformis* A. Agassiz, 1872, *Metalia nobilis* Verrill, 1867, *Rhyncholampas pacificus* (A. Agassiz, 1863), *Diadema mexicanum* A. Agassiz, 1863, *Astropyga pulvinata* (Lamarck, 1816), *Arbacia stellata* (Blainville, 1825; ?Gmelin, 1791), *Mellitella stokesii* (L. Agassiz, 1841), and *Echinothrix diadema* (Linnaeus, 1758), as well as various species of *Mellita* and *Encope* for which barcode fragments of COI have already been published (Coppard & Lessios, 2017; Coppard et al., 2013).

The larval OTUs from the Caribbean were identified as belonging to *Tripneustes ventricosus* (Lamarck, 1816) (1 larva), *Echinometra lucunter* (10 larvae), *Echinometra viridis* (22 larvae), *Brissus unicolor* (Leske, 1778) (19 larvae), *Eucidaris tribuloides* (Lamarck, 1816) (33 larvae), *Meoma ventricosa ventricosa* (Lamarck, 1816) (1 larva), *Mellita quinquiesperforata* (Leske, 1778) (16 larvae), *Clypeaster subdepressus* (Gray, 1825) (4 larvae) and an OTU of 133 larvae of *Lytechinus variegatus* or *L. williamsi*, most of which cannot confidently be distinguished based on mitochondrial genes (Zigler & Lessios, 2004). In addition, there were four larval OTUs (one with 10 larvae, one with 6 larvae and 2 singletons) that did not match any of the sequences generated from our shallow-water adult echinoid collections. One of these matched a deep-water *Brissopsis* sp. from 125 m collected by the RV "Miguel Oliver" off the coast of Costa Rica (Table 1; Figure 2). Reference sequences from adults collected in coastal Caribbean waters which could be eliminated as potential matches for the 3 remaining unidentified OTUs were: *Plagiobrissus grandis* (Gmelin, 1791), *Brissopsis atlantica* Mortensen, 1907, *Leodia sexiesperforata* (Leske, 1778), *Diadema antillarum* Philippi, 1845, *Astropyga magnifica* A.H. Clark, 1934, *Arbacia punctulata* (Lamarck, 1816), and *Clypeaster rosaceus* (Linnaeus, 1758).

One of the singleton larvae was indexed in BOLD with the same Barcode Index Number (BIN; Ratnasingham and Hebert 2013) as *Pseudoboletia indiana* (Michelin, 1862) and other unidentified *Pseudoboletia* species from Australia, Hawaii and the Atlantic (Figure 3). There is little sequence divergence between what are presumably different species based on the current understanding of *Pseudoboletia* taxonomy and biogeography (Lopes, Ferreira, & Ventura, 2017; Zigler, Byrne, Raff, Lessios, & Raff, 2012;). The COI barcode sequence from this OTU was >98% identical to a COI sequence (KC626175) reported as *Lytechinus euerces* H.L. Clark, 1912 in Bribiesca-Contreras, Solís-Marín, Laguarda-Figueras, & Zaldívar-Riverón (2013). However, based on its position in the Neighbor-joining tree presented in Figure 3, KC626175 is clearly misidentified in GenBank. In addition, the 16S sequence we generated from a true adult *L. euerces* (identified by HAL) was not similar (divergence = 9.7%) to the 16S sequences from this singleton larva, further supporting the conclusion that this larva does not belong to *L. euerces*. Unfortunately, no published 16S sequences from *Pseudoboletia* species are available to make additional comparisons. Sequences of the 3' end of COI from our larva failed to amplify

and could therefore not be put into the context of the most recent molecular phylogeny of *Pseudoboletia* (Lopes et al., 2017).

Further investigation of the two remaining unidentified OTUs included sequencing of the 3' end of the COI gene, which is most commonly used in studies of echinoid genetics and phylogeography, and a section of the 18S gene. Nevertheless, these markers did not help in identifying our unknowns. OTU C1 could not be identified with any confidence using BLASTn searches, as the Folmer fragment of COI has 83% identity with a species of *Araeosoma* in GenBank, while the 16S fragment has 84% identity with *Echinolampas crassa* (Bell, 1880), showing that this OTU could not be identified even to family. The photograph of the single larva attributable to this OTU is difficult to interpret as the animal was undergoing metamorphosis and no definitive features are visible. OTU C2 appears most closely related to OTU P1 in our dataset and BLASTn searches indicate that their COI barcode fragment is ~84% identical to an *Encope* species, and the 16S fragment is ~88% identical to *Brisaster* and *Schizaster* species in GenBank. The distinctive morphology of the larvae of OTUs P1 and C2, with fenestrated arm rods and a posterior process, supports their identity as spatangoids (Figure 2). Despite collecting several larvae with large rudiments (Figure 2), we did not find any with a 5th pair of arms or with a pair of lateral processes, suggesting that these features, which are often but not always present in spatangoid larvae (Rees 1953; Nunes & Jangoux, 2007), are absent in these two species.

Discussion

The sea urchin fauna of the Caribbean is well-known and well-studied. Nevertheless, sequences of the DNA barcode fragments of COI and 16S for many of the abundant species were not present in BOLD and/or GenBank. The efficacy of studies relying on barcodes to identify unknown material depends on good taxonomic coverage within a reference database (Ekram et al., 2007). This weakness in the reference set for tropical echinoids is a common hurdle in studies seeking to identify wild-caught larvae (e.g., Collin et al., 2019a,b,c; Webb, Barnes, Clark, & Bowden, 2006). Our addition of 43 species from the Caribbean and Pacific of Central America should improve the barcode reference set, which now includes both the 5' end of COI and a fragment of 16S for most of the abundant species from the region. Missing from our set of data are other shallow-water taxa that are rare or may be absent from Panama, such as the following Caribbean species *Stylocidaris affinis* (Philippi, 1845), *Echinoneus cyclostomus* Leske, 1778,

Cassidulus caribaearum Lamarck, 1801, *Clypeaster luetkeni* Mortensen, 1948, *Clypeaster chesheri* Serafy, 1970, *Moira atropos* (Lamarck, 1816), *Schizaster floridiensis* Kier & Grant, 1965, *Schizaster doederlein, Brissopsis elongata* Mortensen, 1907, *Genocidaris maculata* A. Agassiz, 1869 , *Homolampas fragilis* (A. Agassiz, 1869); and the eastern Pacific species *Lytechinus panamensis* Mortensen, 1921, *Plagiobrissus pacificus* H.L. Clark, 1940, *Hesperocidaris asteriscus* H.L. Clark, 1948, *H. perplexa* (H.L. Clark, 1907), *Arbacia spatuligera* (Valenciennes, 1846), *Clypeaster elongatus* H.L. Clark, 1948, *Clypeaster europacificus* H.L. Clark, 1914, *Clypeaster ochrus* H.L. Clark, 1914, *Clypeaster rotundus* (A. Agassiz, 1863), *Clypeaster speciosus* Verrill, 1870, and *Brissopsis pacifica* (A. Agassiz, 1898). In addition, upon comparison with our adult sequence data we noticed a few taxa for which GenBank sequences seem to be assigned to the wrong species. These include some sequences of *Echinometra lucunter* that were identified as *Arbacia punctulata* (KC626142), *Pseudoboletia* sp. identified as *Lytechinus euerces* (KC626175) and an *E. lucunter* sequence that belongs to *E. vanbrunti* (AY262883). As there are so few reference sequences available for tropical echinoids such misidentifications could cause confusion, as they have been known to do in other groups (Tixier, Hernandes, Guichou & Kreiter, 2012; Beaz-Hidalgo, Hossain, Liles & Figueras, 2015; Li et al. 2018).

Larval diversity is not expected to reflect perfectly the known or actual resident adult diversity. Species with direct or lecithotrophic development, narrow reproductive seasons, or demersal larvae are likely to be under-sampled in plankton surveys. This could explain why the known species diversity of the Caribbean coast of Panama is under-represented in our larval samples. However, this may also be due to our sampling at a single location within a semi-enclosed bay. Our larval samples represent some of the most abundant shallow-water species in Bahia Almirante, including both species of *Echinometra* and both species of *Lytechinus*, as well as *Eucidaris tribuloides* and *Clypeaster subdepressus*. These samples also captured an appreciable number of *Brissus unicolor* and *Mellita quinquiesperforata*, species that have not been collected in Bahia Almirante, but which are likely abundant, as there are large areas with appropriate soft-bottom habitats. It is not surprising that larvae of *C. rosaceus* and *A. punctulata* were not collected, as the former has short-lived, facultatively feeding larvae whilst the latter is an errant species that has not been observed in the bay. It *is* surprising though that *D. antillarum* larvae were not detected because this species is fairly abundant (for post mass-mortality

population levels), the larvae are particularly long-lived and distinctive (Eckert, 1998), and reproduction in Panama, though lunar, is not seasonal (Lessios, 1981). Our sampling strategy was designed to be evenly spaced across the year, but could have missed species with very narrow reproductive periods, if such periods occurred between our quarterly sampling campaigns.

Larval barcoding can improve detection and identification of species that (1) occur below easy-collecting depths, (2) are fragile and difficult to collect intact as adults, (3) live deeply buried in the sediment, or (4) live far away but have particularly long-lived larvae which may occasionally arrive but fail to recruit to an area. Two of our unidentified OTUs appear to be spatangoids. Spatangoids live fully covered by sediment and can be particularly challenging to collect intact. Our samples also include a number of other spatangoids which are not commonly encountered as adults without specialized collecting equipment. The few other studies that have compared diversity discovered through barcoding of larvae and adults have also demonstrated that larval barcoding usually documents the presence of species that have not been recovered from studies of the local adult fauna (e.g., Barber & Boyce, 2006; Brandão, Freire & Burton, 2016; Collet et al., 2018). In the poorly-studied, diverse tropics this appears likely to result from inadequate sampling of adult diversity. However, a recent study from Chukchi Sea shows that current-driven patterns of larval occurrences combined with limited distribution of appropriate adult habitats in this unique region leads to a geographic mis-match between larval and adult communities, with larvae of coastal species occurring far offshore (Ershova et al., 2019).

Another OTU not identified by our shallow-water adult reference set is a species of *Pseudoboletia*, a genus of toxopneustids that resembles *Lytechinus* in their pale color (Lopes et al., 2017; Zigler et al., 2012). *Pseudoboletia maculata* Troschel, 1869 has been reported from the southeastern US and the Gulf of Mexico north of Yucatan, whereas *Pseudoboletia occidentalis* H.L. Clark, 1921 has been reported from Barbados and Antigua (Lopes et al., 2017). Neither of these species have DNA barcode sequences in GenBank or BOLD, they are rarely reported in the tropical western Atlantic, and their taxonomy is in dire need of revision. Species within the genus are distinguished by differences in skeletal morphology, but their inter-specific genetic divergence between their COI sequences is very low, causing sequences from *P. indiana* collected from Hawaii and Australia to occur in the same BIN as our larval sequences. The COI sequence of our larval OTU is 0.01-0.6% divergent from BOLD sequences obtained from adult

animals collected in São Tomé by Endre Willassen and colleagues (Figure 3). The 3' end of COI was sequenced for different individuals from São Tomé (Zigler et al., 2012) and found to be the same as animals from southern Brazil (Lopes et al., 2017). This raised the intriguing possibility of the presence of a wide-spread, but virtually undocumented, species of sea urchin in the tropical Atlantic. Such wide distributions through the tropical Atlantic are known for *E. lucunter* and *E. tribuloides* (Lessios, Kessing, Robertson & Paulay, 1999; McCartney, Keller & Lessios, 2000). Although we were not able to identify our larva to species, this observation is the first record of any *Pseudoboletia* between Mexico and Barbados. The larva collected from Bocas del Toro was relatively early in development, without a developed rudiment, suggesting that its parental source was nearby. The sequence KC626175, reportedly from *Lytechinus euerces*, appears to represent an additional observation of *Pseudoboletia* from eastern Yucatan (Bribiesca-Contreras et al., 2013). Therefore, despite the inability of larval sampling to detect several species that are locally abundant as adults, it can nevertheless produce surprising discoveries.

Acknowledgements

This work was supported by the Smithsonian Institution and an anonymous donor. Work with the larvae was performed with permission from the Panamanian Ministry of the Environment (MiAmbiente) permit numbers SC/AP-5-15 and SEX/P-58-15 (2015), SE/S-79-16 (2016), and SEX/P-33-17 and from the ARAP Collecting permit No. 47 in 2013 and No. 06 in 2014 and export permit Nos. 37 and 80 (2013-2014) to RC. We thank Leyre Villotta Nieva and the students of the Larval Invertebrate Diversity, Form and Function short-course for help collecting larvae, Ross Robertson (STRI) and the crew of the RV "Miguel Oliver" for collecting the off-shore animals, and Chris Mah (USNM) for identifying them. We thank Gustav Paulay and Endre Willassen for sharing unpublished sequences of *Pseudoboletia* with us. Numerous collectors have contributed adult specimens over a number of years: P. Blackwell, Axel Calderón, Bailey Kessing, Javier Jara, Shannon Lee, Carlos Prada, D. Ross Robertson, Felix Rodriguez, Astrid Terry, Craig Young, and Kirk Zigler. Some molecular work was completed in and supported by the Laboratories of Analytical Biology, National Museum of Natural History. This publication is Smithsonian Marine Station Contribution No. 1137.

Literature Cited

Arnedo, M.A., Orom, P., & Ribera, C. (2001). Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. *Cladistics, 17*, 313-353.

Asgaard, U., & Bromley, R.G. (2008). Echinometrid sea urchins, their trophic styles and corresponding bioerosion. pp. 279-303. In. M. Wisshak and L. Tapanila (Eds). *Current Developments in Bioerosion*. Springer Berlin Heidelberg, pp. 499.

Bak, R.P.M. (1994). Sea urchin bioerosion on coral reefs: place in the carbonate budget and relevant variables. *Coral Reefs, 13*, 99-103.

Barber, P., & Boyce, S.L. (2006). Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. *Proceedings of the Royal Society, London B: Biological Sciences, 273*, 2053-2061.

Beaz-Hidalgo, R., Hossain, M.J., Liles, M.R. & Figueras, M.J. (2015). Strategies to avoid wrongly labelled genomes using as example the detected wrong taxonomic affiliation for *Aeromonas* genomes in the GenBank database. *PloS one, 10*(1), e0115813.

Berry, O., Bulman, C., Bunce, M., Coghlan, M., Murray, D. C., & Ward, R. D. (2015). Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series, 540*, 167-181.

Birkeland, C. (1989). The influence of echinoderms on coral-reef communities. *Echinoderm Studies, 3*, 1-79.

Brandão, M.C., Freire, A.S. & Burton, R.S., (2016). Estimating diversity of crabs (Decapoda: Brachyura) in a no-take marine protected area of the SW Atlantic coast through DNA barcoding of larvae. *Systematics and Biodiversity, 14*(3), 288-302.

Bribiesca-Contreras, G., Solís-Marín, F. A., Laguarda-Figueras, A., & Zaldívar-Riverón, A. (2013). Identification of echinoderms (Echinodermata) from an anchialine cave in Cozumel Island, Mexico, using DNA barcodes. *Molecular Ecology Resources, 13*(6), 1137-1145.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. (2011). DNA barcoding of marine metazoa. *Annual Review of Marine Science, 3*, 471-508.

Bucklin, A., Lindeque, P.K., Rodriguez-Ezpeleta, N., Albaina, A., & Lehtiniemi, M. (2016). Metabarcoding of marine zooplankton: prospects, progress and pitfalls. *Journal of Plankton Research, 38*(3), 393-400.

Collet, A., Durand, J.D., Desmarais, E., Cerqueira, F., Cantinelli, T., Valade, P. & Ponton, D., (2018). DNA barcoding post-larvae can improve the knowledge about fish biodiversity: an example from La Reunion, SW Indian Ocean. *Mitochondrial DNA Part A, 29*(6), 905-918.

Collin, R., Venera-Pontón, D.E., Driskell, A.C., Macdonald, K.S., & Boyle, M.J. (2019a). Planktotrophic brachiopod larvae from the Pacific and Caribbean of Panama. *Diversity, 11*, 2.

Collin, R., Venera-Pontón, D.E., Driskell, A.C., Macdonald, K.S., & Boyle, M.J. (2019b). Unexpected molecular and morphological diversity of hemichordate larvae from the Neotropics. *Invertebrate Biology*, DOI: 10.1111/ivb.12273

Collin, R., Venera-Pontón, D.E., Driskell, A.C., Chan, K-Y.K. Macdonald, K.S. & Boyle, M.J. (2019). Documenting Neotropical diversity of phoronids with DNA barcoding of planktonic larvae. *Invertebrate Biology*, 138, e12242. https ://doi.org/10.1111/ivb.12242.

Coppard, S.E. & Lessios, H.A. (2017). Phylogeography of the sand dollar genus *Encope*: implications regarding the Central American Isthmus and rates of molecular evolution. *Scientific Reports*, 7, (1):https://doi.org/10.1038/s41598-017-11875-w

Coppard, S.E., Zigler, K.S., & Lessios, H.A. (2013). Phylogeography of the sand dollar genus *Mellita*: cryptic speciation along the coasts of the Americas. *Molecular Phylogenetics and Evolution, 69(3),* 1033–1042. https://doi.org/10.1016/j.ympev.2013.05.028

Davidson, T. M., & Grupe, B. M. (2015). Habitat modification in tidepools by bioeroding sea urchins and implications for fine-scale community structure. *Marine Ecology, 36*(2), 185-194.

Eckert, G. L. (1998). Larval development, growth and morphology of the sea urchin *Diadema antillarum*. *Bulletin of Marine Science*, *63*(2), 443-451.

Ekrem, T., Willassen, E., & Stur, E. (2007). A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution, 43(2),* 530-542.

Ershova, E., Descoteaux, R., Wangensteen, O., Iken, K., Hopcroft, R., Smoot, C., Grebmeier, J.M. & Bluhm, B.A. (2019). Diversity and distribution of meroplanktonic larvae in the Pacific Arctic and connectivity with adult benthic invertebrate communities. *Frontiers in Marine Science, 6,* 490.

Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K. & Creer, S. (2010). Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature Communications, 1*, p.98.

Fonseca, G., Fontaneto, D., & Di Domenico, M. (2018). Addressing biodiversity shortfalls in meiofauna. *Journal of Experimental Marine Biology and Ecology*, *502*, 26-38.

Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution, 14(7),* 685-695.

Geller, J., C. Meyer, M. Parker, & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources, 13*, 851-861.

Hendler, G., Miller, J., Pawson, D. & Kier, P. (1995). Sea Stars, Sea Urchins, and Allies: Echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington pp. 391.

Heck, K.L. & Valentine, J.F. (1995). Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *Journal of Experimental Marine Biology and Ecology, 189*, 205-217.

Hoareau, T.B. & Boissin, E. (2010). Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. *Molecular Ecology Resources, 10*, 960-967.

Kroh, A. & Mooi, R. (2020). World Echinoidea Database. Accessed at http://www.marinespecies.org/echinoidea on 2020-04-02. doi:10.14284/355

Kuempel, C.D. & Altieri, A. H. (2017). The emergent role of small-bodied herbivores in pre-empting phase shifts on degraded coral reefs. *Scientific Reports*, *7*, 39670.

Kvist, S. (2013). Barcoding in the dark?: a critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. *Molecular Phylogenetics and Evolution*, *69*(1), 39-45.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T. & Machida, R.J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(1), p.34.

Lessios, H.A. (1981). Reproductive periodicity of the echinoids *Diadema* and *Echinometra* on the two coasts of Panama. *Journal of Experimental Marine Biology and Ecology, 50*(1), 47-61.

Lessios, H.A., Kessing, B.D., Robertson, D.R. & Paulay, G. (1999). Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. *Evolution, 53*(3), 806-817.

Lessios, H.A. (2005). Echinoids of the Pacific waters of Panama: Status of knowledge and new records. *Revista de Biología Tropical, 53 (Supplement 3)*, 147–170.

Lessios, H.A. (2016). The great *Diadema antillarum* die-off: 30 years later. *Annual Review of Marine Science, 8*, 267-283.

Lessios, H.A., Kessing, B.D. & Pearse, J.S. (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution*, 55(5), 955-975.

Lessios, H.A., Kane, J., & Robertson, D.R. (2003). Phylogeography of the pantropical sea urchin Tripneustes: contrasting patterns of population structure between oceans. *Evolution*, *57(9),* 2026-2036.

Lessios, H.A., Kessing, B.D., Wellington, G.M. & Graybeal, A. (1996). Indo-Pacific echinoids in the tropical eastern Pacific. *Coral Reefs, 15,* 133-142.

Lessios, H.A., Lockhart, S., Collin, R., Sotil, G., Sanchez-Jerez, P., Zigler, K.S., Perez, A.F., Garrido, M.J., Geyer, L.B., Bernardi, G., Vacquier, V.D., Haroun, R., & Kessing, B.D. (2012). Phylogeography and bindin evolution in *Arbacia*, a sea urchin genus with an unusual distribution. *Molecular Ecology, 21(1),* 130–144.

Li, X., Shen, X., Chen, X., Xiang, D., Murphy, R.W. & Shen, Y. (2018). Detection of potential problematic Cytb gene sequences of fishes in GenBank. *Frontiers in Genetics*, *9,* 30.

Ling, S.D., Scheibling, R.E., Rassweiler, A., Johnson, C.R., Shears, N., Connell, S.D., ... & Clemente, S. (2015). Global regime shift dynamics of catastrophic sea urchin overgrazing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370(1659),* 20130269.

Lopes, E.M., Ferreira, C.E.L., & Ventura, C.R.R. (2017). Occurrence of the genus *Pseudoboletia* (Echinoidea: Toxopneustidae) in the South-Western Atlantic: morphological and molecular approaches. *Revista de Biología Tropical*, *65*(1-1), S299-S308.

Mahon, A.R., Thornhill, D.J., Norenburg, J.L., & Halanych, K.M. (2010). DNA uncovers Antarctic nemertean biodiversity and exposes a decades-old cold case of asymmetric inventory. *Polar Biology, 33(2),* 193-202.

McCartney, M.A., Keller, G. & Lessios, H.A. (2000). Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. *Molecular Ecology*, *9*(9), 1391-1400.

Medlin, L., Elwood, H.J., Stickel, S., & Sogin, M.L. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene, 71,* 491-499.

Meyer, C.P. (2003). Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society, 79,* 401–459.

Moura, C.J., Harris, D.J., Cunha, M.R. & Rogers, A.D. (2008). DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zoologica Scripta*, *37*(1), 93-108.

Nunes, C.D.A.P & Jangoux, M. (2007). Larval growth and perimetamorphosis in the echinoid *Echinocardium cordatum* (Echinodermata): the spatangoid way to become a sea urchin. *Zoomorphology*, *126*(2), 103-119.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., & Grabowski, G. (1991). *The Simple Fool's Guide to PCR, Version 2.* Department of Zoology and Kewalo Marine Laboratory, University of Hawaii. Honolulu.

Palumbi, S.R., & Benzie, J. (1991). Large mitochondrial DNA differences between morphologically similar Penaeid shrimp. *Molecular Marine Biology and Biotechnology, 1,* 27-34.

Perkins, N.R., Hill, N.A., Foster, S.D., & Barrett, N.S. (2015). Altered niche of an ecologically significant urchin species, *Centrostephanus rodgersii*, in its extended range revealed using an Autonomous Underwater Vehicle. *Estuarine, Coastal and Shelf Science, 155*, 56-65.

Pruski, S. & Miglietta, M.P. (2019). Fluctuation and diversity of Hydromedusae (Hydrozoa, Cnidaria) in a highly productive region of the Gulf of Mexico inferred from high frequency plankton sampling. *PeerJ, 7,* e7848.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology, 21,* 1864-1877.

Ratnasingham, S., & Hebert, P.D.N. (2013). A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS One, 8(7),* e66213. https://doi.org/10.1371/journal.pone.0066213

Rees, C.B. (1953). The larvae of the Spatangidae. *Journal of the Marine Biological Association of the U.K., 32,* 477–490.

Schubart, C.D., Cuesta, J.A., & Felder, D.L. (2002). Glyptograpsidae, a new Brachyuran family from Central America: Larval and adult morphology, and a molecular phylogeny of the Grapsoidea, *Journal of Crustacean Biology, 22(1),* 28-44.

Stat, M., Huggett, M.J., Bernasconi, R., DiBattista, J.D., Berry, T.E., Newman, S.J., Harvey, E.S., & Bunce, M. (2017). Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports, 7(1),* 12240.

Telford, M., Mooi, R., & Harold, A.S. (1987) Feeding activities of two species of *Clypeaster* (Echinoides, Clypeasteroida): further evidence of clypeasteroid resource partitioning. *Biological Bulletin, 172,* 324-336.

Tixier, M.S., Hernandes, F.A., Guichou, S. & Kreiter, S. (2012). The puzzle of DNA sequences of Phytoseiidae (Acari: Mesostigmata) in the public GenBank database. *Invertebrate Systematics, 25*(5), 389-406.

Valentine, J.F. & Heck, K.L. (1991). The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology, 154,* 215-230.

Vences, M., Thomas, M., Bonett, R.M., & Vieites, D.R. (2005). Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society, Series B, 360,* 1859–1868.

Webb, K.E., Barnes, D.K., Clark, M.S., & Bowden, D.A. (2006). DNA barcoding: a molecular tool to identify Antarctic marine larvae. *Deep Sea Research Part II: Topical Studies in Oceanography*, *53*(8-10), 1053-1060.

Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., ... & Miya, M. (2017). Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*, *7*, 40368.

Zaiko, A., Schimanski, K., Pochon, X., Hopkins, G.A., Goldstien, S., Floerl, O. & Wood, S.A., (2016). Metabarcoding improves detection of eukaryotes from early biofouling communities: implications for pest monitoring and pathway management. *Biofouling, 32*(6), 671-684.

Zigler, K.S. & Lessios, H.A. (2004). Speciation on the coasts of the New World: Phylogeography and the evolution of bindin in the sea urchin genus *Lytechinus*. *Evolution, 58,* 1225-1241.

Zigler, K.S., Byrne, M., Raff, E.C., Lessios, H.A., & Raff, R.A. (2012). Natural hybridization in the sea urchin genus *Pseudoboletia* between species without apparent barriers to gamete recognition. *Evolution: International Journal of Organic Evolution*, *66*(6), 1695-1708.

Table 1: Summary of the new GenBank sequences from adults and larvae resulting from this study. BIN numbers and GenBank numbers in **bold** indicate that the sequences are the first for the species in the respective databases. The first 5 GenBank numbers are given for larval OTUs with more than 5 sequences.

OTU/Species*	Life Stage	Genbank # COI	Genbank # 16S	BIN	Location$
Caribbean taxa with adults and larvae					
Echinometra lucunter	adult	MN683905			Punta Galeta, Panama
	larvae	MN708596, MN708630, MN708664, MN708666, MN708682,	MN701242, MN701277, MN701314, MN701334, MN701376,	ABA2440	Bahia Almirante
Echinometra viridis	adult	MN683909-11			Punta Galeta, Panama
	larvae	MN708573, MN708602, MN708623, MN708635, MN708647,	MN701201, MN701215, MN701222, MN701227, MN701247,	ABA2023	Bahia Almirante
Lytechinus variegatus	adult	MN683942-45	-	ACC6921	Punta Galeta, Panama
Lytechinus williamsi	adult	MN683946-47			Bahia Almirante
Lytechinus variegatus/ L. williamsi	larvae	MN708558- 62, MN708565, MN708568-69	MN701200, MN701202-04, MN701207, MN701210-11	ACC6921	Bahia Almirante
Eucidaris tribuloides	adult	MN683933-35			Punta Galeta, Panama
	larvae	MN708563, MN708566-67, MN708578-79, MN708581, MN708588,	MN701205, MN701208-09, MN701221, MN701223, MN701225,	ABA4414	Bahia Almirante
Tripneustes ventricosus	adult	MN683990-91			Nalunega, San Blas, Panama and Carrie Bow Cay, Belize
	larvae	MN708626	MN701272	ACG7657	Bahia Almirante
Brissus unicolor	adult	MN683889-92			Cayos Cochinos, Honduras

	larvae	MN708570, MN708591, MN708631, MN708641, MN708680,	MN701212, MN701237, MN701278, MN701288, MN701332,	ADE4265	Bahia Almirante
Clypeaster subdepressus	adult	MN683897-99			San Blas, Panama
	larvae	MN708614, MN708617, MN708736, MN708740	MN701259, MN701263, MN701393, MN701397	ABA2438	Bahia Almirante
Meoma ventricosa	Adult	MN683972-93			San Blas, Panama
	larvae	MN708672	MN701322	AAX4591	Bahia Almirante
Mellita quinquiesperforata *	larvae	MN708594, MN708598, MN708600, MN708601, MN708645,	MN701240, MN701244, MN701246, MN701294, MN701306,	ACH4624	Bahia Almirante
Brissopsis sp.	Adult	MN868996		ACG7856	Costa Rica, Caribbean Sea
	larvae	MN708577, MN708587, MN708620, MN708640, MN708660,	MN701220, MN701233, MN701266, MN701287, MN701309,	ACG7856	Bahia Almirante
Caribbean Taxa with Adults only					
Diadema antillarum	adult	MN683900-01			Punta Galeta, Panama
Astropyga magnifica	adult	MN683886			Portobello, Panama
Arbacia punctulata	adult	MN683883			Carrie Bow Cay, Belize
Lytechinus euerces	adult	MN683940-41			Bahamas
Clypeaster rosaceus	adult	MN683895-96			Bahia Almirante
Paraster doederleini	adult	MN683977-78			Punta Galeta, Panama,
Araeosoma sp.	adult	MN868991-95	MN868937-39	ACG7722, ACG7724	Panama, Caribbean Sea
Brissopsis atlantica	adult	MN868997	MN868940-41		Panama, Caribbean Sea
Clypeaster euclastus	adult	MN868998-9000	MN868942-44	ACG7892	Costa Rica and Panama, Caribbean Sea

Plagiobrissus grandis	adult	MN683979-80			San Blas, Panama, Caribbean
Caribbean Taxa with Larvae only					
Pseudoboletia	larva	MN708704	MN701356	AAX7025	Bahia Almirante
OTU C1	larva	MN708608	MN701253	ADE4471	Bahia Almirante
OTU C2	larvae	MN708564, MN708604, MN708713, 78	MN701206, 26, 49, 91, MN701367, MN701439	ADE4267	Bahia Almirante
Pacific Taxa with adults and larvae					
Echinometra vanbrunti	adult	MN683907-08			Genovesa, Galapagos Islands
	larvae	MN708685	MN701199, MN701337	ADE4472	Bay of Panama
Toxopneustes roseus	adult	MN683984-86			Bay of Panama
	larvae	MN708764	MN701422		Bay of Panama
Agassizea scrobiculata	adult	MN683880-82			Panama, Eastern Pacific
	larvae	MN708572, MN708638	MN701214, 85	ADE4266	Bay of Panama
Pacific Taxa with adults only					
Centrostephanus coronatus	adult	MN683893-94			California, USA and Baja California, Mexico
Eucidaris thouarsi	adult	MN683930-35			Bay of Panama
Tripneustes depressus	adult	MN683987-89			Pacheca and Taboguilla Islands, Panama
Lovenia cordiformis	adult	MN683938-39			Bay of Panama
Metalia nobilis	adult	MN683974-75			Bay of Panama
Rhyncholampas pacificus	adult	MN683981-83			Veracruz and Chumical, Panama
Astropyga pulvinata	adult	MN683887			Panama

Arbacia stellata	adult	MN683884-85			Bay of Panama
Diadema mexicanum	adult	MN683902-04			Bay of Panama
Echinothrix diadema	adult	MN683912-13			Clipperton Island, Eastern Pacific
Pacific Taxa with larvae only					
OTU P1	larvae	MN708580, MN708622, MN708697, MN708710, MN708731,	MN701224, MN701268, MN701348, MN701362, MN701389,	ADE4473	Bay of Panama

***** Adult sequences available from Coppard et al 2013.

Figure Legends

Figure 1. Circular neighbor-joining tree of cytochrome c oxidase c subunit I DNA sequences from echinopluteus larvae collected in this study. Operational taxonomic units (OTUs) are labelled with the species name of adult sequences that either were indexed with our sequences under the same Barcode Index Number (BIN) in the Barcode of Life Database (BOLD) or fell within this OTU when both adult and larvae were analyzed together in a larger NJ tree. Unidentified OTUs from the Caribbean and Pacific are indicated with the notations C# and P# respectively. Branches with bootstrap support > 95% are labelled with black dots. The length of the branches is proportional to their divergence in substitutions per site, calculated with Kimura's two parameter distances.

Figure 2. Neighbor-joining tree of cytochrome c oxidase subunit I (COI) sequences from *Brissopsis* larvae and adults from this study, from a 2011 cruise of the RV “Miguel Oliver”, and from other *Brissopsis* adults available in GenBank. *Brissus unicolor* larvae and adults from this study were included to root the tree. Sequences from GenBank are labelled with the Accession Number and the species name. Sequences from this study and from the 2011 RV “Miguel Oliver” cruise are labeled with the specimen identification number, life stage (larvae or adult), the collection site, and are highlighted in bold. Bootstrap support values > 70% are shown at their corresponding branches. The branch lengths are proportional to the genetic divergence between sequences, measured as substitutions per site, based on the Kimura's two parameters model. To the right: **A**, ventral view of a larva of our unidentified *Brissopsis* species. **B**, dorsal view of the larva of *Brissus unicolor*. Both images were captured from live specimens. al, anterolateral arm; da, dorsal arch; dt, dorsal transverse rod; lo, lobe; pd, posterodorsal arm; pi, pigment; po, postoral arm; pl, posterolateral arm; pp, posterior process; pr, preoral arm; vt, ventral transverse rod. Scale bar = 500 μm.

Figure 3: Neighbor-joining tree of cytochrome c oxidase subunit I (COI) sequences from *Pseudoboletia* indexed in the Barcode of Life Database (BOLD) under the Barcode Index Number (BIN) AAX7025. One sequence from this BIN was collected from a larva in this study (bold). Sequences are labeled with their specimen identification number, species name provided in their study, and their collection site. Bootstrap support values > 70% are shown at their corresponding branches. The branch lengths are proportional to the genetic divergence between sequences, measured as substitutions per site, based on the Kimura's two parameters model. The tree was rooted with an outgroup sequence from to the closest BIN in BOLD.

Figure 4. Unidentified eight-armed echinopluteus larval representatives of two DNA barcode operational taxonomic units (OTUs) from Panama. **A-C**, dorsal views of three plutei from OTU P1 collected near Taboguilla Island in the Bay of Panama. **D-F**, ventral (D, F) and lateral (E) views of three plutei from OTU C2 collected in Almirante Bay of Bocas del Toro province. All images were captured from live specimens. al, anterolateral arm; da, dorsal arch; dt, dorsal transverse rod; lo, lobe; pd, posterodorsal arm; pi, pigment; po, postoral arm; pp, posterior process; pr, preoral arm; vt, ventral transverse rod. Scale bar = ~500 µm.