How I wonder what you are: Can DNA barcoding identify the larval asteroids of Panama?

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Abstract
As part of a project to document the diversity of larval invertebrates on both coasts of Panama, we collected and photographed 141 larval asteroids and sequenced fragments of their mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal DNA. We uncovered 10 Caribbean operational taxonomic units (OTUs) and five Pacific OTUs. We could identify six of the 15 OTUs based on >99% similarity with reference sequences in GenBank: The Pacific species Astropecten verrilli and Pentaceraster cumingi and the Caribbean species Astropecten marginatus, Astropecten antillensis, Oreaster reticulatus, and Mithrodia clavigera. Two other OTUs were placed in BINs in the Barcode of Life Database (BOLD) with unpublished sequences that were identified as Pharia pyramidata from the Pacific and Valvaster striatus, now known from the Caribbean as well as the Indo-West Pacific. The remaining seven species appear likely to belong to Luidia, as 16S sequences from each have 87%-95% identity with various species of Luidia, and the sequences nest among species of Luidia in neighbor-joining trees. The low diversity of asteroid larvae reflects ~10% of the diversity of adult sea stars reported from Panama, and highlights the need for broader collection efforts and improved coverage of DNA barcode reference sequences for Luidia and other soft-bottom species.

KEYWORDS
brachiolaria, Caribbean, meroplankton, Panama

1 INTRODUCTION

Surveys of marine invertebrate larvae are a powerful tool for the detection of hidden diversity (Barber & Boyce, 2006; Collin et al., 2019; Collin et al., 2019a, 2019b; Mahon et al., 2010). They may be particularly effective at capturing deep-water, small, or infaunal species that are difficult to collect as adults by use of standard sampling techniques, and thus provide an independent assessment of diversity that complements species lists based solely on studies of adults (Collin et al., 2020; Mahon et al., 2010; Sewell & Jury, 2011). In some cases, larval sampling can uncover unexpectedly high diversity (Collin et al., 2019; Mahon et al., 2010).

Here we apply this approach to compare the diversity of adult and larval asteroids from the coastal waters of Panama. A review of Caribbean biodiversity from the Census of Marine Life documented 116 species of adult asteroids in the wider Caribbean (Miloslavich et al., 2010: supplemental data table S8). Twenty-five species were reported for the Caribbean coast of Panama, less...
than the diversity reported for Cuba (35), Mexico (44), Honduras (30), Nicaragua (29), Colombia (51), and Venezuela (36), and similar to the diversity reported for Puerto Rico (22), Dominican Republic (22), Haiti (19), and Jamaica (20) using the same methodology (Miloslavich et al., 2010). Panama has been a center of marine biology research for over 50 years (Robertson et al., 2009), and it seems unlikely that the low reported diversity along the Caribbean coast of Panama is the result of less sampling effort than is typical for the region. A similar methodology was used to report 45 asteroid species for the Tropical Eastern Pacific (Miloslavich et al., 2011). Coppard and Alvarado (2013) reported 56 species for the Pacific coast of Panama alone. Unfortunately, neither of these publications on the Pacific fauna includes a list of the species reported to belong to the fauna. An earlier paper reported 51 asteroid species (Alvarado et al., 2010), but included North Pacific cold-temperate species such as Luidia foliolata (Grube 1866) and Pisaster ochraceus (Brandt 1835), whose presence in Panama would result in an extraordinary disjunct geographic distribution for each species. Regardless of these details, it seems clear that, unlike the situation in a number of other taxa, the Pacific coast supports a greater diversity of asteroids than the Caribbean coast.

As part of a survey of the diversity of marine invertebrate larvae in the coastal waters of Panama, we documented asteroid larvae from Bocas del Toro on the Caribbean coast and from the Bay of Panama on the Pacific coast. We applied a DNA barcoding approach to identify asteroid larvae with the objectives of (a) determining whether surveying larvae uncovers unexpected asteroid diversity as it has for other groups (Barber & Boyce, 2006; Collin, et al., 2019; Mahon et al., 2010); (b) determining whether larval asteroids are more diverse on the Pacific coast than the Caribbean coast, as are the adults; and (c) determining whether variation in larval morphology can be used to distinguish species at these sites.

2 | METHODS

2.1 | Sample Collection

Asteroid larvae from three distinct sets of samples were photographed and sequenced. Caribbean samples were collected from Bahía Almirante in Bocas del Toro Province with a 0.5-m diameter 125-µm mesh plankton net towed horizontally behind a small boat drifting in the current, with the engine alternating in and out of gear, and sampling the water column by gently bouncing the net through the middle third of the water column (from 10 to 20 m depth). In 2013, larvae were collected incidentally as part of the short-course on Larval Invertebrate Diversity, Form and Function at the Bocas del Toro Research Station (BRS) of the Smithsonian Tropical Research Institute. Samples were collected over 2 weeks from various sites around Bahía Almirante in July 2013 and sorted by a team of 12 students. Larvae were then selected for barcoding based on student interests. In 2015–2016, structured sampling involved a campaign of four plankton surveys evenly spaced throughout a year (August 2015, November 2015, February–March 2016, and June 2016). Each survey consisted of 3–5 tows conducted on different days over 9 days, between 7 a.m. and 9 a.m. Tows were conducted at a single 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 flow meter was attached to the mouth of the net to determine the volume of water sampled, and a depth meter recorded that tows did not drop below 20 m. The depth at this site is ~25 m.

Pacific samples were collected in the northern part of the Bay of Panama, between Taboga and Contadora Islands, from 2013 to 2016 (August 2013, March 2014, April 2014, May 2014, June 2014, November 2014, December 2015, March 2016). Pacific surveys were exploratory, not quantitative, and included multiple locations on each sampling date. Pacific samples were collected by towing the net horizontally, but small changes in speed were used to induce a bouncing depth profile to obtain samples from ~10 to 40 m.

Samples were sorted using a stereomicroscope and asteroid larvae were moved to dishes of filtered seawater. The 2015–2016 Caribbean samples were sorted exhaustively, providing data on larval density; however, for morphologically similar larvae we prepared no more than eight individuals of a similar morphotype from each tow for sequencing. Even when there were small numbers of larvae collected, we could not always sequence all of the individuals, because animals that were damaged during collection often did not survive long enough to be photographed. Therefore, larval counts did not always match the number of individuals sequenced from the same tow. Individual larvae were photographed alive, often moving, in a depression slide under a dissecting microscope prior to preservation for DNA sequencing. During the course in 2013, larvae were relaxed with MgCl2 isotonic with seawater prior to photographing under a compound microscope with differential interference contrast, resulting in fewer processed samples with higher resolution photographs. Notes were taken on the overall appearance, morphological details, and approximate size of each larva before they were preserved for sequencing.

2.2 | 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. Plates with 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 ~600 bp 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. The 10-µl PCR cocktail for COI included 5 µl GoTaq Hot Start Mix (Promega), 0.1 µl BSA,
<table>
<thead>
<tr>
<th>Species or OTU (number of larvae sequenced)</th>
<th>GenBank match Sequence identity</th>
<th>New COI sequences&lt;sup&gt;a&lt;/sup&gt;</th>
<th>New 16S sequences&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BIN#</th>
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<td>&quot;Oreasteridae sp.&quot; MN730656, MN730659, MN730664, MN730665, MN730673</td>
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<td></td>
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<td>16S: &gt;99%</td>
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<td>COI: &gt;99%</td>
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<td>COI: 84%</td>
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<td>COI: &gt;99%</td>
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<td>COI: &gt;99%</td>
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<td>MN730878, MN730883, MN730913</td>
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<td>16S: 94%</td>
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<td>16S: &gt;99%</td>
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<td>COI: &gt; 99%</td>
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<td><strong>Pacific Ocean</strong></td>
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<td>COI: &gt;99%</td>
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and 0.3 µl of each 10-mM primer. For amplification and sequencing of a ~490 bp fragment of 16S, the primer pairs 16SAR/16SBR (Palumbi et al., 1991) or 16SL2/16SH2 (Armedo et al., 2001; Schubart et al., 2002) were used. The cocktail for 16S used Biolase Taq (Bioline) with the addition of 0.5 µl 50 mM MgCl₂. The annealing temperature was 48°C for COI and 50°C for 16S.

### 2.3 Analysis

Sequences were screened for quality and used to generate 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 analyses. To check for potential contamination, sequences were compared internally with all larvae sequenced in our project within the BOLD project workbench (www.boldsystems.org) and to sequences available in GenBank using BLASTn searches. For COI we followed the suggestions of Song et al. (2008) to check for the presence of pseudogenes in our datasets. No gaps were identified, nor were there any stop codons in the COI sequences.

Neighbor-joining trees (BIONJ, Gascuel, 1997) with Kimura two-parameter distances were constructed from our sequences, trimmed to the length of the shortest sequence to aid in OTU visualization. COI alignments were inferred with the BOLD aligner (amino acid based Hidden Markov Model; Ratnasingham & Hebert 2007) using the default settings; 16S was aligned using ClustalX (www.clustal.org) followed by manual editing in McClade (www.mcclade.org). OTUs were identified with the Automatic Barcode Gap Discovery (ABGD) method \( P_{\text{min}} = 0.001; P_{\text{max}} = 0.1; \) Steps = 10; \( X \) (relative gap width) = 1.5; Kimura (K80) distance; TS/TV = 2) (Puillandre et al., 2012). To try to confirm the genus or family identity of the unidentified larval OTUs, they were analyzed with all available 16S sequence data for species of Luidia as above.

DNA sequences generated by this project have been deposited in GenBank (accession numbers: MN730654–MN730776 for COI; MN730777–MN730916 for 16S), and the COI dataset can be found at dx.doi.org/10.5883/DS-ASTEROID. Alignments are available at FigShare: 10.25573/data.c.5084306.

### 3 RESULTS AND DISCUSSION

Asteroid larvae were fairly abundant in Bocas del Toro, with densities from 0.4 to 8.8 larvae/m³ (mean = 2.7; SD = 2.9 larvae/m³) in the sampled seawater. Overall, we preserved 154 larvae for DNA sequencing and obtained 123 COI and 140 16S sequences from them. Asteroid larvae were uncommon in the Pacific, and only 16 larvae were collected for sequencing.

Using ABGD, we detected 15 larval OTUs, with 5 from the Pacific and 10 from the Caribbean (Table 1; Figure 1). They are
easily visualized as distinct in a neighbor-joining tree, and the OTUs identified both ways were concordant and the same OTUs were obtained with 16S and COI. The most abundant OTUs were found in the Caribbean and had 46, 32, and 20 larvae, respectively. The remaining OTUs contained between 1 and 8 larvae (Figure 1). Each COI OTU corresponded to a distinct Barcode Index Number (BIN) in BOLD (Ratnasingham & Herbert, 2013). OTUs differed from each other by at least 10% divergence in COI and with no more that 2% intra-OTU divergence (Figure 1). Thus, we infer that each of these OTUs corresponds to a different species.

We identified 6 of the 15 OTUs based on >99% similarity with reference sequences in GenBank using blast searches of the COI and 16S sequences (Table 1). These were the Pacific species Astropecten verrilli de loriol 1899 and Pentaceraster cumingi (Gray 1840) and the Caribbean species Astropecten marginatus Gray 1840, Astropecten antillensis Lütken 1859, Oreaster reticulatus (Linnaeus 1758), and Mithrodia clavigera (Lamarck 1816) (Table 1). Two other OTUs were placed in BINs with unpublished sequences. One of these was identified as Pharia pyramidata (Gray 1840) from Pacific samples (Table 1). The other OTU, collected in the Caribbean, was identified as Valvaster striatus (Lamarck 1816), a species that was previously thought to be endemic to the Indo-West Pacific (IWP) (see Collin et al., 2020 for details). The remaining seven OTUs, which could not be identified to species, are likely to belong to Luidia, as they each have 87%–95% identity in 16S with various species of Luidia (Table 1; Figure 2), and neighbor-joining trees constructed with our 16S sequences and these sequences from GenBank place them closer to species of Luidia than to species of Astropecten. Unfortunately, there are too few COI barcodes for species of Luidia available from the Neotropics to adequately perform a similar comparison with our COI sequences.

Although Knott et al. (2003) indicate that asteroid larvae cannot be identified to species with any certainty based on morphology, we present some of our observations of the larval morphology from our Pacific (Figure 3) and Caribbean (Figures 4 and 5) OTUs in the hopes of illustrating the variation among species and shining light on potentially useful features that may help to distinguish the major groups of Neotropical asteroid larvae. For each group, we provide a discussion of their molecular identification and any relevant information on their reproduction and development in the Neotropics.

3.1 Astropecten

We found three species of Astropecten in our larval samples and identified them primarily by comparisons with sequences generated as part of a global molecular phylogeny of 117 specimens representing ~40 of the ~150 known species of Astropecten (Zulliger & Lessios, 2010). These sequences include (following their taxonomy) A. antillensis, A. articulatus (Say 1825), A. cingulatus Sladen 1883, A. marginatus, and A. oerstedi Lütken 1859 collected from the Caribbean of Panama, and Astropecten alligator Perrier 1881, A. americanus (Verrill 1880), A. comptus Verrill 1915, A. duplicatus Gray 1840, and A. nitidus Verrill 1915 from other sites in the Caribbean and Gulf of Mexico. Of these 10 Caribbean species, only 2, A. antillensis and A. marginatus, were identified among our larval OTUs. Specimens of A. articulatus were collected from Bocas del Toro as adults (Zulliger & Lessios, 2010), but surprisingly, we did not collect larvae with corresponding sequences. On the Pacific coast, A. erinaceus Gray 1840, A. sideralis Verrill 1914, and A. verrilli were previously sequenced.
shown in Figures 3C,D and 5C,D,F,G). The four larvae of A. marginatus we collected were similar to the larvae of A. antillensis, although two of them showed a more pronounced scattering of mesenchyme cells across the epithelium (Figure 5C). This was not as evident in the other larvae of the genus. Additionally, the preoral lobes and bipinnarial arms of A. antillensis were more defined and protruded further than in their congeneric species, which may reflect species-specific or developmental differences (Figure 5F,G).

3.2 | Luidia

Seven OTUs were assigned to Luidia based on larval morphology and the fact that the closest blastn matches were species of Luidia. In addition, in the neighbor-joining trees we generated that included sequences of Luidia from GenBank, our sequences occurred mixed among the other species, without generating particularly long branches (Figure 2). There were two OTUs from the Pacific (e.g., Figure 3E,F) and five from the Caribbean (e.g., Figures 4E–G and 5A,B,E). The Caribbean fauna is widely considered to be composed of six species of Luidia, many of which are attributed to subspecies of species with extraordinarily wide latitudinal ranges. These species include records of adults of Luidia clathrata (Sav 1825), L. alternata (Sav 1825), L. barbadensis Perrier 1881, L. ludwigi Fisher 1906, L. senegalensis (Lamarck 1816), and L. sarsi Düb en & Koren in Düb en 1844, which range from the southeastern US, through the Gulf of Mexico and the Caribbean, and south to Brazil (Lawrence Durán-Gonzalez et al., 2013). Two other species, L. lawrencei Hopkins & Knott 2010 and L. sagamina Doderlein 1920, occur along the east coast of the US and the Gulf of Mexico but are not known to extend southward into the Caribbean. Of these eight species, Milaslovich et al. (2010) reported only L. alternata, L. clathrata, and L. senegalensis for the Caribbean coast of Panama. Recently collected vouchers at the Florida Museum of Natural History, University of Florida (UF), confirmed the presence of L. alternata, and one of us (RC) has observed L. senegalensis in Bahia Almirante. Regarding this Caribbean fauna, GenBank contains sequence data only for L. clathrata (COI and 16S) and L. sarsi (COI; from the North Sea). In addition, UF has unpublished COI sequences from L. senegalensis, L. lawrencei, and L. alternata from the Caribbean (G. Paulay, unpublished data; pers. com.). None of the five OTUs corresponding to Luidia we identified from our Caribbean samples showed high sequence identity (>95%) with any sequences from these five species of Luidia. These results suggest that larvae from our collections either belong to other species reported from the region, which lack available sequence data, or that regional subspecies within these widespread taxa are genetically dissimilar enough to be considered distinct species.

from Panama, and A. regalis Gray 1840 from the Pacific of Costa Rica (Zulliger & Lessios, 2010). Our larval samples were all attributable to A. verrilli (distinct from A. californicus Fisher 1906; see Zulliger & Lessios, 2010 for discussion of the taxonomy of these species).

The development and larvae of Astropecten armatus Gray 1840 from California has been recently described in detail (Pernet et al., 2017). The larvae from the three OTUs we collected were similar to larvae of A. armatus. All had five pairs of bipinnarial arms with orange pigment on their tips and the juvenile rudiment, when present, was white. We did not observe any indication of cloning in the larvae we collected. As expected for this genus, no larvae had brachiolarial arms. None of the larvae we collected had bipinnarial arms as long as those figured by Pernet et al. (2017), who indicated that arm length may reflect egg size.

Larvae of A. verrilli were notable as their bipinnarial arms were very short, even in larvae with a developing juvenile (Figure 3C,D), and their orange pigment was less distinct than it was in the other two species of Astropecten we identified (Figure 5C,D,F–H). All six of the larvae of A. verrilli were collected in the Bay of Panama on December 7, 2015, with the largest larva measuring 1.2-mm long. The largest of the 20 larvae of A. antillensis we collected were 1.1 mm, and, like the larvae of A. armatus, larvae of A. verrilli, A. marginatus, and A. antillensis each had a noticeable blue tint to their stomach and/or intestine (Figures 3C,D and 5C,D,F,G).
The Tropical Eastern Pacific (TEP) fauna is composed of eight species of Luidia (Alvarado et al., 2010; excluding L. foliolata, which is clearly in error), although none of these have sequences in GenBank. Luidia armata Ludwig 1905 and L. asthenosoma Fisher 1906 from Southern California have sequences in BOLD, but neither one shares a BIN with our larvae (i.e., are not conspecific with our larvae). In addition, the COI of L. latiradiata (Gray 1871) has been sequenced by UF and does not match our larvae (G. Paulay, unpublished data; pers. com.). We can therefore eliminate the possibility that these three species are present in our larval samples, but the other five species reported to occur in the Pacific fauna have not been sequenced and therefore remain as possible matches to our larvae.

Little is known about the reproduction and development of Luidia in the Caribbean, and nothing has been published on the life histories of Luidia from the TEP. In the Caribbean, L. clathrata and L. senegalensis both show a single seasonal peak in gonad index, which occurs either in the fall or very early spring in Florida (Dehn, 1980; Miller & Lawrence, 1999). All of the larvae we assigned to Luidia were morphologically consistent with descriptions by Komatsu et al. (1991) of three species from Florida: typical bipinnaria larvae that did not exceed 1.5 mm, with five pairs of bipinnarial arms, and two anterior processes of the anterior lobe. None of our larvae were brachiolaria (a larval stage that is not known to occur in species of Luidia). In L. sarsi, larvae are highly derived and giant, sometimes >3 cm in length (Domanski, 1984). We did not find this type or size of larvae in our samples. Luidia sp. C3 (Figure 4E) was represented by three larvae, the largest of which was 1.1 mm and had orange-tipped bipinnarial arms and a rudiment. Luidia sp. C4 (Figure 4F,G) was represented by two larvae, the largest of which was a bipinnaria with an orange esophagus and pink gut. This larva was notable because there was some orange pigment on the body (Figure 4G). Luidia sp. C6 was represented by eight larvae, which also had orange-tipped bipinnarial arms and a clear gut (Figure 5A,B). Luidia sp. C7 (Figure 5E) was represented

FIGURE 3 Asteroid larval representatives of four DNA barcode operational taxonomic units (OTUs) from the Bay of Panama. All larvae are oriented with anterior to the top. A,B. Ventral views of a bipinnaria (A) and brachiolaria (B) of Pentaceraster cumingi. C,D. Ventral and left-lateral views of two bipinnaria of Astropecten verrilli, with juvenile rudiments. E,F. Dorsal and right-lateral views of the unidentified bipinnaria of Luidia sp. P3. G,H. Dorsolateral and lateral views of the brachiolaria larva of Pharia pyramidata, with juvenile rudiment. All images were captured from live specimens. ad, anterodorsal arm; adk, adhesive disk; af, anal field; an, anus; ap, adhesive papilla; bia, bipinnaria arm; bra, brachiolar arm; es, esophagus; ff, frontal field; in, intestine; jr, juvenile rudiment; js, juvenile skeletal element; mo, mouth; pd, posterodorsal arm; pl, posterolateral arm; po, postoral arm; poc, postoral ciliary band; pr, preoral arm; prc, preoral ciliary band; prl, preoral lobe; st, stomach. Scale bars ~250 µm
by four larvae. Three of these showed evidence of cloning, with at least one missing posterolateral arm tip on each larva. These may be the same species as the cloning larvae of *Luidia* reported by Knott et al. (2003), which were placed close to *L. clathrata* in the phylogeny of Galac et al. (2016). BOLD records indicate that the nearest BIN to our OTU is an unpublished BIN identified as *L. clathrata*. Unfortunately, the sequence fragment used by Knott et al. (2003) does not overlap sufficiently with either of our sequences to support direct comparisons between our datasets.

*Luidia* sp. C9 (not shown) was represented by three larvae, one of which was a large bipinnaria with orange tips on the arms. There were two OTUs that corresponded to species of *Luidia* in our Pacific samples, each represented by a single larva. *Luidia* sp. P3 was a large bipinnaria with orange-tipped arms, a pinkish gut, and a white-tipped rudiment flanking the stomach (Figure 3E,F). *Luidia* sp. P4 was an unpigmented bipinnaria with a white rudiment (not shown). None of these larvae of *Luidia* are easily distinguishable from each other, and the possibility of larval plasticity in response to different food rations, which has been demonstrated in the North Pacific species *L. foliolata* (George, 1994), further complicates the interpretation of any subtle differences in shape or allometry.

### 3.3 Valvatacea

The remaining five larval OTUs could all be identified based on DNA sequence data. In the Caribbean, these included the well-documented larvae of three shallow-water species. The most common larva we encountered was *V. striatus* (Lamarck 1816) (46 larvae; Figure 4A,B). These cloning larvae have been discussed in detail elsewhere (Collin, et al., 2020; Galac et al., 2016; Janies et al., 2019; Knott et al., 2003). *Valvaster striatus* were the most abundant larvae overall and included small early stages as well as brachiolaria with developing rudiments. There may be a seasonal pattern to their appearance in the plankton, with this species representing the majority of asteroid larvae collected in August and November, but with few individuals collected in March and June. The other most abundant larvae belonged to *O. reticulatus* (Linnaeus 1758).
1758) (32 larvae; Figure 4C,D), an abundant species found in benthic habitats of Bocas del Toro Province. A,B. Ventral views of two bipinnaria of *Luidia* sp. C6. C,D. Dorsal and right-lateral views of a bipinnaria of *Astropecten marginatus*. E. Ventral view of a bipinnaria of *Luidia* sp. C7. F–H. Ventral view (F) and paired ventral and right-lateral views (G,H) of bipinnaria larvae of *Astropecten antillensis*. All images were captured from live specimens. ad, anterodorsal arm; af, anal field; an, anus; ax, axocoel; cb, ciliary band; es, esophagus; ff, frontal field; in, intestine; jr, juvenile rudiment; js, juvenile skeletal element; jsp, juvenile spine; mo, mouth; pd, posterodorsal arm; pi, pigment; pl, posterolateral arm; po, postoral arm; poc, postoral ciliary band; pr, preoral arm; prc, preoral ciliary band; prl, preoral lobe; so, somatocoel; st, stomach. Scale bars ~250 µm

It should be noted that, like *V. striatus*, *M. clavigera* is primarily an IWP species, but has been recently reported from the Caribbean (Bribiesca-Contreras et al., 2013; Clark & Downey, 1992). Our COI and 16S sequences revealed high-percent identity with sequences of adults of *M. clavigera* collected from the Caribbean and with some sequences from the Indian Ocean (G. Paulay, pers. com.). Specimens of *M. clavigera* collected from other parts of the IWP fall into different clades based on COI barcode data (G. Paulay, unpublished data; pers. com.).

Only two valvatacean OTUs were collected from the Pacific coast of Panama. The most abundant larvae were identified as *P. cumingi* (Gray 1840) (Figure 4H). As described by Galac et al. (2016; referencing Knott et al., 2003), these larvae also have a colored gut, similar in color to, but darker than, the larvae of *V. striatus*. Galac et al. (2016) were able to identify specimens of “larval group 4” from Knott et al. (2003) as cloning larvae belonging to this species, although they observed that cloning was uncommon, and none of the four larvae of *M. clavigera* that we collected showed evidence of cloning.

We also collected four larvae identified as *M. clavigera* (Lamarck 1816) (Figure 4H). As described by Galac et al. (2016; referencing Knott et al., 2003), these larvae also have a colored gut, similar in color to, but darker than, the larvae of *V. striatus*. Galac et al. (2016) were able to identify specimens of “larval group 4” from Knott et al. (2003) as cloning larvae belonging to this species, although they observed that cloning was uncommon, and none of the four larvae of *M. clavigera* that we collected showed evidence of cloning.
rudiment was yellow-orange. These larvae were all collected during the non-upwelling season in the Bay of Panama (May, June, August, and November). The other Pacific valvatacean OTU was represented by a single brachiolaria stage larva of *P. pyramidata* (Gray 1840) (Figure 3G,H). The bipinnarial arms were stubby and the round rudiment was a rusty brown color. This larva was collected in November, which is consistent with the spawning season of July–September reported from the Mexican tropical Pacific (Benítez-Villalobos & Martínez-García, 2012). There is no other published information on the reproduction of this species.

4 | CONCLUSIONS

The larval asteroid fauna of Panama collected during this investigation was not particularly diverse, with only 15 OTUs detected, compared to a total of ~80 species reported to occur as adults in Panamanian waters. From the Caribbean our intense sampling documented 10 OTUs from ~130 samples compared to an adult fauna of 25 species. In the Pacific, we documented five OTUs from 16 samples, compared to the adult fauna of 56 species. This strongly suggests that additional plankton sampling efforts, with broader spatial and temporal coverage, would likely increase the larval diversity reported for the Pacific. It is interesting to note that we found no forcipulatacean larvae in our samples, yet several forcipulatacean species have been reported in Panamanian waters. Additionally, half of the larval diversity reported in our study belong to a single genus, *Luidia*. These soft-bottom specialists are in need of taxonomic revision and improved representation in genetic databases such as GenBank and BOLD. Importantly, our efforts succeeded in collecting and characterizing larval stages of 15 distinct asteroids, providing evidence, for the first time for most of them, of their indirect life history patterns, their respective larval morphologies, and by extension, their reproductive populations along Pacific and Caribbean coasts of Panama.

ACKNOWLEDGMENTS

This work was supported by the Smithsonian Institution, Mr. Paul Peck, and an anonymous donor. Work 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 Lyre Villotta Nieva and the students of the Larval Invertebrate Diversity, Form and Function short-course for help collecting larvae and Gustav Paulay for providing information on Indo-West Pacific species. 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. 1148.

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