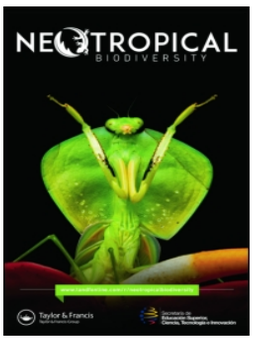

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An integrative identification guide to the Hydrozoa (Cnidaria) of Bocas del Toro, Panama

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ABSTRACT

This work is the first attempt to assess the biodiversity of the Hydrozoa in the Archipiélago de Bocas del Toro (Panamá, Caribbean Sea) using morphology and molecular taxonomy, and to produce field identification tools to help future identification and monitoring efforts in the area.

We sampled, identified, vouchered, and barcoded 112 specimens of Hydrozoa from shallow coastal waters (0–22 m depth) in the Archipiélago de Bocas del Toro. The specimens belong to 70 taxa, of which 53 were identified at the species level, and 17 were identified at the genus or family level. We produced 64 sequences of the large ribosomal subunit of the mitochondrial RNA (mt lsu-rRNA, 16S), the genetic marker generally used for barcoding Hydrozoa. We updated the local checklist that now comprises 118 species, and produced 87 detailed taxon identification tables that display species descriptions augmented with pictures, geographic distribution (worldwide and in Bocas del Toro), GenBank accession numbers for the 16S mitochondrial gene, and a synopsis of the families they belong to.

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KEYWORDS
Hydrozoa; Bocas del Toro; identification tools; barcoding; Caribbean; 16S; biodiversity

Introduction

Hydrozoa are an inconspicuous and often overlooked class of the phylum Cnidaria [1]. Most hydrozoans have a complex life cycle characterized by a progression of three life stages: a short-living larva (the planula), generally metamorphosing into a benthic colonial stage (the polyp), and a pelagic sexual stage (the medusa stage) asexually budded off from the polyp [2]. The life cycle can be shortened into a biphasic cycle, by reduction or complete suppression of either the polyp or the medusa stage [3]. With more than 3,700 described species [4] hydrozoans are structurally and functionally important members of benthic and planktonic communities [5; 1]. Work on Hydrozoa has been hampered by the scarcity of taxonomic expertise, which has dramatically declined over the past two decades [6,7]. Also, because polyps and medusae require different expertise and each follow their own identification rules, linking both to a single species has proven difficult and has further hampered cohesive taxonomic revisions. In the last 10 years molecular tools have contributed significantly to hydrozoan taxonomy and have shown that selected gene sequences may be necessary, in combination with traditional taxonomy, to correctly identify cryptic species and disentangle taxonomic confusion [8–12].

Hydrozoa from the Atlantic/Caribbean coast of Panama are abundant but scarcely studied. The Archipiélago de Bocas del Toro, Panamá is located on the NW Caribbean shore of Panama, close to the Costa Rican border. It consists of more than 68 small islands and mangrove keys and is characterized by diverse ecosystems, from mangrove dominated shallow water to coral reefs and sea grass meadows (see Figure 1 for a map). To date, 79 nominal species have been reported in the Bocas del Toro region [13]. However, proper descriptions and species identification tools are lacking, inadequate, or scattered in old and hard to access

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Supplemental data can be accessed here.

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Figure 1. Map of the Bocas del Toro archipelago, with sampling localities.

articles, thus hindering the ability of nonexperts to identify species of interest in this area.

This work is a result of the Taxonomic Training workshop held in July 2015 in Bocas del Toro, Panama, organized by Smithsonian Tropical Research Institute as part of a National Science Foundation Advancing Revisionary Taxonomy and Systematics (ARTS) grant. During the workshop, in which 14 students participated, we collected shallow water Hydrozoa around the Archipiélago de Bocas del Toro, Panamá. Collections targeted both pelagic medusa with plankton tows and benthic polyps with snorkeling. In this paper, we present an updated checklist of hydrozoan biodiversity in the Archipiélago de Bocas del Toro,, augmented by [I] a DNA barcoding database consisting of sequences of a ~ 600bp gene fragment of the mitochondrial large ribosomal RNA subunit (mt lsu-rRNA, 16S), and [II] taxon identification tables. The 16S gene has been successfully used for taxonomic revisions and is considered the Hydrozoa "barcoding" molecule [10,11,14–17]. Both the barcoding data and the taxonomic identification sheets were produced to aid future biodiversity inventory efforts in the region.

Material and methods

Collection

Hydrozoa were collected during the Tropical Taxonomy Training course on systematics and

biology of Hydrozoa (Cnidaria) held at the Smithsonian Tropical Research in Bocas del Toro, Panama from 7/7/2015 to 7/21/2015. A total of 16 people (two instructors and 14 students) were in the field during every sampling effort. A total of 11 sites were sampled. Localities sampled were: Bocas del Toro Marine Station docks/weather station, Punta Hospital, Crawl Cay, Bocatorito Bay vicinity, San Cristobal, Vicinity of Manuguar Cay, Punta Caracol, Swans Cay, Bastimento vicinity of "Casa Verde", Bocas del Drago, Pandora, Almirante (Quary's point) (Table 1 and Figure 1). Polyps were collected by snorkeling (0–8 m depth) or SCUBA diving (18–22 m depth), and medusae by plankton towing using a net with 280µm mesh size. Plankton tows were carried out near the Bocas del Toro Station docks and weather station.

Polyps and medusae were sorted in the laboratory and identified to the lowest taxonomic level using appropriate taxonomic literature [e.g. 16–20]. When possible, polyps from each colony where divided in two vials and preserved in both formalin (for morphological analyses) and 99% ethanol (for genetic analyses). Vouchered specimens were deposited at the Museum of Universidad the Panamá, Panama City, Panama. When little tissue was available, specimens were preserved in ethanol only and used for molecular analyses. DNA samples are currently at the University of Texas A&M at Galveston. Pictures of live specimens featured colony, polyps, medusae (or equivalent reproductive structures), and other morphological structures useful for identification.

Barcoding

Genomic DNA was extracted using standard techniques followed by ethanol precipitation [8]. A ~ 600bp fragment of the large ribosomal subunit of the mitochondrial RNA (lsu-rRNA, 16S) was amplified using SHA and SHB primers [23], Polymerase Chain Reaction (PCR), and conditions as described in Miglietta [15]. The PCR product was run on a 2% agarose gel stained with SybrGreen I nucleic acid gel stain (Sigma-Aldrich) to assay its quantity and quality (i.e. accessory bands). PCR products were purified using exoSAP-it (Affymetrix), following manufacturer's instruction and used as a template for double stranded sequencing with the amplification primers. The purified DNA was sequenced at the Texas A&M Corpus Christi Genomics Core Laboratory. Sequences were analyzed in Geneious R9 (http://www.geneious.com [24]) and deposited in Genbank (accession numbers MH361321 to MH361381). For definition of anatomical terms used in the ID tables, see online taxonomic glossary for Hydrozoa at https://stricollections.org/portal/misc/glossarycover.php [25].

Results

We collected, vouchered, and fixed in ethanol and/or formalin 112 specimens of Hydrozoa (Table 1). We identified a total of 53 species. An additional 17 taxa could be identified at the genus or family level only due to the lack of fertile structures, small size of the colony, or, in the case of planktonic specimens, early age of the medusae (Table 2 for a complete species list).

We sequenced the 16S gene for 64 out of 112 specimens belonging to 44 species. Some of the 112 specimens did not yield sufficient DNA for Polymerase Chain Reaction and thus could not be barcoded. For some species, however, multiple sequences were produced. All sequences were ~ 600bp in length and were deposited in GenBank (accession numbers **MH361321-MH361332, MH361334-MH361359, MH361361-MH361381, MH374630**).

Morphological, ecological, and barcoding data where assembled in Taxon Identification Tables. We produced 87 tables featuring 28 families (13 in the order Anthoathecata; 12 in the order Leptothecata, 2 in the order Trachymedusae and 1 in the Order Limnomedusae) and 55 species (Appendix 1). These 55 species represent the most common Hydrozoa found in Bocas del Toro during the workshop. For the most speciose taxa we supply identification keys (to genera and/or to species). One of the most abundant families found in the area was the Campanulariidae, with 3 genera and 9 species. Because the polyps of the three genera sampled (*Clytia, Gastroblasta*, and *Obelia*) can be easily confused we also supply identification tables for each genus.

Discussion

Check list of Bocas del Toro Hydrozoa

A comprehensive inventory of the Hydrozoa from the Caribbean coast of Panama was previously produced by Calder and Kirkendale [13], who gathered three different collections acquired in 1969, 2002, and 2004, mostly from the Bocas del Toro region. They recorded 79 nominal species (of which were 17 identified at the genus or family level only) belonging to 22 families. We found 53 species, of which only 31 were in common with Calder and Kirkendale [13]. We merged our own inventory with that of Calder and Kirkendale [13],to produce an updated checklist of the Hydrozoa of Bocas del Toro that now comprises 118 taxa, of which 86 identified at the species level and 32 identified at the genus or higher level (Table 3). Given such a small geographical area (250 km^2), this is an impressive number. For comparison, 118 species constitutes about ¼ of the total known

Table 1. Samples collected during the 2015 Hydrozoa workshop held in Bocas del Toro, Panama. Date of collections, sample ID, species identified, type of fixative used for preservation (ethanol or formalin), GenBank accession number, and location within the Bocas del Toro archipelago are reported. In the table N = No, Y = Yes.

	Date	Sample ID	Species	Fertile	Ethanol	Formalin	Sequenced/ GenBank accession #	Location
1	7/7/2015	BTH.15.1	*Kirchenpaueria halecioides*	Y	Y	N	N	STRI BDT docks/weather station
2	7/7/2015	BTH.15.2	*Halecium* cf. *bermudense*	Y	Y	N	N	STRI BDT docks/weather station
3	7/7/2015	BTH.15.3	*Nemalecium lighti*	N	Y	Y	N	STRI BDT docks/weather station
4	7/7/2015	BTH.15.4	*Nemalecium lighti*	N	Y	Y	MH361321	STRI BDT docks/weather station
5	7/7/2015	BTH.15.5	*Kirchenpaueria halecioides*	N	Y	Y	N	STRI BDT docks/weather station
6	7/7/2015	BTH.15.6	*Clytia linearis*	Y	Y	N	MH374630	STRI BDT docks/weather station
7	7/7/2015	BTH.15.7	*Dynamena crisioides*	Y	Y	N	MH361322	STRI BDT docks/weather station
8	7/7/2015	BTH.15.8	*?Cytaeis*	N	Y	Y	N	STRI BDT docks/weather station
9	7/7/2015	BTH.15.9	*Dynamena crisioides*	Y	Y	N	MH361323	STRI BDT docks/weather station
10	7/7/2015	BTH.15.10	*Halecium bermudense*	Y	Y	N	N	STRI BDT docks/weather station
11	7/8/2015	BTH.15.11	*Myrionema amboinense*	N	Y	Y	N	Punta hospital
12	7/8/2015	BTH.15.12	*Plumularia* sp.	N	Y	Y	N	Punta hospital
13	7/8/2015	BTH.15.13	*Antennella secundaria*	Y	Y	N	MH361324	Punta hospital
14	7/8/2015	BTH.15.14	*Halopteris alternata*	N	Y	N	MH361325	Punta hospital
15	7/8/2015	BTH.15.15	*Eudendrium carneum*	N	Y	Y	MH361326	Punta hospital
16	7/8/2015	BTH.15.16	*Salacia desmoides*	Y	Y	Y	MH361327	Punta hospital
17	7/8/2015	BTH.15.17	*Eudendrium bermudense*	Y	Y	Y	N	Punta hospital
18	7/8/2015	BTH.15.18	*Antennella secundaria*	Y	Y	Y	MH361328	Punta hospital
19	7/8/2015	BTH.15.19	*Obelia dichotoma*	Y	Y	Y	N	Punta hospital
20	7/8/2015	BTH.15.20	*Aglaophenia latecarinata*	N	Y	Y	N	BDT docks/weather station
21	7/8/2015	BTH.15.21	*Kirchenpaueria halecioides*	Y	N	Y	N	Punta hospital
22	7/8/2015	BTH.15.22	*Plumularia* sp.	N	Y	Y	N	Punta hospital
23	7/9/2015	BTH.15.23	*Pennaria disticha*	Y	Y	Y	MH361329	Crawl Cay
24	7/9/2015	BTH.15.24	*Ralpharia gorganiae*	Y	Y	N	MH361330	Crawl Cay
25	7/9/2015	BTH.15.25	*Stylaster roseus*	N	Y	Y	MH361331	Crawl Cay
26	7/9/2015	BTH.15.26	*Thyroscyphus marginatus*	Y	Y	Y	MH361332	Crawl Cay
27	7/9/2015	BTH.15.27	*Gastroblasta raffaelei*	N	Y	Y	N	Crawl Cay
28	7/9/2015	BTH.15.28	*Pennaria disticha*	Y	Y	N	N	Crawl Cay
29	7/9/2015	BTH.15.29	*Thyroscyphus marginatus*	N	Y	N	MH361334	Crawl Cay
30	7/9/2015	BTH.15.30	*?Obelia dichotoma*	N	Y	Y	N	Crawl Cay
31	7/9/2015	BTH.15.31	*Millepora alcicornis*	N	Y	N	MH361335	Crawl Cay
32	7/9/2015	BTH.15.32	*Dynamena disticha*	N	Y	Y	MH361336	Crawl Cay
33	7/9/2015	BTH.15.33	*Dynamena crisioides*	N	Y	N	N	Crawl Cay
34	7/9/2015	BTH.15.34	*Obelia dichotoma*	Y	N	Y	N	Crawl Cay
35	7/9/2015	BTH.15.35	*Eudendrium capillare*	Y	Y	Y	MH361337	Crawl Cay
36	7/9/2015	BTH.15.36	*Clytia hemisphaerica*	Y	Y	N	N	Crawl Cay
37	7/9/2015	BTH.15.37	*Clytia hemisphaerica*	N	N	Y	N	Crawl Cay
38	7/9/2015	BTH.15.38	*Halecium* sp.2	Y	Y	Y	MH361338	Crawl Cay
39	7/9/2015	BTH.15.39	*Hincksella formosa*	N	Y	Y	MH361339	Crawl Cay
40	7/10/2015	BTH.15.40	*Sphaerocoryne* cf. *agassizii*	N	Y	Y	MH361340	Near Bocatorito Bay
41	7/10/2015	BTH.15.41	*Clytia hemisphaerica*	N	Y	Y	N	Near Bocatorito Bay
42	7/10/2015	BTH.15.42	*Monotheca margaretta*	Y	Y	Y	N	Near Bocatorito Bay
43	7/10/2015	BTH.15.43	*Sertularia distans*	N	Y	Y	MH361341	Near Bocatorito Bay
44	7/10/2015	BTH.15.44	*Gastroblasta raffaelei*	N	Y	Y	MH361342	Near Bocatorito Bay
45	7/10/2015	BTH.15.45	*Halecium* cf. *nanum*	N	Y	Y	MH361343	Near Bocatorito Bay
46	7/10/2015	BTH.15.46	*Gastroblasta raffaelei*	N	Y	Y	N	Near Bocatorito Bay
47	7/10/2015	BTH.15.47	*Kirchenpaueria halecioides*	N	Y	N	MH361344	Near Bocatorito Bay

(*Continued*)

Table 1. (Continued).

	Date	Sample ID	Species	Fertile	Ethanol	Formalin	Sequenced/ GenBank accession #	Location
48	7/10/2015	BTH.15.48	*Gastroblasta raffaelei*	N	Y	N	N	Near Bocatorito Bay
49	7/10/2015	BTH.15.49	*Gastroblasta raffaelei*	N	Y	N	N	Near Bocatorito Bay
50	7/10/2015	BTH.15.50	*Halopteris alternata*	N	Y	Y	MH361345	Near Bocatorito Bay
51	7/10/2015	BTH.15.51	*Monotheca margaretta*	Y	Y	Y	MH361346	Near Bocatorito Bay
52	7/10/2015	BTH.15.52	*Halecium* sp. [sensu 17]	N	Y	N	MH361347	Near Bocatorito Bay
53	7/10/2015	BTH.15.53	*Halopteris alternata*	N	Y	N	MH361348	Near Bocatorito Bay
54	7/10/2015	BTH.15.54	*Kirchenpaueria halecioides*	Y	Y	Y	MH361349	Near Bocatorito Bay
55	7/11/2015	BTH.15.55	*Gastroblasta raffaelei*	N	Y	Y	N	San Cristobal
56	7/11/2015	BTH.15.56	Oceaniidae indet	N	Y	Y	N	San Cristobal
57	7/11/2015	BTH.15.57	*Halecium* sp.1	N	Y	N	MH361350	San Cristobal
58	7/11/2015	BTH.15.58	*Nemalecium lighti*	N	Y	N	MH361351	San Cristobal
59	7/11/2015	BTH.15.59	Oceaniidae indet	N	Y	N	N	San Cristobal
60	7/11/2015	BTH.15.60	*Obelia bidentata*	N	Y	N	MH361352	San Cristobal
61	7/11/2015	BTH.15.61	*Amphinema dinema*	Medusa	N	N	N	San Cristobal
62	7/11/2015	BTH.15.62	*Clytia gracilis*	Y	Y	Y	N	San Cristobal
63	7/11/2015	BTH.15.63	*Liriope tetraphylla*	Medusa	Y	Y	MH361353	San Cristobal
64	7/11/2015	BTH.15.64	*Thecocodium* sp.	N	Y	N	MH361354	San Cristobal
65	7/12/2015	BTH.15.65	*Plumularia floridana*	Y	Y	Y	MH361355	Vicinity of Manuguar Cay
66	7/12/2015	BTH.15.66	*Cirrholovenia tetranema*	N	N	Y	N	Vicinity of Manuguar Cay
67	7/12/2015	BTH.15.67	*Clytia hummelincki*	Y	Y	Y	N	Vicinity of Manuguar Cay
68	7/12/2015	BTH.15.68	*Clytia linearis*	N	Y	N	MH361356	Vicinity of Manuguar Cay
69	7/12/2015	BTH.15.69	*Sphaerocoryne* cf. *agassizii*	N	Y	N	N	Vicinity of Manuguar Cay
70	7/12/2015	BTH.15.70	*Halecium tenellum*	Y	Y	Y	N	Vicinity of Manuguar Cay
71	7/12/2015	BTH.15.71	*Egmundella* sp. (on *Clytia linearis*)	N	N	Y	N	Vicinity of Manuguar Cay
72	7/12/2015	BTH.15.72	*Clytia hummelincki*	Y	Y	N	MH361357	Vicinity of Manuguar Cay
73	7/12/2015	BTH.15.73	*Dynamena crisioides*	Y	Y	Y	MH361358	Vicinity of Manuguar Cay
74	7/12/2015	BTH.15.74	*Dynamena crisioides*	N	Y	N	MH361359	Vicinity of Manuguar Cay
75	7/12/2015	BTH.15.75	*Obelia bidentata*	N	Y	Y	N	Vicinity of Manuguar Cay
76	7/12/2015	BTH.15.76	*Clytia linearis*	N	Y	Y	MH361361	Vicinity of Manuguar Cay
77	7/12/2015	BTH.15.77	*Nemalecium lighti*	Y	Y	N	MH361362	Vicinity of Manuguar Cay
78	7/13/2015	BTH.15.78	*Pteroclava krempfi*	N	Y	Y	MH361363	Punta Caracol
79	7/13/2015	BTH.15.79	*Sphaerocoryne* cf. *agassizi*	N	Y	N	MH361364	Punta Caracol
80	7/13/2015	BTH.15.80	*Eudendrium capillare*	N	Y	N	N	Punta Caracol
81	7/13/2015	BTH.15.81	*Pteroclava krempfi*	N	Y	Y	N	Punta Caracol
82	7/13/2015	BTH.15.82	*Eudendrium capillare*	Y	Y	Y	N	Punta Caracol
83	7/13/2015	BTH.15.83	*Halopteris alternata*	N	Y	Y	N	Punta Caracol
84	7/13/2015	BTH.15.84	*Clytia noliformis*	Y	Y	N	N	Punta Caracol
85	7/13/2015	BTH.15.85	Bougainvilliidae/?*Bimeria*	N	Y	Y	MH361365	Punta Caracol
86	7/13/2015	BTH.15.86	*Codonorchis* sp.	N	Y	N	N	Punta Caracol
87	7/14/2015	BTH.15.87	*Solanderia gracilis*	Y	Y	Y	MH361366	Swans Cay
88	7/14/2015	BTH.15.88	*Eudendrium bermudense*	Y	Y	Y	MH361367	Swans Cay
89	7/14/2015	BTH.15.89	*Thyroscyphus ramosus*	N	Y	Y	MH361368	Swans Cay
90	7/14/2015	BTH.15.90	*Pennaria disticha*	Y	Y	Y	MH361369	Swans Cay
91	7/14/2015	BTH.15.91	*Eudendrium carneum*	Y (Female)	Y	Y	MH361370	Swans Cay
92	7/14/2015	BTH.15.92	*Stylaster roseus*	N	Y	Y	MH361371	Swans Cay
93	7/14/2015	BTH.15.93	*Stauridiosarsia nipponica*	N	Y	Y	MH361372	Swans Cay
94	7/14/2015	BTH.15.94	*Sertularia marginata*	N	Y	Y	MH361373	Swans Cay
95	7/14/2015	BTH.15.95	*Eudendrium bermudense*	Y (male)	Y	Y	N	Swans Cay

(*Continued*)

Table 1. (Continued).

	Date	Sample ID	Species	Fertile	Ethanol	Formalin	Sequenced/ GenBank accession #	Location
96	7/14/2015	BTH.15.96	*Pennaria disticha*	N	Y	N	MH361374	Swans Cay
97	7/14/2015	BTH.15.97	*Macrorhynchia grandis*	N	Y	Y	N	Swans Cay
98	7/14/2015	BTH.15.98	Bougainvilliidae 2/?*Bimeria*	N	Y	Y	MH361375	Swans Cay
99	7/14/2015	BTH.15.99	*Clytia* sp.	Y	Y	N	MH361376	Swans Cay
100	7/16/2015	BTH.15.100	*Antennella secundaria*	Y	Y	Y	N	Bastimento vicinity of "Casa Verde"
101	7/15/2015	BTH.15.101	*Dentitheca dendritica*	N	Y	N	MH361377	The Wall (25mt)/Pandora (20m)
102	7/17/2015	BTH.15.102	Filifera (on hermit crab)/?*Turritopsoides*	N	Y	N	MH361378	Almirante (Quary's point)
103	7/17/2015	BTH.15.103	*Persa incolorata*	Medusa	Y	N	N	Plankton tow near BDT docks
104	7/17/2015	BTH.15.104	*Bougainvillia ?pyramidata*	Medusa	Y	N	MH361379	Plankton tow near BDT docks
105	7/17/2015	BTH.15.105	*Cubaia aphrodite*	Medusa	N	Y	N	Plankton tow near BDT docks
106	7/18/2015	BTH.15.106	*Sertularia rugosissima*	N	Y	Y	N	Bocas del Drago
107	7/18/2015	BTH.15.107	*Sertularia rugosissima*	N	Y	N	N	Bocas del Drago
108	7/18/2015	BTH.15.108	*Rhizogeton sterreri*	N	Y	Y	MH361380	Bocas del Drago
109	7/15/2015	BTH.15.109	*Sertularella diaphana*	N	Y	Y	MH361381	The wall (25mt)/Pandora (20m)
110	7/10/2015	No Voucher	*Turritopsis* sp.1	Y	N	N	MH029856, MH029857	Close to Bocatorito Bay
111	7/7/2015	No Voucher	*Zanclea alba*	N	N	N	N	STRI BDT docks/weather station
112	7/7/2015	No Voucher	*Millepora complanata*	N	N	N	N	STRI BDT docks/weather station
113	7/7/2015	No Voucher	*Turritopsis dohrnii*	Y	N	N	MH029858, MH029859	Multiple locations

Hydrozoa species from the Mediterranean Sea [26], and more than a half of the species known from the Arctic [27] or the Antarctic [28]. Seven families and 12 genera are also reported for the first time in Bocas del Toro. Genera added to the checklist are: *Amphynema, Cytaeis, Codonorchis, Rhizogeton, Thecocodium, Turritopsoides* (?), *Gastroblasta, Egmundella, Pteroclava, Cubaia, Lyriope*, and *Persa*. Families new to the Bocas del Toro region are: Pandeidae, Cytaeididae, Cladocorynidae, Ptilocodidae, Olindiidae, Geryoniidae, Rhopalonematidae. Of the new species added to the list, of particular interest is *Thecocodium* sp. The genus *Thecocodium* was never reported in the Caribbean and was only recently recorded for the first time in the Atlantic Ocean [29]. *Thecocodium* sp. (specimen BTH 15.64) presents unique morphological features and may represent a new species. Another species of interest is a species of the genus *Coryne* (specimen 15.93) found in Swans Key. The colony could not be identified at the species level; however in GenBank its 16S sequence showed 100% identity with *Coryne japonica* (AY512540) from New Zealand. *C. japonica* has been reported from the Pacific Ocean but never from the Atlantic Ocean. Our record from Bocas del Toro is the first in the Atlantic Ocean and may represent an introduced species.

Taxon identification sheets

Taxon Identification Sheets collate the taxonomic description of 56 species found during the Hydrozoa Taxonomy course and confidently identified at the species level. They also include a brief synopsis of the 28 families they belong to (see Tables 1–88 in Supplementary Materials. For each family we included authorship, corresponding Order, number of species (worldwide and in Bocas del Toro), morphologically similar taxa that could be mistakenly identified as member of the family of interest, and their key diagnostic characters. The species identification sheets include author, diagnostic characters of the colony and their reproductive structures (medusae, eumedusoids, or fixed gonophores), species ecology, species distribution in Bocas del Toro, number of specimens collected, pictures and, when available, GenBank accession numbers for their 16S sequence(s). These species identification sheets assemble in simple format information that can be used for a correct identification. The morphological description, pictures, and link to their 16S barcoding sequence represent a comprehensive display of information that integrates traditional and modern taxonomy into a practical tool to aid identification of the most commons species of Hydrozoa in the shallow waters of the Archipiélago de Bocas del Toro, Panamá.

Conclusive remarks

Knowledge on the local biodiversity is an essential prerequisite for the monitoring and management of

Table 2. List of species found in Bocas del Toro during the Hydrozoa Taxonomy course in 2015. For each species we report voucher name(s), number of barcoding sequences (mt lsu-rRNA, 16S) produced per species, and whether their taxonomic description is in the Taxon Identification Tables. A total of 53 taxa were identified at the species level. At the bottom, 17 taxa identified at the genus or higher taxonomic level.

	Species	Voucher Name	Family	Barcoding (n. of sequences)	Taxon Identification Table
1	*Turritopsis dohrnii*	Yes	Oceaniidae	Yes (2)	Yes
2	*Turritopsis* sp. 1	BTH.15.110	Oceaniidae	Yes (2)	Yes
3	*Turritopsis* sp. 4	No	Oceaniidae	No	Yes
4	*Rhizogeton sterreri*	BTH.15.108	Oceaniidae	Yes (1)	Yes
5	*Bougainvillia* cf. *pyramidata* medusae	BTH.15.104	Bougainvilliidae	Yes (1)	No
6	*Amphinema dinema*	BTH.15.61	Pandeidae	No	Yes
7	*Stylaster roseus*	BTH.15.25; BTH.15.92	Stylasteridae	Yes (2)	Yes
8	*Eudendrium bermudense*	BTH.15.17; BTH.15.95; BTH.15.88	Eudendriidae	Yes (1)	Yes
9	*Eudendrium capillare*	BTH.15.80; BTH.15.82	Eudendriidae	No	Yes
10	*Eudendrium carneum*	BTH.15.91; BTH.15.15; BTH.15.35	Eudendriidae	Yes (3)	Yes
11	*Myrionema amboinense*	BTH.15.11	Eudendriidae	No	Yes
12	*Ralpharia gorgoniae*	BTH.15.24	Tubulariidae	Yes (1)	Yes
13	*Millepora alcicornis*	BTH.15.31	Milleporidae	Yes (1)	Yes
14	*Millepora complanata*	Yes Not sampled.	Milleporidae	No	No
15	*Solanderia gracilis*	BTH.15.87	Solanderiidae	Yes (1)	Yes
16	*Pennaria disticha*	BTH.15.23; BTH.15.90; BTH.15.96	Pennariidae	Yes (3)	Yes
17	*Sphaerocoryne* cf. *agassizii*	BTH.15.40; BTH.15.79; BTH.15.69	Sphaerocorynidae	Yes (2)	Yes
18	*Pteroclava krempfi*	BTH.15.78; BTH.15.81	Cladocorynidae	Yes (1)	Yes
19	*Zanclea alba*	Yes Not Vouchered	Zancleidae	No	Yes
20	*Aglaophenia latecarinata*	BTH.15.20	Aglaopheniidae	No	Yes
21	*Macrorhynchia grandis*	BTH.15.97	Aglaopheniidae	No	Yes
22	*Antennella secundaria*	BTH.15.13; BTH.15.100; BTH.15.18	Halopteridiidae	Yes (2)	Yes
23	*Halopteris alternata*	BTH.15.50; BTH.15.53; BTH.15.83; BTH.15.14	Halopteridiidae	Yes (3)	Yes
24	*Kirchenpaueria halecioides*	BTH.15.1; BTH.15.5; BTH.15.47; BTH.15.21; BTH.15.54	Kirchenpaueriidae	Yes (2)	Yes
25	*Dentitheca dendritica*	BTH.15.101	Plumulariidae	Yes (1)	Yes
26	*Plumularia margaretta*	BTH.15.42; BTH.15.51	Plumulariidae	Yes (1)	Yes
27	*Plumularia floridana*	BTH.15.65	Plumulariidae	Yes (1)	Yes
28	*Thyroscyphus marginatus*	BTH.15.26; BTH.15.29	Thyroscyphidae	Yes (2)	Yes
29	*Thyroscyphus ramosus*	BTH.15.89	Thyroscyphidae	Yes (1)	Yes
30	*Hincksella formosa*	BTH.15.39	Syntheciidae	Yes (1)	Yes
31	*Dynamena crisioides*	BTH.15.7; BTH.15.9; BTH.15.33; BTH.15.73; BTH.15.74	Sertulariidae	Yes (4)	Yes
32	*Dynamena disticha*	BTH.15.32	Sertulariidae	Yes (1)	Yes
33	*Sertularella diaphana*	BTH.15.109	Sertulariidae	Yes (1)	Yes
34	*Sertularia rugosissima*	BTH.15.106; BTH.15.107	Sertulariidae	No	Yes
35	*Sertularia marginata*	BTH.15.94	Sertulariidae	Yes (1)	Yes
36	*Sertularia distans*	BTH.15.43	Sertulariidae	Yes (1)	Yes
37	*Salacia desmoides*	BTH.15.16	Sertulariidae	Yes (1)	Yes
38	*Gastroblasta raffaelei*	BTH.15.27; BTH.15.44; BTH.15.46; BTH.15.48; BTH.15.49; BTH.15.55	Campanulariidae	Yes (1)	Yes
39	*Clytia gracilis*	BTH.15.62	Campanulariidae	No	Yes
40	*Clytia hemisphaerica*	BTH.15.36; BTH.15.37; BTH.15.41	Campanulariidae	No	Yes
41	*Clytia linearis*	BTH.15.6; BTH.15.76; BTH15.68	Campanulariidae	Yes (3)	Yes
42	*Clytia noliformis*	BTH.15.84	Campanulariidae	No	Yes
43	*Clytia hummelincki*	BTH.15.62; BTH.15.67; BTH.15.72	Campanulariidae	Yes (1)	Yes
44	*Obelia bidentata*	BTH.15.60; BTH.15.75	Campanulariidae	Yes (1)	Yes
45	*Obelia dichotoma*	BTH.15.30?, BTH.15.19; BTH.15.34	Campanulariidae	No	Yes
46	*Cirrholovenia tetranema*	BTH.15.66	Lovenellidae	No	Yes
47	*Halecium* cf. *nanum*	BTH.15.45	Haleciidae	Yes (1)	Yes
48	*Halecium tenellum*	BTH.15.70	Haleciidae	No	Yes
49	*Halecium bermudense*	BTH.15.2, BTH.15.10	Haleciidae	No	Yes
50	*Nemalecium lighti*	BTH.15.77; BTH.15.3; BTH.15.4; BTH.15.58	Haleciidae	Yes (3)	Yes
51	*Cubaia aphrodite* medusa	BTH.15.105	Olindiidae	No	Yes
52	*Liriope tetraphylla* medusa	BTH.15.63	Geryoniidae	Yes (1)	Yes
53	*Persa incolorata* medusa	BTH.15.103	Rhopalonematidae	No	Yes
1	Filifera (on hermit Crab: ? *Turritopsoides*)	BTH.15.102	?	Yes (1)	No
2	*?Cytaeis* sp.	BTH.15.8	Cytaeididae	No	No
3	*Codonorchis* sp.	BTH.15.86	Pandeidae	No	Yes
4	Oceaniidae indet	BTH.15.56	Oceaniidae	No	No
5	Oceaniidae indet	BTH.15.59	Oceaniidae	No	No
6	Bougainvilliidae 2/*Bimeria*?	BTH.15.98	Bougainvilliidae	Yes (1)	No
7	Bougainvilliidae/*Bimeria*?	BTH.15.85	Bougainvilliidae	Yes (1)	No
8	*Stauridiosarsia nipponica*	BTH.15.93	Corynidae	Yes (1)	Yes
9	*Thecocodium* sp.	BTH.15.64	Ptilocodiidae	Yes (1)	No
10	*Halecium* cf. *bermudense*	BTH.15.2	Haleciidae	No	No
11	*Halecium* sp. 1	BTH.15.57	Haleciidae	Yes (1)	No
12	*Halecium* sp. 2	BTH.15.38	Haleciidae	Yes (1)	No

(*Continued*)

Table 2. (Continued).

	Species	Voucher Name	Family	Barcoding (n. of sequences)	Taxon Identification Table
13	*Halecium* sp. [sensu 17]	BTH.15.52	Haleciidae	Yes (1)	No
14	*Plumularia* sp.1	BTH.15.22	Plumulariidae	No	No
15	*Plumularia* sp.2	BTH.15.12	Plumulariidae	No	No
16	*Clytia* sp.	BTH.15.99	Campanulariidae	Yes (1)	No
17	*Egmundella* sp. (on *Clytia linearis*)	BTH.15.71	Campanulinidae	No	Yes

Table 3. Updated checklist of the Hydrozoa of Bocas del Toro. The list includes species reported in Calder and Kirkendale [13], and this paper. For the species in this paper, the voucher number is reported. At the bottom, taxa identified at the genus or higher taxonomic level.

	Family	Species	Calder & Kirkerdale [13]	This paper
1	Pandeidae	*Amphinema dinema*	No	BTH.15.61
2	Cordylophoridae	*Corydendrium parasiticum*	Yes	No
3	Oceaniidae	*Turritopsis dohrnii*	No	Yes
4	Oceaniidae	*Turritopsis* sp. 1	No	BTH.15.110
5	Oceaniidae	*Turritopsis* sp. 4	No	No
6	Oceaniidae	*Turritopsis nutricula*	Yes	No
7	Oceaniidae	*Rhizogeton sterreri*	No	BTH.15.108
8	Bougainvilliidae	*Bimeria vestita*	Yes	No
9	Bougainvilliidae	*Bougainvillia ?pyramidata*	No	BTH.15.104
10	Bougainvilliidae	*Parawrightia robusta*	Yes	No
11	Bougainvilliidae	*Silhouetta uvacarpa*	Yes	No
12	Stylasteridae	*Stylaster roseus*	Yes	BTH.15.25; BTH.15.92
13	Eudendriidae	*Eudendrium bermudense*	Yes	BTH.15.17; BTH.15.95; BTH.15.88
14	Eudendriidae	*Eudendrium capillare*	Yes	BTH.15.80; BTH.15.82
15	Eudendriidae	*Eudendrium carneum*	Yes	BTH.15.91; BTH.15.15; BTH.15.35
16	Eudendriidae	*Eudendrium* sp., aff. *album*	Yes	No
17	Eudendriidae	*Myrionema amboinense*	Yes	BTH.15.11
18	Tubulariidae	*Ectopleura mayeri*	Yes	No
19	Tubulariidae	*Ralpharia gorgoniae*	Yes	BTH.15.24
20	Tubulariidae	*Zyzzyzus calderi*	Yes	No
21	Sphaerocorynidae	*Sphaerocoryne* cf. *agassizii*	No	BTH.15.40; BTH.15.79; BTH.15.69
22	Sphaerocorynidae	*Sphaerocoryne bedoti*	Yes	No
23	Cladocorynidae	*Pteroclava krempfi*		BTH.15.78; BTH.15.81
24	Corynidae	*Stauridiosarsia nipponica*	No	BTH.15.93
25	Zancleidae	*Zanclea alba*	Yes	Yes, Not vouchered.
26	Solanderiidae	*Solanderia gracilis*	Yes	BTH.15.87
27	Pennariidae	*Pennaria disticha*	Yes	BTH.15.23; BTH.15.28; BTH.15.90; BTH.15.96
28	Milleporidae	*Millepora alcicornis*	Yes	BTH.15.31
29	Milleporidae	*Millepora complanata*	Yes	No
30	Milleporidae	*Millepora squarrosa*	Yes	No
31	Laphoeinidae	*Cirrholovenia tetranema*	Yes	BTH.15.66
32	Haleciidae	*Halecium lightbourni*	Yes	No
33	Haleciidae	*Halecium nanum*	Yes	No
34	Haleciidae	*Halecium* cf. *nanum*	No	BTH.15.45
35	Haleciidae	*Halecium tenellum*	Yes	BTH.15.70
36	Haleciidae	*Halecium bermudense*	No	BTH.15.2, BTH.15.10
37	Haleciidae	*Nemalecium lighti*	Yes	BTH.15.77; BTH.15.3; BTH.15.4; BTH.15.58
38	Haleciidae	*Sagamihydra dyssymetra*	Yes	No
39	Kirchenpaueriidae	*Kirchenpaueria halecioides*	Yes	BTH.15.1; BTH.15.5; BTH.15.47; BTH.15.21; BTH.15.54
40	Plumulariidae	*Dentitheca dendritica*	Yes	BTH.15.101
41	Plumulariidae	*Monotheca margaretta*	Yes	BTH.15.42; BTH.15.51
42	Plumulariidae	*Plumularia floridana*	Yes	BTH.15.65
43	Plumulariidae	*Plumularia setacea*	Yes	No
44	Plumulariidae	*Plumularia strictocarpa*	Yes	No
45	Halopteridiidae	*Antennella curvitheca*	Yes	No
46	Halopteridiidae	*Antennella secundaria*	Yes	BTH.15.13; BTH.15.100; BTH.15.18
47	Halopteridiidae	*Halopteris alternata*	Yes	BTH.15.50; BTH.15.53; BTH.15.83; BTH.15.14
48	Halopteridiidae	*Halopteris carinata*	Yes	No
49	Aglaopheniidae	*Aglaophenia dubia*	Yes	No
50	Aglaopheniidae	*Aglaophenia latecarinata*	Yes	BTH.15.20
51	Aglaopheniidae	*Macrorhynchia philippina*	Yes	No
52	Aglaopheniidae	*Macrorhynchia grandis*		BTH.15.97
53	Thyroscyphidae	*Thyroscyphus marginatus*	Yes	BTH.15.26; BTH.15.29
54	Thyroscyphidae	*Symmetroscyphus intermedius*	Yes	No
55	Thyroscyphidae	*Thyroscyphus ramosus*	Yes	BTH.15.89
56	Syntheciidae	*Hincksella formosa*	Yes	BTH.15.39
57	Syntheciidae	*Synthecium tubithecum*	Yes	No
58	Sertulariidae	*Diphasia tropica*	Yes	No

(*Continued*)

Table 3. (Continued).

	Family	Species	Calder & Kirkerdale [13]	This paper
59	Sertulariidae	*Dynamena crisioides*	Yes	BTH.15.7; BTH.15.9; BTH.15.33; BTH.15.73; BTH.15.74
60	Sertulariidae	*Dynamena disticha*	Yes	BTH.15.32
61	Sertulariidae	*Dynamena quadridentata*	Yes	No
62	Sertulariidae	*Sertularella cylindritheca*	Yes	No
63	Sertulariidae	*Sertularella diaphana*	No	BTH.15.109
64	Sertulariidae	*Sertularella hartlaubi*	Yes	No
65	Sertulariidae	*Sertularia rugosissima*	No	BTH.15.106; BTH.15.107
66	Sertulariidae	*Sertularia loculosa*	Yes	No
67	Sertulariidae	*Sertularia marginata*	Yes	BTH.15.94
68	Sertulariidae	*Tridentata subtilis*	Yes	No
69	Sertulariidae	*Sertularia turbinata*	Yes	No
70	Sertulariidae	*Sertularia distans*	No	BTH.15.43
71	Sertulariidae	*Sertularia vervoorti*	Yes	No
72	Sertulariidae	*Salacia desmoides*	No	BTH.15.16
73	Campanulariidae	*Gastroblasta raffaelei*	No	BTH.15.27; BTH.15.44; BTH.15.46; BTH.15.48; BTH.15.49; BTH.15.55
74	Campanulariidae	*Clytia gracilis*	Yes	BTH.15.62
75	Campanulariidae	*Clytia hemisphaerica*	Yes	BTH.15.36; BTH.15.37; BTH.15.41
76	Campanulariidae	*Clytia linearis*	Yes	BTH.15.6 ; BTH.15.76; BTH15.68
77	Campanulariidae	*Clytia paulensis*	Yes	No
78	Campanulariidae	*Clytia stolonifera*	Yes	No
79	Campanulariidae	*Clytia gracilis*	No	BTH.15.62
80	Campanulariidae	*Clytia noliformis*	No	BTH.15.84
81	Campanulariidae	*Clytia hummelincki*	No	BTH.15.62; BTH.15.67; BTH.15.72
82	Campanulariidae	*Obelia bidentata*	Yes	BTH.15.60; BTH.15.75
83	Campanulariidae	*Obelia dichotoma*	Yes	BTH.15.30?, BTH.15.19; BTH.15.34
84	Olindiidae	*Cubaia aphrodite* medusa	No	BTH.15.105
85	Geryoniidae	*Liriope tetraphylla* medusa	No	BTH.15.63
86	Rhopalonematidae	*Persa incolorata* medusa	No	BTH.15.103
	Family	**Species**	**Calder**	**This paper**
1	?	Filifera (on Hermit Crab) (? *Turritopsoides*)	No	BTH.15.102
2	Cytaeididae	?*Cytaeis* sp.	No	BTH.15.8
3	Pandeidae	*Codonorchis* sp.	No	BTH.15.86
4	Cordylophoridae	*Rhizodendrium* sp.	Yes	No
5	Oceaniidae	Oceaniidae indet.	No	BTH.15.56
6	Oceaniidae	Oceaniidae indet.	No	BTH.15.59
7	Bougainvilliidae	Bougainvilliidae 2/?*Bimeria*	No	BTH.15.98
8	Bougainvilliidae	Bougainvilliidae/?*Bimeria*	No	BTH.15.85
9	Bougainvilliidae	Bougainvilliidae indet.	Yes	No
10	Eudendriidae	*Eudendrium* sp.	Yes	No
11	Corynidae	*Coryne* sp.	Yes	No
12	Corynidae	Corynidae indet.	Yes	No
13	Zancleidae	*Zanclea* sp.	Yes	No
14	Hydrocorynidae	*Hydrocoryne* sp.	Yes	No
15	Ptilocodiidae	*Thecocodium* sp.	No	BTH.15.64
16	Haleciidae	*Halecium* cf. *bermudense*	No	BTH.15.2
17	Haleciidae	*Halecium* sp.	Yes	No
18	Haleciidae	*Halecium* sp. 1	No	BTH.15.57
19	Haleciidae	*Halecium* sp. 2	No	BTH.15.38
20	Haleciidae	*Halecium* sp. [sensu 17]	No	BTH.15.52
21	Haleciidae	*Hydranthea* sp.	Yes	No
22	Plumulariidae	*Plumularia* sp.	No	BTH.15.22
23	Plumulariidae	*Plumularia* sp.	No	BTH.15.12
24	Campanulariidae	*Clytia* sp., aff. *kincaidi*	Yes	No
25	Campanulariidae	*Clytia* sp.	No	BTH.15.99
26	Campanulariidae	*Clytia* sp. A	Yes	No
27	Campanulariidae	*Clytia* sp. B	Yes	No
28	Campanulariidae	*Clytia* sp. C	Yes	No
29	Campanulariidae	*Orthopyxis* sp.	Yes	No
30	Halammohydridae	*Halammohydra* sp.	Yes	No
31	Otohydridae	*Otohydra* sp.	Yes	No
32	Campanulinidae	*Egmundella* sp. (on *Clytia linearis*)	No	BTH.15.71

environmental assets and ecosystem health worldwide. The present inventory of the marine hydrozoan fauna in the Bocas del Toro shallow water is far to be exhaustive due to the inherent limitation of our sampling efforts, based mostly on snorkeling and more rarely on SCUBA diving collections. However, the high number of recorded taxa suggests that the Caribbean Sea should be considered a region of high hydrozoan diversity. Paradoxically, taxonomy is a science at brink of extinction. The ARTS courses have been devoted not only to increase knowledge on local biodiversity, but towards the conservation and promotion of biodiversity

expertise. More generally, training a new generation of taxonomists is a current challenge and a mandatory urge to understanding ecosystem functioning in face of local and global changes, and to address the needs of sustainability of humankind activities.

Author contributions

MPM and S. Piraino designed the experiments; S. Pruski produced the barcoding sequences, all authors collected samples and contributed the Taxon Identification Tables: MPM wrote the paper.

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