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Research Article

Fouling ascidians (Chordata: Ascidiacea) of the Galápagos: Santa Cruz and Baltra Islands

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Abstract

The Galápagos Marine Bioinvasions Expedition (April 24–May 4, 2016) carried out a survey of the biofouling communities on a number of floating docks and pilings, as well as numerous previously deployed settlement panels both uncaged and caged, on two of the Galápagos Islands: Santa Cruz and neighboring Baltra. The suspended panels had been submerged for either 3 months or 14 months. Studies were concentrated at two sites on Santa Cruz Island, which is the location of the main town Puerto Ayora and has the most vessel traffic. The small adjacent Baltra Island contains the airport and there is frequent boat traffic there, as well as a Navy base with a floating dock. Ascidians were the dominant foulers on most panels, especially the caged ones, with a dramatic difference in coverage between caged and uncaged which is presumed to result from differential fish predation pressure since the panels were all suspended with none touching the benthos. There were schools of small fish around all the docks observed to be constantly nipping at the dock surfaces. Eighteen species of ascidians were collected, comprising 12 new records. Of the 14 identified to species level, 11 are considered introduced and 3 cryptogenic. The most common species included *Didemnum perlucidum* Monniot, 1983, *Diplosoma listerianum* (Milne Edwards, 1841), *Polyclinum constellatum* Savigny, 1816, *Ascidia sydneiensis* Stimpson, 1855, *Ascidia ceratodes* (Huntsman, 1912), an *Ascidia* sp. with long tunic spines, *Polyandrocarpa zorritensis* (Van Name, 1931), *Styela canopus* (Savigny, 1816), and *Pyura haustor* (Stimpson, 1864). Other foulers included small colonies of *Botrylloides niger* Herdman, 1886, a few small colonies of *Symplegma brakenhielmi* (Michaelsen, 1904), a dark *Didemnum* sp. and another unidentified didemnid, a pink *Symplegma rubra* Monniot, 1972, three very tiny *Molgula* sp. and a single specimen of *Microcosmus exasperatus* Heller, 1878. Several colonies of *Botrylloides giganteus* (Pérès, 1949) were retrieved from plates on the Baltra Navy dock. Two large specimens (~ 6 cm) of *Halocynthia dumosa* (Stimpson, 1855) (usually incorrectly referred to as *H. hispida* (Herdman, 1881)) were collected from the Baltra dock and one by SCUBA at 12.2 m off of Bartolome Island. A complete species list is presented, with a comparison with previous published and unpublished records.

Key words: floating docks, non-indigenous, introduced, biofouling, anthropogenic, tunicates

Introduction

Floating docks, pilings, boat hulls and other non-natural benthic surfaces (called artificial substrates) are well documented as sites of first arrival and establishment of many introduced marine species, transported by anthropogenic vectors (Ruiz et al. 2000; Fofonoff et al. 2003; Wonham and Carlton 2005). Such vectors are responsible for the majority of ascidian introductions, because ascidians have a short-lived non-feeding larva and on their own have very limited dispersal ability (Lambert 2005). Once established in harbors and marinas, some of these non-native species may subsequently spread and become established on the natural benthos, disrupting the community structure and biodiversity of native communities (Bumbeer and Rocha 2016). An extreme example of this is the ascidian *Didemnum vexillum* Kott, 2002, now established worldwide (Lambert 2009).The Galápagos Islands are a World Heritage Site and the location of many unique endemic species; establishment of introduced species is thus an important threat to this protected area.

The 2015 and 2016 Marine Bioinvasions Expeditions to the Galápagos led by scientists from the Smithsonian Environmental Research Center (SERC), Williams College and the Charles Darwin Research Foundation (CDRF) surveyed biofouling communities on a number of floating docks and pilings on two of the Galápagos Islands, Santa Cruz and Baltra. Numerous experimental settlement plates deployed in 2015 and early 2016 on these islands were retrieved and examined in 2016. Studies were concentrated on Santa Cruz Island because this is the location of the main town, Puerto Ayora, with the majority of Archipelago vessel traffic. The small adjacent Baltra Island has frequent boat traffic due to the location of the airport there as well as a Navy base and dock with a floating pontoon.

There have been very few studies of the shallow-water ascidians of the Galápagos Islands. Van Name (1945) stated that "the ascidians of the Galápagos are as yet unknown". Tokioka (1967) reported *Didemnum candidum* Savigny, 1816 from Isabela (Albemarle) Island from collections made during an Allan Hancock *Velero* Expedition to the Galápagos in 1934. The single colony was on the carapace of the crab *Dromidia sarraburei* Rathbun, 1910 (now *Moreiradromia sarraburei*) taken in "shallow water, coral", station 189-34 (Fraser 1943). As noted below, the name *D. candidum* may represent any of a number of species; without reexamination it cannot be known what Tokioka examined. Millar (1988) listed 6 species from the Galápagos collected intertidally and by shallow SCUBA and dredging during a Smithsonian Southeast Pacific 1965–66 expedition. Duclaux et al. (1988) collected colonies of the didemnid *Polysyncraton bilobatum* Lafargue, 1968 and *Cystodytes* sp. containing the symbiont *Prochloron* in a loose non-obligate association. Monniot and Monniot (1989) recorded 8 species from the Galápagos, collected by a manned submersible from 300–800 m, 5 of them new species; another two may be new species. There appears to be no overlap with shallow water species except possibly for *Halocynthia dumosa* (Stimpson, 1855) (listed as *H. hispida* (Herdman, 1881); see comments below) and *Aplidium* *californicum* (Ritter and Forsyth, 1917) which they reported as very common intertidally plus a few deepwater small immature colonies which they could not verify as that species. Iturralde (1991, *unpublished*; see his Table 4) collected 16 species in Tagus Cove, Isabela Island during 1988–1989 but due to taxonomic difficulties most were identified only to family, a few to genus, and three to species. They belonged to six families: Polycitoridae (*Cystodytes dellechiajei*), Polyclinidae, Didemnidae (*Didemnum candidum*), Ascidiidae, Styelidae and Pyuridae (*Halocynthia hispida*). Bustamante et al. (2002) added *Leptoclinides* sp. and *Botryllus tuberatus* Ritter and Forsyth, 1917, based upon 2000–2001 rocky sublittoral (6 and 15 m) collections throughout the Islands. Witman and Smith (2003) recorded 8 spp., two identified only to genus, from Rocas Gordon, a Marine Reserve off northeast Santa Cruz Island. Banks et al. (2009), also working at 6 and 15 m from 2005–2007 on Wolf, Darwin, and Marchena Islands, recorded several species previously reported, newly adding *Tridemnum* spp. The Charles Darwin Foundation in Puerto Ayora, Santa Cruz Is. lists 13 unverified ascidian species (Chiriboga et al. 2016). Most of the above records are summarized in Table 1.

Materials and methods

In February 2015 divers on both snorkel and SCUBA collected ascidians from biofouled dock and piling communities and from adjacent sublittoral waters on two of the Galápagos Islands, Santa Cruz and Baltra. From April 24–May 4, 2016 the Marine Bioinvasions Expedition members analyzed the numerous settlement plates, both uncaged and caged, that had been deployed (February 2015 and January 2016, 14 months and 3 months submergence respectively), and resurveyed the supporting floating docks and pilings. The sites chosen were the Puerto Ayora floating passenger docks (0°44′52.18″S; 90°18′45.23″W) and a private floating dock in Franklin's Bay (0°45′18.70″S; 90°18′45.55″W) on Santa Cruz Island, and the Navy floating dock on Baltra Island (0°26′10.91″S; 90°17′50.89″W) (Supplementary material Table S1). The 14 × 14 cm, 0.5 cm thick, grey high-impact polyvinyl chloride (PVC) plates had been lightly sanded on one side to optimize attachment conditions, and suspended horizontally at one meter depth below floating docks attached to bricks with the roughened settlement surface facing down. Some of the plates were enclosed in heavy-duty 6 mm Vexar mesh cages (Figure 1A).

Upon retrieval from each site, the plates were placed in Ziploc bags filled with seawater and brought to the lab in a large cooler where they were then examined live under a dissecting microscope while immersed in a shallow pan of sea water. Voucher samples were removed and preserved in either 10% seawater formalin or 95% ethanol in numbered vials; additional samples of ascidians for morphological analysis were relaxed in menthol in

Table 1. Shallow-water ascidians from the Galápagos Islands. Status: I, Introduced; C, Cryptogenic.

	Status	New record	Present study	Chiriboga et al. 2016	Millar 1988	Iturralde 1991 (*unpubl.*)	Bustamante et al. 2002	Witman and Smith 2003	Banks et al. 2009
Year of Collections:			2015–2016	not given	1965–1966	1988–1989	2000–2001	1999–2000	2005–2007
TAXON:									
Aplousobranchia									
Aplidium californicum	C				x				
Aplidium solidum	C			x				x	
Aplidium sp.						x			
Aplidium spp.									x
Cystodytes dellechiajei	C			x	x	x			
Didemnum candidum						x			
?Didemnum carnulentum					x				
Didemnum cineraceum	C			x				x	x
Didemnum sp.			x						
Didemnum perlucidum	I		x	x				x	
Didemnum sp.								x	
Didemnum sp.									x
Didemnid A			x						
Diplosoma listerianum	I		x	x				x	
Eudistoma sp./spp.					x	x			
Leptoclinides sp.				x			x		
Polyclinum constellatum	I	x	x						
?Polyclinum planum					x				
Tridemnum spp.									x
Phlebobranchia									
Ascidia ceratodes	C		x	x					
Ascidia sydneiensis	I	x	x						
Ascidia sp.		x	x						
Ascidia sp.						x			
Stolidobranchia									
Botrylloides giganteus	I	x	x						
Botrylloides niger	I	x	x						
Botryllus tuberatus	C			x			x		
Eusynstyela tincta				x					
Eusynstyela sp.								x	
Metandrocarpa manina				x					
Polyandrocarpa sagamiensis				x					
Polyandrocarpa zorritensis	I	x	x						
Polyandrocarpa sp.						x			
Polycarpa ecuadorensis	C			as *Polyandrocarpa ecuadorensis*				x	
Styela canopus	I	x	x						
Symplegma brakenhielmi	I	x	x						
Symplegma rubra	I	x	x						
Halocynthia dumosa	C		x	x	as *H. hispida*	as *H. hispida*			
Microcosmus exasperatus	I	x	x						
Pyura haustor	C		x					x	x (as cf.)
Molgula sp./spp.		x	x						

sea water followed by preservation in 10% seawater formalin buffered with sodium borate (formula/liter: 850 ml seawater, 50 ml tap or distilled water, 100 ml full strength 37% formaldehyde, 1 gram sodium borate). Photographs were taken of all plates before removal of any organisms. After a sample of every species was vouchered from each plate, the plates were then scraped, washed and dried.

Results

Eighteen species of ascidians were collected during the present study (Table 1, Figures 1–4). Based on a combination of taxonomic, historical, biogeographic and ecological (including habitat) data, each species in the present study, as well as in the published papers by Millar (1988) and Witman and Smith (2002) was assigned a status of native, introduced, or cryptogenic (see Chapman and Carlton 1991 and Carlton 1996 for the criteria used for determining status). The identifications are still incomplete for a few species; nevertheless, 12 of the species are new records for the Galápagos. Some of the samples were sequenced using CO1, resulting in a 99% match for *Botrylloides niger* and *Polyandrocarpa zorritensis* (J.B. Geller, *personal communication*). In this study, those identified to species level are considered introduced except *Ascidia ceratodes*, *Halocynthia dumosa* and *Pyura haustor*, which are considered cryptogenic.

During the 2015 survey of artificial substrates, most of the ascidians remained unidentified and did not include any identified species different from the much more extensive 2016 survey. The ascidian species checklist (Chiriboga et al. 2016; entries unchanged from the 2014 checklist) compiled at the Charles Darwin Research Foundation from earlier collections contains some species not recorded by this expedition (Table 1), but those identifications have not been verified and thus their status (Introduced, Cryptogenic, Native), as well as for the species in the unpublished thesis of Iturralde (1991), are not included.

Systematic Account

Aplousobranchia

Didemnidae

Didemnum perlucidum Monniot, 1983

Figure 1B, C

Collected during 2015 and 2016 expeditions. Franklin's Bay dock fouling and settlement panels especially those caged for 14 months (e.g. 16613b, 30241), and Baltra Is., abundant on settlement plates and cages, including samples 30234, 30251, 30261, 30268, 30287. This species is worldwide in distribution in tropical and subtropical waters, especially as a fouler of artificial substrates in harbors (Dias et al. 2016). It was well described by F. Monniot (1983a) from Guadeloupe Island in the Caribbean, who recognized that it was most likely not native to that region. Its native region remains unknown. *D. perlucidum* was earlier reported from the Galápagos by Witman and Smith (2003) based on 1999–2000 collections.

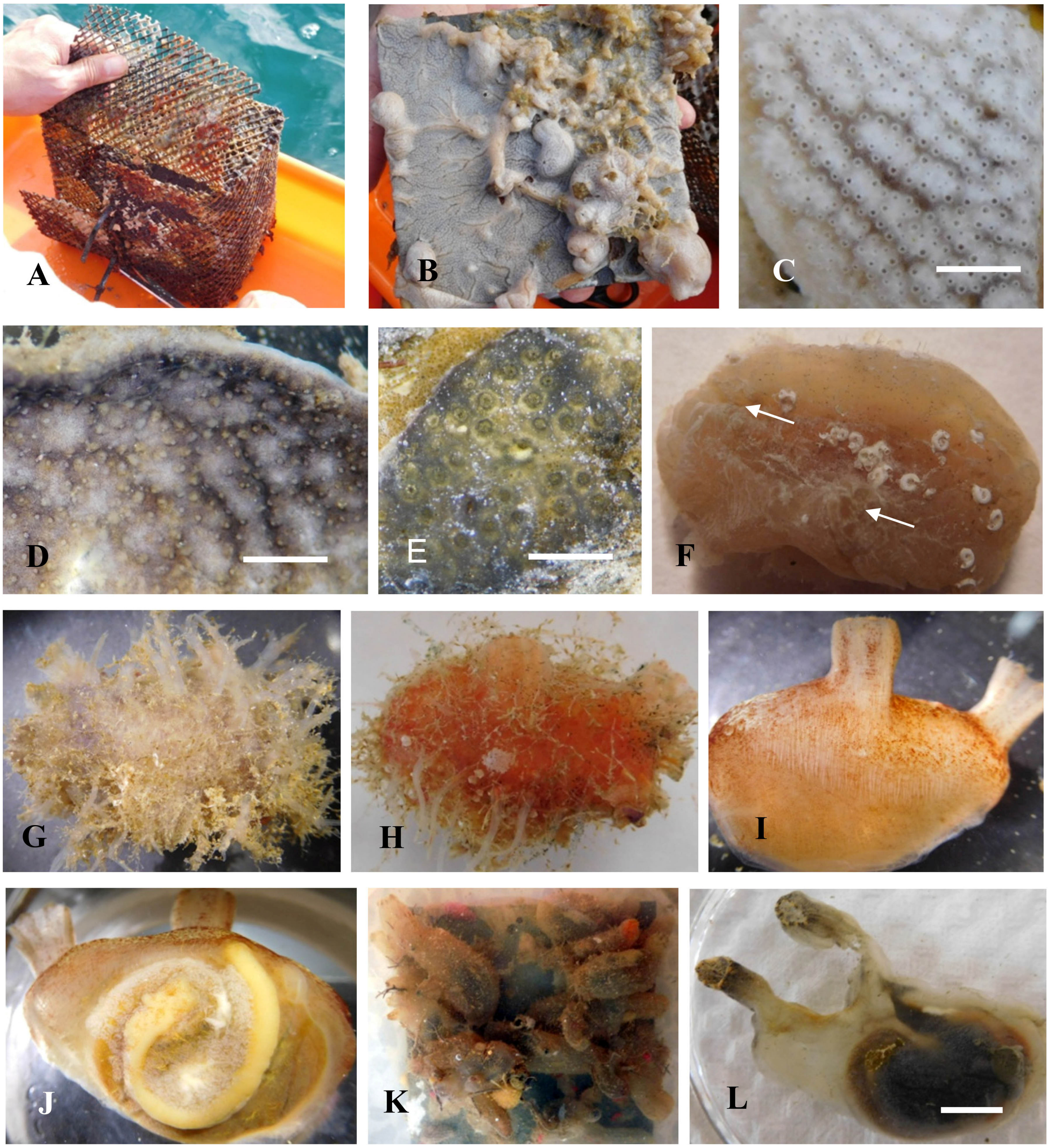

Figure 1. A: cage; B: 14 month caged panel from Baltra Is. Navy dock covered with *Didemnum perlucidum*; C: closeup of part of *D. perlucidum* colony; D: *Didemnum* sp.; E: *Polyclinum constellatum*; F: *Ascidia ceratodes* 3 cm in length, very contracted, from 14 month caged plate, Puerto Ayora town dock: left arrow oral siphon, right arrow atrial siphon; G–J: *Ascidia* sp.: G, H: two color morphs 3 cm in length; I: right side body of red individual (out of tunic) showing musculature; J: left side body; K: *Ascidia sydneiensis* covering 14 month caged plate from Franklin's Bay; L: left side body of another *A. sydneiensis* showing expanded posterior intestine. Scale bars: C, 3 mm; D, 4.25 mm; E, 2.7 mm; L, 1 cm. Photos by G. Lambert.

***Didemnum* sp.**

Figure 1D

Puerto Ayora town dock 4-25-16, settlement plate 234096; Franklin's Bay 4-27-16, on rock, collected during snorkeling by N. de Voogd. Tunic with a pavement pattern, very dark due to abundant black pigment (retained when fixed in seawater formalin); dark channels between groups of zooids. Resembles morphologically *D. cineraceum* (Sluiter, 1898) (Monniot F 1983a, 1995; Rodrigues et al. 1998) but DNA sequencing does not match (R. Rocha, *personal communication*). *D. cineraceum* was reported from the Galápagos by F. Monniot (1995) as an unpublished comment, and by Witman and Smith (2003), and is included in the Chiriboga et al. (2016) checklist.

Didemnid A

Several tiny colonies on tunic of *Halocynthia dumosa* collected 4-29-2016 by G. Ruiz on Bartolome Is., 12.2 m depth. Black with dense spherical spicules. Zooids too tiny, contracted and immature to determine even the genus.

***Diplosoma listerianum* (Milne Edwards, 1841)**

Franklin's Bay dock and settlement plates, and Baltra Is. Navy dock settlement plates and cages, collected during 2015 and 2016 expeditions. See Van Name (1945) for description (as *D. macdonaldi* Herdman, 1886) and distribution. The species occurs now worldwide, its origin unknown, and most likely is a species complex only partially understood and analyzed (Monniot C et al. 1985; Pérez-Portela et al. 2013). It was reported from the Galápagos by Witman and Smith (2003), based on 1999–2000 collections.

Polyclinidae

***Polyclinum constellatum* Savigny, 1816**

Figure 1E

New record for Galápagos. Several small colonies on Puerto Ayora dock; three colonies at end of short rope attached to dock; one colony on plate 30242; 4-25-2016. Franklin's Bay 4-27-2016, dock fouling and on several settlement plates, two color morphs: tan, and dark greenish. This is a very common fouling species, worldwide in distribution in all warm-water oceans; origin unknown (Monniot C et al. 1985). For descriptions see Van Name (1945) and Monniot F (1983b).

Phlebobranchia

Ascidiidae

***Ascidia ceratodes* (Huntsman, 1912)**

Figure 1F

Puerto Ayora town dock 4-30-2016, two individuals, largest one 3 cm in length (Figure 1F), on caged settlement plate submerged 14 months; Franklin's Bay 4-27-2016, caged settlement plate, 4 individuals, largest one 2 cm in length, with several commensal amphipods in pharyngeal sac (not counted); Baltra Island Navy dock 4-28-2016, several small individuals on cages covering settlement plates. Fairly smooth thick tunic, with longitudinal dorsal furrow almost always present in this species when contracted, as in Figure 1F. No tunic extensions. Flattened on underside. Oral siphon with 8 lobes, atrial siphon with 6 on all specimens regardless of size. Oral siphon anterior, atrial siphon well separated, about halfway back to posterior end. Dorsal tubercle horseshoe shaped, opening anteriorly; neural ganglion several mm posterior to tubercle; oral tentacles filiform, very dense and numerous. No intermediate branchial papillae; branchial sac undulated, not possible to count number of stigmata per mesh but similar to range in number given by Van Name (1945). Intestine isodiametric. Oocytes in oviduct somewhat dark, appearance indicates they were probably red in life, a very rare character but present in this species (Lambert and Lambert 1998). These specimens agree in all characters for *A. ceratodes*, well described by Van Name (1945). Known distribution prior to this study was the coast of California south to Costa Rica and Panama (Van Name 1945; Tokioka 1972; Carman et al. 2011; Bonnet et al. 2013). It is considered cryptogenic here. Chiriboga et al. (2016) reported *A. ceratodes* from Santiago Island, but without a date of collection, and the identification has not been confirmed.

***Ascidia sydneiensis* Stimpson, 1855**

Figure 1K, L

New record for Galápagos. Numerous on settlement plates especially at Franklin's Bay, 4-27-2016, largest and most abundant individuals on caged plates (e.g. plate 16456, 14 months submersion, Figure 1K). Up to 10 cm in length. Convoluted dorsal tubercle; posterior intestine dark, very distended. Anterior intestine narrow. A cosmopolitan species recorded worldwide in warm inshore waters especially on manmade structures in harbors. For descriptions and distribution see Van Name (1945) and Bonnet and Rocha (2011).

***Ascidia* sp.**

Figure 1G–J

Common on Baltra Is. settlement plates and cages 4-28-2016, up to 3.3 cm in length, either whitish or reddish, color retained in seawater formalin. Tunic densely covered with long extensions. Intestine isodiametric. Right side muscles longitudinal only, extend about halfway to posterior end. Dorsal tubercle heart shaped, opening anteriorly; ganglion is directly behind it. Numerous long filiform oral tentacles. No intermediate branchial papillae. In specimen 3 cm in length, oral siphon with 7 lobes, atrial siphon with 6 lobes, minutely fringed. Considered a new record for Galápagos because of its unusual exterior appearance; it does not match the *Ascidia* sp. previously recorded by Iturralde (1991) (Table 1).

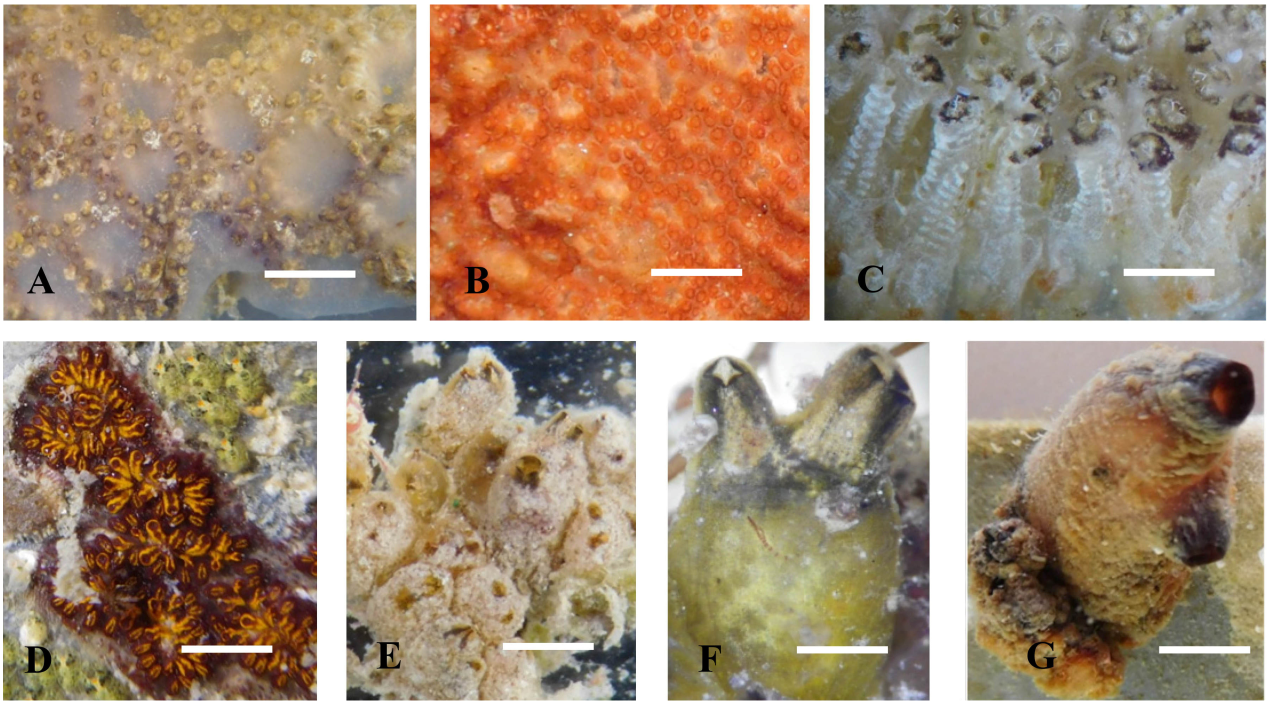

Figure 2. Styelidae. A, B: two color morphs of *Botrylloides giganteus*; C: a row of the very long zooids of *B. giganteus*; D: *Botrylloides niger*, bordered by two small greenish colonies of *Symplegma brakenhielmi*; E: *Polyandrocarpa zorritensis,* group of zooids covered with sediment, Franklin's Bay settlement plate 2016*;* F*:* single *P. zorritensis* zooid, Tortuga Bay 2015; G: *Styela canopus*. Scale bars: A, 5 mm; B, 10 mm; C, 1.6 mm; D, 5 mm; E, 1 cm; F, 4 mm; G, 2.5 mm. Photo 2F by J. Mallinson; others by G. Lambert.

Stolidobranchia
Styelidae

***Botrylloides giganteus* (Pérès, 1949)**

Figure 2A–C

New record for Galápagos. Two color morphs collected during 2015 and 2016 expeditions, purple and red. Sample #158509 from Tortuga Bay mangrove roots 2-21-2015, Baltra Is. Navy dock settlement plates and cages 4-28-16, common. Very long zooids with 18+ rows of stigmata, arranged in rows with areas of zooid-free pale thick tunic between. The species is well described by Pérès (1949) from Senegal and by Brunetti and Mastrototaro (2012, from Italy as a new species *B. pizoni* later synonymized under *B. giganteus:* R.M. Rocha et al., *in preparation*). Although the species has been listed in many publications as *B. giganteum*, the correct spelling must be *giganteus*, following the guidelines of Ryland (2015). Distribution appears to be expanding worldwide and includes southern California (Lambert and Lambert 2003 as *B. perspicuum*), Brazil, New Zealand and Australia (R.M. Rocha et al., *in preparation*).

***Botrylloides niger* Herdman, 1886**

Figure 2D

New record for Galápagos. Collected during 2015 and 2016 expeditions, small but common colonies. Sample #158505 from Tortuga Bay mangrove roots 2-21-2015, Puerto Ayora dock 4-25-2016 general scraping, and settlement plate 30242; also Franklin's Bay 4-27-2016, dock fouling and settlement plates and Baltra Is. Navy dock 4-28-2016, settlement plates and cages. See Van Name (1945) for description. Many publications have listed this species as *B. nigrum* (the original spelling), but Ryland (2015) noted that under the rules of the International Code on Zoological Nomenclature, the generic ending *–oides* is masculine and thus certain species in this genus must have a changed spelling to agree in gender. He chose *niger*, the other spelling that has long been used by various authors for this species. Distribution worldwide in warm waters; considered native to the western Atlantic (Sheets et al. 2016).

Vial 234250 gave 99% CO1 sequence match to Genbank HF548550 *Botryllus nigrum*, and vial 310416 gave 99% CO1 match to Genbank HF548550 *Botryllus nigrum* (J.B. Geller, *personal communication*). The name *Botryllus nigrum* is a junior synonym of *Botrylloides niger* (http://www.marinespecies.org/aphia.php?p=taxdetails&id=252322).

***Polyandrocarpa zorritensis* (Van Name, 1931)**

Figure 2E, F

New record for Galápagos; collected during 2015 and 2016 expeditions. Several colonies on Puerto Ayora town docks and on settlement plates and cages (e.g. plate #16587, April 2016); also Franklin's Bay dock and plates, and Baltra Is. Navy dock and plates both caged and uncaged. At least one colony was collected from Tortuga Bay mangrove roots in February 2015, a site that was not revisited in 2016; see Supplementary material Table S1. For description see Van Name (1945) and Monniot (2016, 2018). Distribution global (Monniot 2018). Vial 232747 field ID *Polyandrocarpa zorritensis* gave 99% CO1 sequence match to Genbank KF309643 *Polyandrocarpa zorritensis* (J.B. Geller, *personal communication*).

***Styela canopus* (Savigny, 1816)**

Figure 2G

New record for Galápagos. Puerto Ayora dock 4-25-2016, settlement plate 234054. Also common on Franklin's Bay dock 4-27-2016, and settlement plates, and Baltra Is. settlement plates. For description see Van Name (1945, as *S. partita* (Stimpson, 1852)), Lee et al. (2013) and Monniot (2018). Worldwide distribution in warm waters (Lee et al. 2013).

***Symplegma brakenhielmi* (Michaelsen, 1904)**

New record for Galápagos. Collected during 2015 and 2016 expeditions; Tortuga Bay mangrove roots 2-21-2015, and on Puerto Ayora dock and caged settlement plate 30242, 4-25-2016, several small colonies. See Monniot C (1983) and Monniot F (2018) for descriptions. Worldwide distribution in warm waters (Carman et al. 2011).

Symplegma rubra **Monniot, 1972**

New record for Galápagos. Puerto Ayora dock fouling 4-25-2016, also caged settlement plate 30242. Colonies small, zooids immature. Tunic colorless and transparent, zooids bright red. The gonads develop separately in this species. In the largest zooid the male gonads are just beginning to develop but gonads are not visible in most zooids. See descriptions in Monniot C (1972) and Monniot F (2018). Distribution worldwide in warm waters (Carman et al. 2011): Caribbean, Indo-Pacific, Gulf of Mexico, Brazil, Panama (both Caribbean and Pacific sides). No species of *Symplegma* have been reported previously for the Galápagos (Table 1).

Pyuridae

Halocynthia dumosa **(Stimpson, 1855)**

Figure 3A, B

Two on underside of Baltra Navy dock 4-28-2016, one on Bartolome Island at 12.2 m depth on rocky substrate, 4-29-2016, all collected by G. Ruiz. One Baltra specimen dissected, 6 cm in width; 12 pharyngeal folds/side, posterior ones incomplete and small. Large dorsal tubercle inrolled on both sides about 3 times. No siphonal spines internally. The three specimens agree in all morphological characters with Kott (1985), Millar (1988) and the shallow water individuals in Monniot and Monniot (1989).

All previous records for this species worldwide indicate that it is a shallow water species. However, besides the shallow water Galápagos records listed in Table 1, Monniot and Monniot (1989) described a deep-water form differing in many morphological characters from the shallow form; it is possible that it might be a different species. There are no samples from that collection suitable for DNA sequencing. Wetzer (1990) described isopods from one of the Monniots' deep-water specimens from near Fernandina Island (316 m). *H. dumosa* has a very wide Pacific Ocean distribution, including Solomon Is., Australia, Sri Lanka, and New Caledonia (Kott 1985; Millar 1988; Monniot C 1989). Iturralde (1991) recorded it from Tagus Cove; Chiriboga et al. (2016) record it with no location. It may be introduced or native in the Galápagos but is considered cryptogenic in this study because of its wide disjunct distribution. It is usually incorrectly listed as *H. hispida* (Herdman, 1881) following Kott (1985), though she corrected this in a later publication (Kott 1998).

Microcosmus exasperatus **Heller, 1878**

Figure 3C

New record for Galápagos. One on Puerto Ayora dock, 4-25-2016. This is another fouling species in all tropical and subtropical waters worldwide. See Van Name (1945) for description and Nagar and Shenkar (2016, Table S2) for extensive distribution records.

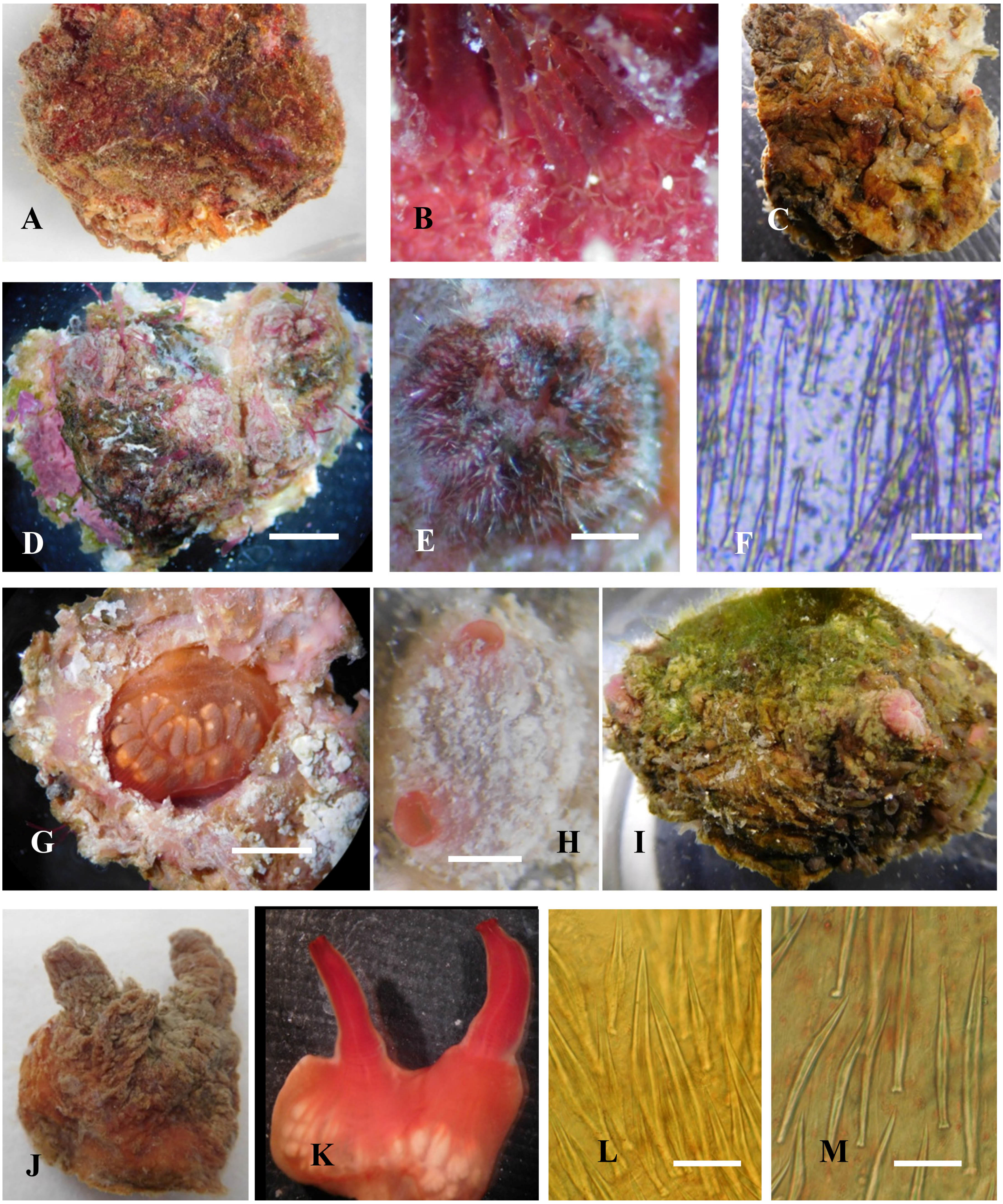

Figure 3. Pyuridae. A: *Halocynthia dumosa* 6 cm wide; B: same specimen oral siphon; C: *Microcosmus exasperatus* 3 cm wide; D–M: *Pyura haustor*: D–H from Puerto Ayora dock settlement plate: D: whole animal 3 cm across siphons; E: oral siphon; F: siphonal spines; G: posterior flattened area of tunic of 2.5 cm animal opened to expose right gonad; H: immature specimen; I–J: specimens from Franklin's Bay dock fouling: I: short-siphoned specimen 3.2 cm in width; J–L: long-siphoned specimen 2.5 cm in width; K: same animal removed from tunic; L: siphonal spines; M; siphonal spines of *P. haustor* from Friday Harbor WA. Scale bars: D, 7 mm; E, 3.7 mm; F, 50 µm; G, 6 mm; H, 2 mm; L, 50 µm; M, 50 µm. Photos by G. Lambert.

***Pyura haustor* (Stimpson, 1864)**

Figure 3D–M

Puerto Ayora dock at end of short rope and on settlement plates, 4-25-2016; Franklin's Bay dock fouling, and small immature specimens on a few of the settlement plates 4-27-2016; settlement plates and cages from Baltra Navy dock 4-28-2016. In small specimens the body is flattened, tunic pink and thinner on posterior attachment surface; siphons short, pink, widely separated, while the siphons are much longer in two of the larger specimens. The 2 largest specimens are oval, 3.0 and 3.2 cm wide measured across the widely separated siphons, tunic thick, dark, lumpy, and leathery. The siphons are covered with dense sharply pointed siphonal spines up to 150–200 µm in length. Animal removed from tunic with bright red body wall anteriorly, fading to pale pink posteriorly. Body wall muscles do not extend all the way to the flattened, in some cases disk-like, posterior end that is the region of attachment. Dorsal tubercle only slightly inrolled; about 18 oral tentacles of various sizes, all with primary branching and largest tentacles with tiny secondary branching. Six pharyngeal folds/side; longitudinal vessel formula of largest specimen: RE 0(10)2(13)4(16)4(18)4(18)4(13)3 DL 3(14)3(16) 3(18)4(17)4(14)4(10)3LE

The first fold on the right side is small, short, and incomplete, present only posteriorly.

The siphonal spines are identical in size and shape to those examined from *P. haustor* specimens from Washington state (Figure 3M; *unpublished observations*), as are all other morphological characters (Van Name 1945). Van Name (1945) stated that while the siphons are usually quite long in this species, they are "very little produced" in others. Both types were collected in the present survey. He also mentioned that the siphonal spines were 250–500 µm in length but this was an estimate utilizing only a hand lens; none of the many specimens I have seen from the U.S. west coast have spines this length as measured utilizing a dissecting microscope. Prior known distribution from Alaska to southern California (Van Name 1945), although it was reported previously from the Galápagos Islands by Witman and Smith (2003) based on 1999–2000 collections. It is considered cryptogenic in the present study.

Molgulidae

***Molgula* sp./spp.**

Figure 4A–C

Puerto Ayora dock 4-25-16, three tiny (1–2 mm) specimens, one with brooded embryos, on plate 234071. New record for Galápagos; no molgulids have previously been reported (Table 1). The tunic is covered with tunic hairs, some almost as long as the body; tiny sand grains densely embedded

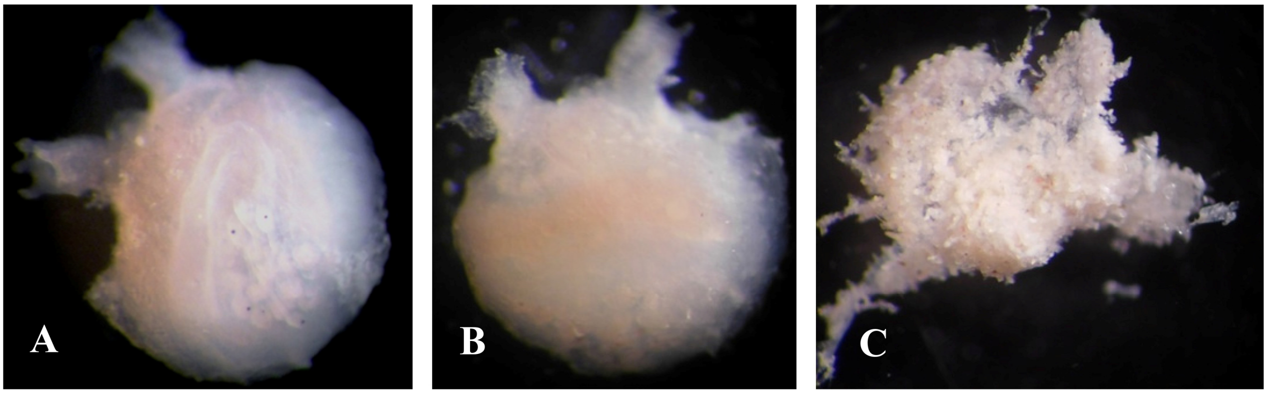

Figure 4. *Molgula* sp., 2 mm in diameter. A, left side of body out of tunic showing gut loop and brooded embryos, oral siphon on the left; B, right side body out of tunic with pinkish horizontal gonad; C, tunic (opened with body removed). Photos by G. Lambert.

in the tunic hairs. The oral siphon has 6 elongated lobes; there are 4 lobes on the atrial siphon. The largest specimen is 2 mm, with the body removed from the tunic only 1 mm in diameter. It was not possible to fully dissect this specimen to record all the morphological characters without completely destroying it, but it has the typical single ovotestis on each side with the testes arranged along the sides of the centrally located ovary. The right gonad (Figure 4B), visible through the body wall, is pale pink, elongated, oriented horizontally, and extends across almost the entire width of the body. There is a very transparent kidney under the right gonad (not visible in the photo). The primary gut loop, on the left side, is large, oriented diagonally and extends almost to the atrial siphon. The secondary loop is deep and narrow, the intestinal apex almost touching the rectum. The left gonad is not visible. There are about 14 brooded embryos on the left side situated over the secondary loop of the intestine; some of them are hatched tadpoles with a single pigment spot.

Discussion

Of the 18 ascidian species collected from floating docks and settlement plates in April 2016, 11 are considered introduced and 3 cryptogenic (Table 1). Twelve of the species are new records. Ascidians were the dominant foulers on most plates (Figure 1B, C, K), especially the caged ones, with a dramatic difference in coverage between caged and uncaged which is presumed to result from differential fish predation pressure. The panels were all suspended with none touching the benthos, and schools of small fish were observed around all the docks constantly nipping at the dock surfaces. A few of the caged plates contained flatworms, small echinoids, and even a small fish in one that had grown too large to escape. Other fouling studies comparing caged and uncaged surfaces have found that ascidian coverage is significantly affected by fish and other predation on uncaged panels (Vieira et al. 2012; Lavender et al. 2014; Kremer and Rocha 2016; Oricchio et al. 2016; Roth et al. 2017), indicating that similar studies should include both treatments of settlement panels.

Many of the shallow-water ascidians recorded prior to this survey were not encountered during this study, which was limited to artificial surfaces (floating docks, pilings and settlement plates). Former studies (Table 1) included surveys of natural benthic substrates, but problems of identification exist for some of the records. *Aplidium californicum*, recorded by Millar (1988) has some differences from the California specimens described by Ritter and Forsyth (1917) and Van Name (1945). Millar (1988) also included ?*Polyclinum planum* (his question mark), indicating a possibility that it may not be that species.

Millar also listed ?*Didemnum carnulentum* (his question mark), a species of the U.S. Pacific coast that was poorly described by Ritter and Forsyth (1917); the type specimen is lost and *Didemnum* specimens collected since then cannot be assigned to that species with any confidence. Tokioka (1967) used the name *Didemnum candidum* Savigny, 1816 to describe a specimen from Isabela (Albemarle) Island in the Galápagos archipelago, however the name *D. candidum* has been incorrectly assigned to many *Didemnum* spp. worldwide and is no longer in use by knowledgeable ascidian taxonomists (F. Monniot, *personal communication*) because the type specimen is lost. Therefore, the Isabela specimens described by Tokioka (1967) need reexamination.

An extended study of the ascidians of the central and southern Ecuador coast and a few offshore islands (but not the Galápagos) is in preparation (F.D. Brown, *personal communication*), but identifications are still tentative and incomplete. Natural benthic areas were surveyed at intertidal to 20 m depths, along with oyster aquaculture tanks, two harbors and a subtidal shipwreck. Representatives of all three orders (Aplousobranchia, Phlebobranchia and Stolidobranchia) are present.

The subtidal fauna of the Galápagos is as yet very incompletely known. More surveys of both biofouling communities and natural benthic areas are needed for this important isolated World Heritage site.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Occurrences of ascidians at sampling locations in the Galápagos Islands in 2015–2016.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2019/Supplements/AI_2019_Lambert_etal_Table_S1.xlsx