Invasion and current distribution of the octocoral Carijoa riisei (Duchassaing & Michelotti, 1860) in the Ecuadorian coast (eastern tropical pacific)

INVASIVESNET

Research Article

Invasion and current distribution of the octocoral *Carijoa riisei* (Duchassaing & Michelotti, 1860) in the Ecuadorian coast (Eastern Tropical Pacific)

Maritza Cárdenas-Calle[1,2,*], Julián Pérez-Correa[1,3], Cecilia Uzca-Sornoza[2], Gregorio Bigatti[3,4], Nardy Diez[5], Mariana Lozada[6], Jorge Coronel[7], Ileana Herrera[3,8], Gladys Torres[9], Telmo De la Cuadra[10], Freddy Espinoza[11], James Mair[1,12] and Inti Keith[13]

[1]Bioelite. Cdla. Bosques el Salado Mz 301 solar 2B frente a Ciudad Colón, Guayaquil, Ecuador
[2]Universidad de Guayaquil, Facultad de Ingeniería Química. Ciudadela Universitaria "Salvador Allende", Av. Delta y Av. Kennedy, Guayaquil, Ecuador
[3]Universidad Espíritu Santo, Km. 2.5 vía La Puntilla, Samborondón, Ecuador
[4]LARBIM (IBIOMAR-CONICET), Bv. Almirante Brown 2915, Puerto Madryn, Chubut, Argentina
[5]Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigación Biotecnológicas del Ecuador-CIBE, Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador
[6]Laboratorio de Microbiología Ambiental (IBIOMAR-CONICET), Bv. Almirante Brown 2915, Puerto Madryn, Chubut, Argentina
[7]Universidad Agraria del Ecuador, Av. 25 de Julio y Pío Jaramillo, Guayaquil, Ecuador
[8]Instituto Nacional de Biodiversidad (INABIO), 170501, Quito, Ecuador
[9]Instituto Oceanográfico y Antártico (INOCAR), Av. 25 de Julio Base Naval Sur, Guayaquil, Ecuador
[10]Instituto Público de Investigación de Acuicultura y Pesca, Letamendi 102 y la Ría. Guayaquil, Ecuador
[11]Dirección Nacional de los Espacios Acuáticos (DIRNEA), Gral. Elizalde 101 y Malecón Simón Bolívar, Guayaquil, Ecuador
[12]Heriot-Watt University, Edinburgh, Scotland, UK EH14 4AS
[13]Charles Darwin Research Station, Charles Darwin Foundation, Santa Cruz, Galápagos, Ecuador

Author e-mails: maritza.cardenasc@ug.edu.ec (MCC), juperez@uees.edu.ec (JPC), cecilia.uzca.s@gmail.com (CU), gbigatti@cenpat-conicet.gob.ar (GB), nardydiez@gmail.com (ND), lozada@cenpat-conicet.gob.ar (ML), jcoronel@uagraria.edu.ec (JC), herrera.ita@gmail.com (IH), gladys.torres@inocar.mil.ec (GT), tdelacuadra@institutopesca.gob.ec (TDC), fespinozah@armada.mil.ec (FE), j.m.mair@hw.ac.uk (JM), inti.keith@fcdarwin.org.ec (IK)

*Corresponding author

Co-Editors' Note: This study was contributed in relation to the 21st International Conference on Aquatic Invasive Species held in Montreal, Canada, October 27–31, 2019 (http://www.icais. org/html/previous21.html). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Copyright: © Cárdenas-Calle et al.

Abstract

Carijoa riisei is a snowflake coral that has aggressively spread across many coastal habitats in the Atlantic and Pacific oceans, threatening a number of tropical ecosystems worldwide, including coral reefs. The aim of this work was to evaluate the distribution and provide an estimation of abundance of the invasive octocoral *C. riisei* along the Ecuadorian coast, as well as the relationship between its abundance and different environmental variables. In a field survey, high abundances of *C. riisei* colonies were reported growing over corals and sessile communities at 5 of 43 sampling sites. The areas with highest relative abundance were found in the Manabí province, at two sites in Jama: Bajo Londres (44.57% coverage) and Vaca Brava 1 (20.25%). Results of ordination and grouping statistical analyses showed no significant differences between invaded and not invaded sites as regards community composition or environmental characteristics, suggesting neither biotic nor abiotic factors could be limiting *C. riisei* dispersal along the Ecuadorian coast. Results from a bibliographic survey covering occurrence data up to 2020 were in accordance, showing that in that period *C. riisei* became an established species to the Ecuadorian coast, being present in at least 22 of the 43 sites, including various sites in Marine Protected Areas. Based on these findings, recommendations are made to promote urgent monitoring programs to detect *C. riisei* in new areas along the coast of Ecuador and in the Galapagos Marine Reserve, in order to develop a mitigation program and to take actions to conserve the ecosystems affected by this invasion.

Key words: invasive species, Eastern Pacific, Ecuador, snowflake coral

Introduction

Carijoa riisei (Duchassaing & Michelotti, 1860), commonly known as snowflake coral, is an octocoral (Octocorallia: Cnidaria) native to the Indo-Pacific, currently present in the Atlantic, Pacific and Caribbean Seas (Sánchez and Ballesteros 2014). This species was reported for the first time, as an invasive species in 1972, in Hawaii (Kahng et al. 2008). It has been reported in areas with rich organic matter, where it inhabits rocky and artificial substrates (Sánchez 1994; Venkataraman et al. 2016). It forms dense, monospecific aggregations, carpeting hard substrata. This octocoral is an aggressive competitor that monopolizes food and space, feeding mainly on phytoplankton, diatoms and zooplankton larvae (Lira et al. 2009). It has few natural predators, therefore no species has been identified as potential for biocontrol (Wagner et al. 2009). This species is usually found to compete successfully with black corals and invertebrates, impacting coral reefs (Kahng and Grigg 2005; Barbosa et al. 2014; Sánchez and Ballesteros 2014). This process can lead to major shifts in species interactions and changes in both nutrient cycles and energy flow, which can result in unpredictable cascading effects (Carlton 2001).

Carijoa riisei was reported for the first time in Ecuador in 2010, in the Machalilla National Park. One year later it was observed in the northern province of Esmeraldas, in the Galera San Francisco Marine Reserve (Instituto Nazca 2013; Keith and Martínez 2017). In 2012, it was detected on the central coast in El Islote Los Ahorcados, Manabí (Instituto Nazca 2013) and south central coast in El Pelado Marine Reserve (Cárdenas-Calle et al. 2018a; Instituto Nazca 2013). In 2013, it was observed at a shallow water site known as El Cuarenta, known for its high diversity of benthic and fish communities (Cárdenas-Calle et al. 2018a). In a short time, this species was reported at several coastal sandy sites as well as in an open water area that is characterized by rocky reefs (Cárdenas-Calle et al. 2018b).

Ecuador is the smallest among the so-called "megadiverse" countries, harboring a wide range of ecosystems and many endemic species distributed in small geographic ranges (Cuesta et al. 2017; Mestanza-Ramón et al. 2019). The Ecuadorian coast is approximately 2,900 km in length, and has been considered a hotspot of species richness due to its location in the great Panamic-Pacific zoogeographic province (Olsson 1961). For example, in a recent survey, a total of 479 macroinvertebrate species and 133 seaweeds were recorded, only in the subtidal (Cárdenas-Calle et al. 2020). Therefore, it is imperative not only to document the extent of the distribution of *C. riisei* along the Ecuadorian coast, but also to analyze the environmental factors that could drive its distribution, in order to have a better understanding of the implications of this invasion and its possible limits. In previous works, *C. riisei* was morphologically identified in the field and in the laboratory, however, molecular identification of the species through the analysis of

mitochondrial markers has been suggested as a way to strengthen its identification as well as to have some insight into its possible geographical origin (Concepción et al. 2010).

Therefore, the aims of this study were: 1) to confirm the identity of the species through molecular analysis; 2) to evaluate the present distribution and provide an estimation of abundance of *C. riisei* along the Ecuadorian coast; 3) to generate an updated list of sites colonized by *C. riisei* 4) to evaluate the relationship between different environmental variables and the presence of *C. riisei*.

Materials and methods

Study area

The Ecuadorian coast is influenced by the Ecuadorian Coastal Current (ECC) (Allauca 1990). The coastal continental zone, excluding the Galapagos archipelago, is home to two sub-regions. The northern coast corresponds to the Panama Bight marine ecoregion which extends from Azuero Peninsula in Panama to Bahía de Caráquez in Ecuador, while the southern coast lies within the Guayaquil marine ecoregion that starts in Bahía de Caráquez and ends in the Peninsula de Illescas in northern Peru (Miloslavich et al. 2011; Sullivan-Sealey and Bustamante 1999).

A total of 43 sites were sampled from April 2015 to October 2015, along the four coastal provinces of Ecuador (Esmeraldas, Manabí, Santa Elena and El Oro). The study area covered 1860 km^2 and the selected sites were chosen considering the representativeness of ecosystems and degree of human intervention. The Marine Protected Areas (MPAs) were: Galeras San Francisco Marine Reserve (RMGSF), Pacoche Wildlife Refuge (RVSMCP), Machalilla National Park (PNM), Bajo Cope Marine Reserve (RMBC), El Pelado Marine Reserve (REMAPE), Puntilla de Santa Elena Fauna Production Reserve (REMACOPSE) and the Santa Clara Island Wildlife Refuge (RVSISC). In addition, three non-protected areas (Jama, Canoa and Ayampe) were sampled for this study. Further details are provided in Supplementary material Table S1.

Taxonomic identification by molecular analysis

Three samples of the octocoral morphologically identified as *C. riisei* were collected by divers in "La Pared" at El Pelado Marine Reserve, the colonies were collected and identified morphologically (Collin et al. 2005). Samples from *C. riisei* were then immersed in liquid nitrogen for rapid freezing, then crushed with the mill "Retsch MM400" to obtain a fine powder and stored in a freezer at −80 °C prior to molecular analysis (Weber et al. 2017).

DNA isolation was performed in the Molecular Biology Laboratory at CIBE-ESPOL with the CTAB-based protocol (Doyle and Doyle 1990). Two pairs of primers were used to amplify fragments of the cytochrome oxidase I

(COI) gene, and one pair for NADPH dehydrogenase subunit 6 (ND6) gene, according to Concepción et al. (2008). The primers used are shown in Table S2. Endpoint PCR reactions were carried out in a Mastercycler Gradient S No. 5345 (Eppendorf), in 50 µl reactions containing 25 µl of 2x-GoTaq® Green Master Mix (Promega), 0.5 µM of each primer, 60 ng (3 µl of a 20 ng/µl solution) DNA template, and the corresponding volume of sterile water for reaching 50 µl. For PCR runs, the following conditions were followed: 3 min at 94 °C (initial denaturation); 36 cycles of [30 s at 94 °C, 30 s at 58 °C (COIF/COIR; COI2F/COI2R) or 30 s at 50 °C (ND6/ND3), 1 min at 72 °C]; and 5 min at 72 °C (final elongation). Five microliters of PCR products were loaded onto agarose gels and electrophoresed to confirm band presence and expected molecular weight, and the remaining 45 µl were purified with the Wizard SV Gel and PCR Clean-Up System kit (Promega). PCR products were stored at −20 °C until sequencing. Sequencing was performed at Macrogen Inc. (Korea) with forward and reverse primers. COI phylogenetic trees were constructed with MEGAX (Kumar et al. 2018), using Maximum Likelihood for tree construction and GTR+I+G as evolutionary model (McFadden and van Ofwegen 2012). For ND6 fragments, a haplotype analysis was carried out based on the work of Concepción et al. (2010). A total of 525 nucleotide positions were analyzed, corresponding to the amplified fragment from this work. A pairwise distance matrix was constructed using MEGAX, which was used to distinguish 100% identical variants or *haplotypes* and match them to the previous work. As the sequence fragments from this work were shorter, some of the haplotypes from Concepción et al. (2010) were not able to be distinguished, and they were grouped for this analysis (24 haplotypes out of 30 identified in Concepción et al. (2010), with an average of 1.6 nucleotide identity among them).

Present distribution

Data were collected in the subtidal zone using SCUBA at a depth of 5–15 m. Each sample was taken by two divers moving along a 50 m transect parallel to the coast conducting visual censuses, one on each side of the transect (Edgar et al. 2011). An area of 2.5 m² was monitored using a PVC quadrat of 0.5 × 0.5 m (0.25 m²). Each quadrat had a grid of 5 × 5 cm, constructed with polypropylene twine with 81 intersection points. Quadrats were placed systematically every 5 m along the same transect. The abundance of mobile species and coverage of sessile species were estimated per quadrat, averaged per site, and transformed to presence/absence data at each site for further analyses (see below). The list of species used is published elsewhere (Cárdenas-Calle et al. 2020). To explain the patterns of occurrence of *C. riisei* over sites in relation to community composition, a Non-Metric Multidimensional Scaling (n-MDS) of all sampled sites was carried out based on presence/absence data of all other species (excluding *C. riisei*). Maps were made to show the sites sampled during 2015 and the types of sediments in

accordance with the subtidal system proposed by Instituto Nazca (2005). Coverage of *C. riisei* was superimposed as bubbles with diameters proportional to percent coverage, using the statistical package *vegan* and other tools in R environment (R Core Team 2020). Additionally, the databases Scopus, Web of Sciences, ScienceDirect and Google Scholar as well as technical reports were reviewed to generate an updated list of sites colonized by *C. riisei* along the coast of Ecuador from 2010 until 2020. The search was done in google scholar, web of science and scopus. The terms of search were "*Carijoa riisei*" "Ecuador" "continental". The search was done as for March 2020. In this case, presence or absence was directly used as an indicator variable of *C. riisei* distribution at each site.

Relationship between environmental variables and the presence of C. riisei *and estimation of colonizable sites*

Seventeen environmental variables were examined to determine the characteristics of the areas inhabited by *C. riisei* using remote sensing from MODIS-AQUA (available in oceancolor.gsfc.nasa.gov and neo.sci.gsfc.nasa.gov) (Table S3). Geomorphic depth, slope and rugosity were derived from *in situ* measurement and downloaded from GEBCO (https://www.gebco.net/) and analyzed using QGIS version 3.8. As variables differ temporarily, monthly values from 2003 to 2016 were subtracted. Principal Component Analysis (PCA) was used in order to visualize the relation of the presence/absence of *C. riisei* and the environmental variables at each site. In the analysis, both the data reported during field work and the data extracted from other studies since 2010 to 2020 were included. PCA routines were assessed using R and the basic package *stats* (R Core Team 2020). For visualization of the analysis the package *factoextra* was used (Kassambara and Mundt 2020). All variables were normalized as all of them obtained a $p < 0.05$ according to the Shapiro-Wilk test.

Finally, for the estimation of colonizable sites, a grouping analysis contrasting the environmental conditions resulting from the PCA was carried out, using as grouping factor Presence or Absence of *C. riisei*. The rationale of this analysis is that if the circles do not have intersected areas, the groups differ from each other, and there are significant differences between conditions where *C. riisei* is present or absent. This can lead to the conclusion that the octocoral is not likely to expand further from its present distribution. To confirm the visual results, an analysis of similarities (ANOSIM) was run to test if differences were significant.

Results

Taxonomic identification by molecular analysis

The molecular analysis of three samples of the octocoral collected in "La Pared" (Site 30) confirmed the morphological identification. A clear grouping
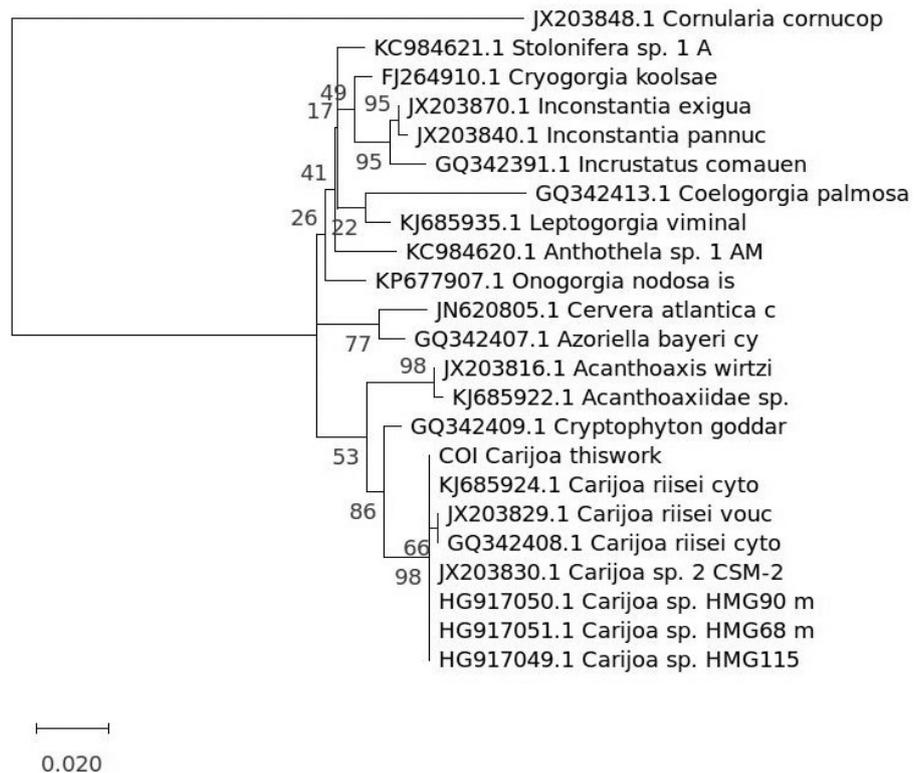
Figure 1. Molecular Phylogenetic analysis of cytochrome oxidase I sequences from this work and various related corals, by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood (−1292.13) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5553)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 51.06% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 450 positions in the final dataset. The outgroup used was the cytochrome oxidase I sequence from *Cornularia cornucopiae*. Evolutionary analyses were conducted in MEGA X.

was observed between the sequences from this work and other *Carijoa* sequences (Figure 1). No resolution was observed inside the cluster, in accordance with results reported for longer COI fragments as well as for other genes (mtMutS and 28S rDNA) (McFadden and Ofwegen 2012).

As regards ND6 gene, haplotype analysis showed that the sequence from this work was identical in the analyzed region to haplotypes 17, 18, 19 and 26 from those of Concepción et al. (2010) (Table S3). Of these, haplotype 18 is the most commonly found, being dominant in the Atlantic zone (Concepción et al. 2010).

Present distribution

Colonies of *C. riisei* were determined morphologically and registered along the Ecuadorian coast during the 2015 surveys. Dense colonies with orange
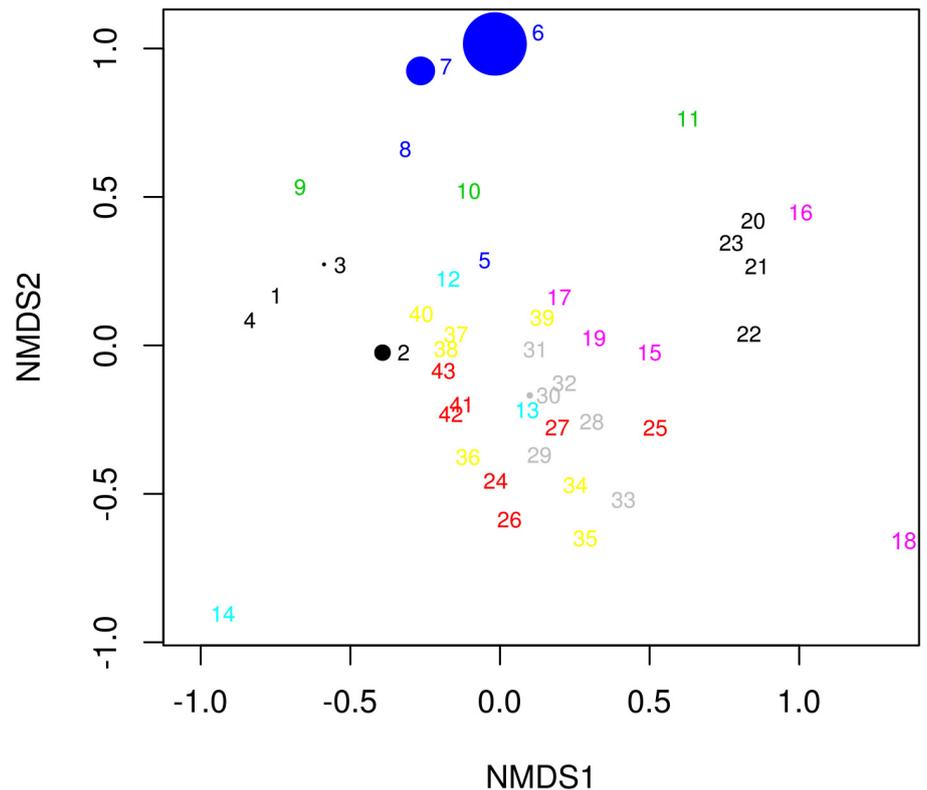
Figure 2. Ordination (non-metric multidimensional scaling, NMDS) based on presence-absence data of all sessile and mobile species recorded (excluding C. *riisei*) at the sites studied. Colors correspond to the different areas (for complete information see Table S1). Bubbles correspond to *C. riisei* coverage values, diameters are proportional to percent coverage.

coloration and white polyps were found on rocky outcrops, cave exteriors, under ledges, on flat rocky areas and on walls. The colonies were observed between 5 and 15 m (average depth 9.32 ± 3.09 m, mean ± SD) whilst the bottom temperature ranged from 22 °C to 27 °C (26.23 ± 1.27 °C) (Table S1). Dense aggregations were observed on the rocky reefboth in exposed and sheltered areas, where they began to grow as mats on the rocky edges and progressively extended on to rocks and sessile organisms. Overgrowth of *Carijoa riisei* was observed on corals and sessile macroinvertebrates communities such as: *Pocillopora* spp, *Pinctada mazatlanica*, *Hyotissa solida*, *Muricea plantaginea*, *Megabalanus* (*Balanus*) *peninsularis* and *Aplysina* sp. However, *Tubastraea coccinea* did not seem to be affected by *C. riisei* (Figure S1).

The highest coverage of *C. riisei* was in in the Province of Manabi, in the area of Jama. The site Bajo Londres had the highest, with 44.6% coverage, followed by Vaca Brava 1, with 20.2%. In contrast, low coverage of *C. riisei* was found at sites in two MPAs: La Pared, in El Pelado Marine Reserve (REMAPE) and Punta Alta, in Galeras San Francisco Marine Reserve (RMGSF) (Figure 2, Table S1).

Accordingly, the literature review conducted for *C. riisei* in Ecuador from 2010 until 2020 showed the successful establishment and expansion of this species along the coast of Ecuador. The results showed that this invasive
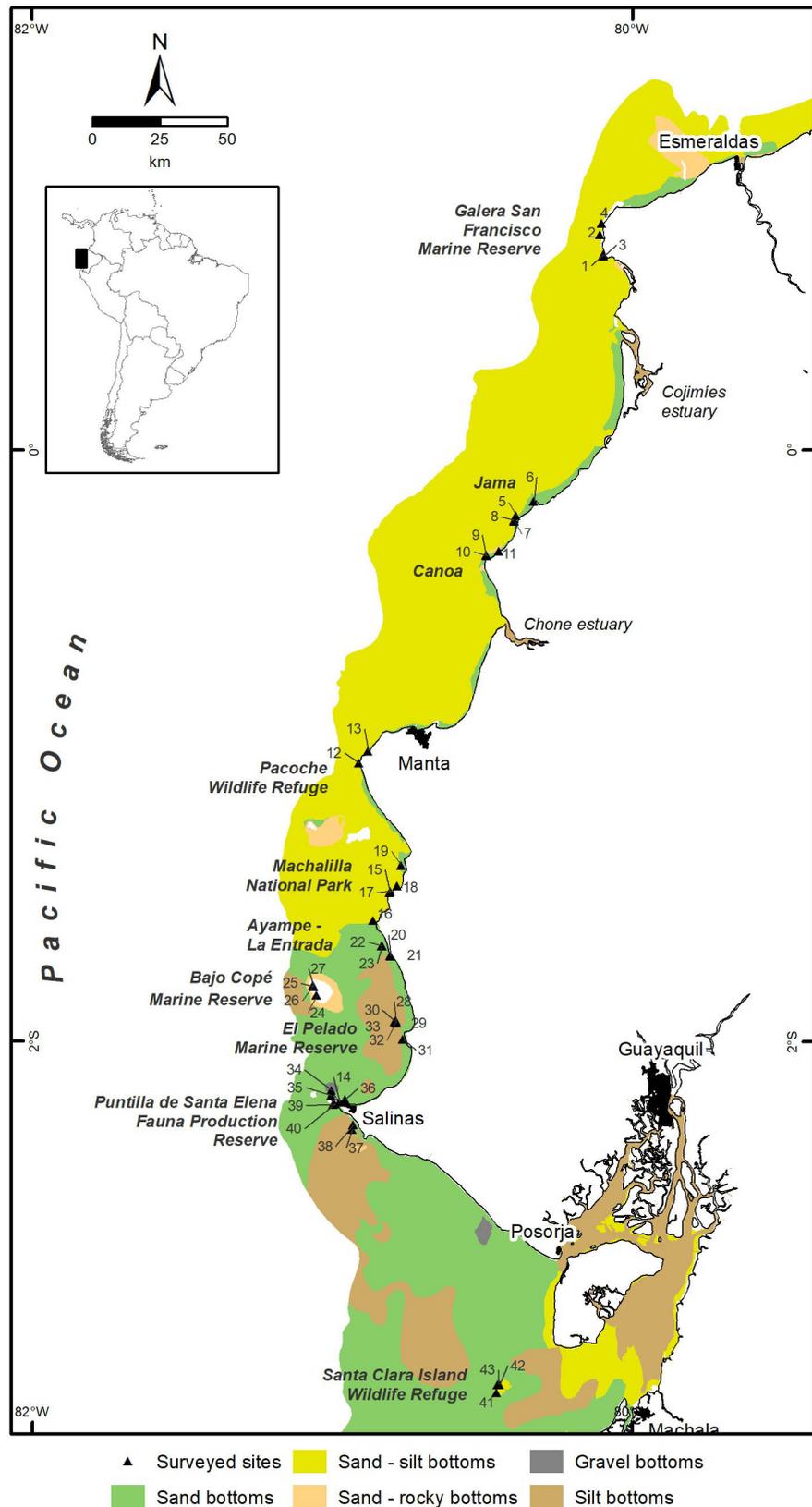
Figure 3. Map showing the location of the 43 sampling sites corresponding to this study. Numbers correspond to sampling sites, complete names are given in Table S1.

species is now present at 22 sites covering four of the five coastal provinces (Figures 3 and 4).
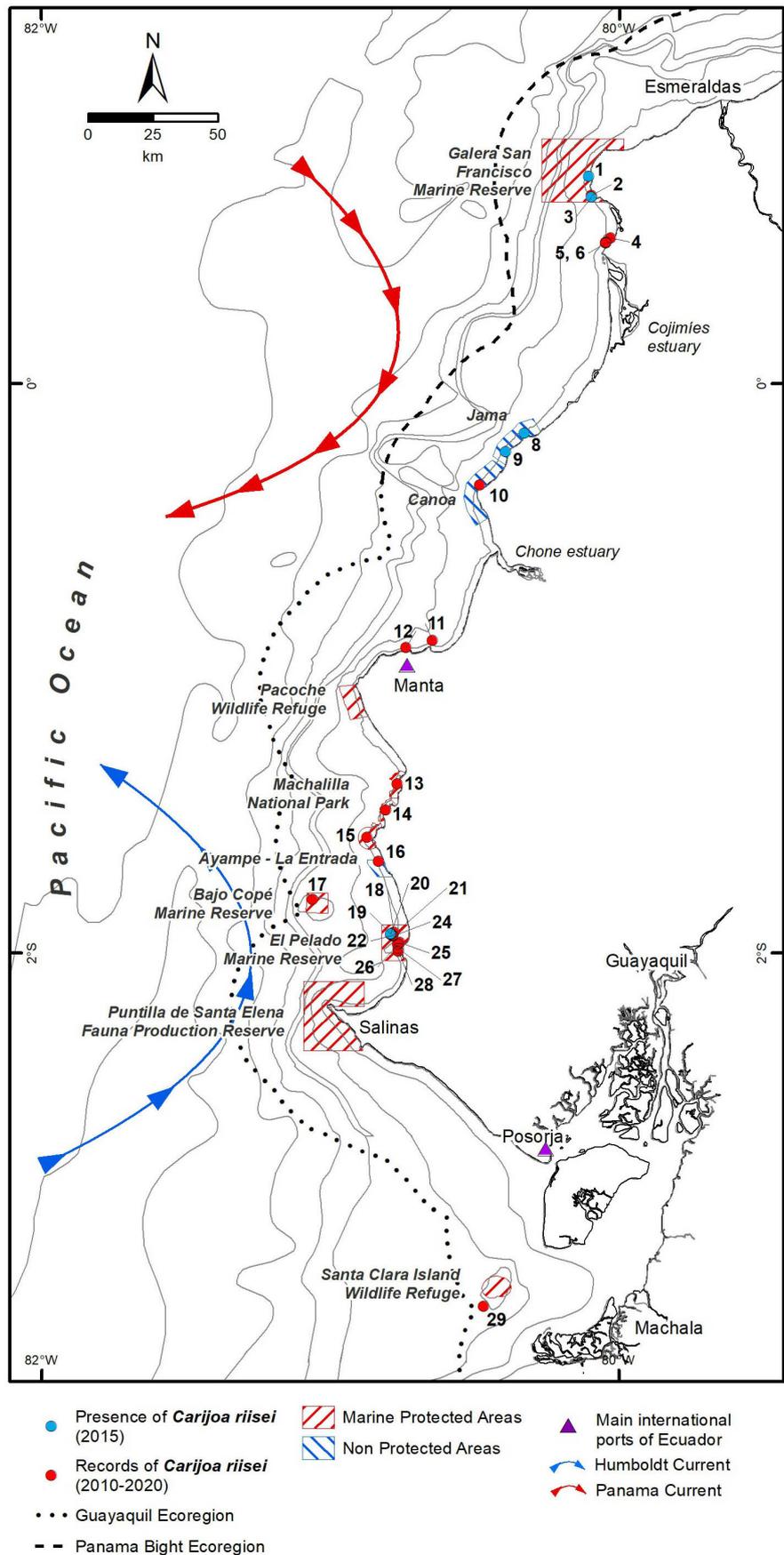
Figure 4. Records of *Carijoa riisei* within the Panama Bight (striped lines) and Guayaquil (dotted lines) Bioregions of Ecuador, since 2010 to 2020. Numbers correspond to sampling sites, complete names are given in Table S1.

Relationship between different environmental variables and the presence of C. riisei, *and estimation of colonizable sites*

PCA ordination allowed the visualization of sites according to their similarity in environmental data (Figure 5). PC1 and PC2 explained ~ 64% of the variation. Sites mostly influenced by the PC1 are positively correlated with standard error of the sea surface temperature (SE_SST), solar radiation and means photosynthetically available radiation (PAR). Contrastingly, the PC1 was negatively correlated with means of sea surface temperature (SST), precipitation (Rain) and standard error of euphotic zone (SE_EUZ). On the other hand, PC2 was mostly explained by positive correlation with the increase of backscattering (Back), organic and inorganic particulate carbon (POC and PIC). A complete list of the environmental variables is shown in Table S4.

A grouping analysis allowed to unravel areas where *C. riisei* had not been reported and compare to those areas where *C. riisei* is currently behaving invasively. The areas where *C. riisei* is present or absent were mixed and superimposed (Figure 5). This implies that the environmental conditions between sites invaded and not invaded do not differ from each other. The ANOSIM revealed that there were no significant differences between groups (R = 0.031, p = 0.279).

Discussion

The results from this study showed the presence of *Carijoa riisei* and its rapid expansion in four coastal provinces along the Ecuadorian coast. High densities were found in turbid waters with high levels of sedimentation and organic matter similar to that recorded in the Mexican Pacific (Galván-Villa and Ríos-Jara 2018). The results of the molecular analysis confirmed the identity of the species and showed genetic similarity with haplotypes found in the Atlantic Ocean (Concepción et al. 2010). However, further studies should be carried out to confirm these results, including more individuals, longer sequences and include other more variable genes such as the nuclear SRP54 (Concepción et al. 2010).

Our study showed that, by 2020, a total of 22 sites have been invaded by this species. Therefore, *C. riisei* represents a great threat for the coral reefs communities mainly in the El Pelado Marine Reserve and Machalilla National Park, where it was seen to rapidly spread, suggesting its high colonization capacity. This aggressive invasive behavior was also found in other places in the Eastern Tropical Pacific (Gutiérrez 2012; Sánchez and Ballesteros 2014; Galván-Villa and Ríos-Jara 2018). *Carijoa riisei* has been recorded from the north coast to the south of Ecuador, suggesting that there are more colonized sites but that they have not yet been reported. For this reason, it is necessary to implement a monitoring program at the harbors and MPAs to understand the growth of colonies, estimate the growth rate, its

A

PCA − Biplot

B Individuals − PCA
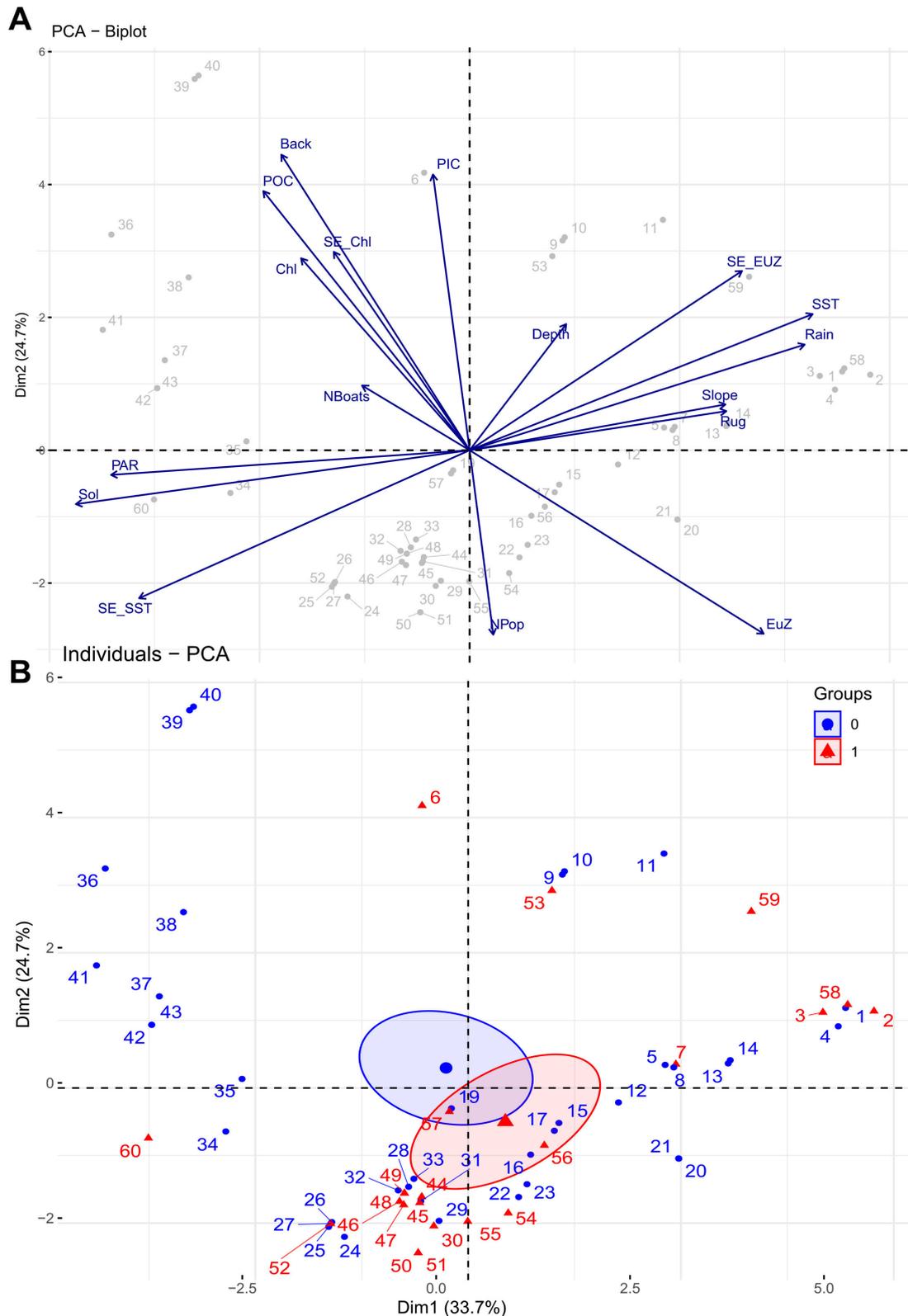
Figure 5. A) Ordination of sites according to their environmental variables, using Principal Component Analysis. The graph shows the first two dimensions. PC1 (37.9% contribution) and PC2 (26.2% contribution) and the relative contribution of environmental variables is shown as arrows. The length of each arrow in the plot represents the relative importance of the variation explained. SST: Sea-surface temperature, Chl: Chlorophyll-*a* concentration; PAR: Photynthetically Active Radiation; EuZ: Euphotic Zone Depth; PIC: Particulate Inorganic Carbon; POC: Particulate Organic Carbon; Back: Backscattering; Rain: Rainfall; SoI: Solar Insolation; SE_SST, SE_CHL, SE_EuZ: Standard Error of the SST, Chl and EuZ respectively; Depth: Depth; Rug: Rugosity; Slo: Slope; The description of environmental variables used for the analysis is shown in Table S4. B) Grouping analysis, discriminating sites where *C. riisei* is present (red triangles) from sites where it is absent (blue circles). Bibliographic survey data from 2010 to 2020 were used for the construction of the matrix.

ecological effect on sessile organisms, to know the type of secondary metabolites that it produces to compete successfully for space and to determine strategies of mitigation that avoid the loss of coral areas and diversity of the microbenthic communities, the decrease of tourist attractions in the dive sites and of the economic sustenance that they provide to the coastal populations that live from dive tourism.

In continental Ecuador, the colonies of *C. riisei* tolerate a wide range of habitats and conditions: shaded ledges with direct light and high current flow, rocky walls, as well as flat surfaces. *Carijoa riisei* was observed growing on rocks, on substrates covered with sediment and even on other organisms such as: sponges, scleractinian corals, octocorals, bivalves and balanus. These have shown to be important substrates for colonization and could drive in a short term the native isolated coral patches on small rocky bottoms actually present in Mainland Ecuador (Cabanillas-Terán et al. 2016). Contrary to what was observed in Hawaii specimens are restricted to cryptic habitats and turbid waters, avoiding direct sunlight (Concepción et al. 2008). The authors, based on the results, suggest that *C. riisei* is mostly opportunistic and the environmental conditions of the Ecuadorian coast do not represent an important driver. Notably, these sites are characterized by high density of native corals (Glynn 2003; Rivera and Martínez 2011) which are highly vulnerable to environmental variability, especially increasing temperature (Glynn et al. 2001). The weakening of native coral reefs in areas with rising temperatures—for example due to climate change—could accelerate the *C. riisei* invasion scenario.

The globalization of maritime traffic plays a key role in the introduction and spread of species since many of these organisms are moved between regions in ballast water, on ships hulls, in sea chests, on chains and even anchors (Kolar and Lodge 2002; Hulme 2009; Seebens et al. 2013). Eighty percent of Ecuador's foreign trade is carried out by sea, there are ports for oil and commercial cargo in Esmeraldas, Manta, La Libertad, Puerto Bolívar (Torres 2017) and Guayaquil; with several fishing and tourist piers along the coast. These larger ports could receive species from around the world and serve as hubs for the transport of non-native species to nearby smaller ports or sites that would not normally receive such organisms (Lockwood et al. 2009). *Carijoa riisei* has been reported as a fouling organism (Padmakumar et al. 2010), making it possible for this species to have been introduced by marine traffic in Ecuadorian coasts.

Due to how this species has behaved and expanded along the coast of Ecuador in a short period of time since its first record, there is a high risk of this species overtaking native species and altering the marine ecosystem as well as the ecosystem services that it provides. However, it is not only the coast of mainland Ecuador that is at risk, the unique marine biodiversity of the Galapagos Marine Reserve is also in danger due to the likelihood of this species being introduced to the Galapagos Islands because of the connectivity

that exists between mainland Ecuador and the archipelago through marine traffic routes and current regimes (Carlton et al. 2019). Currently all vessels that enter the Galapagos Marine Reserve, be it from national or international destinations, must have their hull cleaned prior to entry and inspected on arrival by the Galapagos Biosecurity Agency, marine unit (Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos 2018). Extreme care should be taken during inspections in order to minimize the risk of possible introduction of this species.

The data presented in this work could be used as a reference to continuing monitoring the spreading of *C. riisei* along the Eastern Pacific in order to measure the expanding distribution and abundance of this species. Biosecurity protocols should be socialized with dive centers, fishers and local boat owners as well as with cargo companies and the Ecuadorian Navy. A risk assessment of the impact that this species is causing should be presented to the authorities in order to consider management strategies such as real-time continuous monitoring.

Currently, the Ecuadorian Navy, through the National Directorate of Acuatic Spaces (Dirección Nacional de los Espacios Acuáticos, DIRNEA), is conducting a baseline survey of invasive Spaces in continental and insular Ecuador under funding from the International Maritime Organization (IMO) and the participation of the Permanent Commission of the South Pacific (CPPS).

Ethics and Permits

This paper contains partial information derived from an environmental consultancy carried out by the company BIOELITE for the Subsecretaría de Gestión Marina Costera of the Ministry of the Environment (MAE) during the period 2015–2016 and BIOELITE had the permits to carry out the investigation in the protected areas and coastal areas of Ecuador to carry out the research called "Quantitative subtidal and intertidal marine biodiversity inventories in six marine protected coastal areas and four areas of possible expansion" (CFC-001-2015) and Maritza Cárdenas obtained in 2016 the MAE's permission to use the data to scientific publications.

References

Agencia de Regulación y Control de la Bioseguridad y Cuarentena para Galápagos (2018) Resolución No. D-ABG-0-32-05-2018. Ministerio del Ambiente, Puerto Ayora Galápagos, Ecuador

Allauca S (1990) Presencia de la Corriente Costanera Ecuatoriana. *Acta Oceanográfica del Pacífico* 6(1): 10–17

Barbosa TM, Gomes PB, Bergeron AS, Santos AM, Chagas C, Freitas EMS, Perez CD (2014) Comparisons of sexual reproduction in *Carijoa riisei* (Cnidaria, Alcyonacea) in South Atlantic, Caribbean, and Pacific areas. *Hydrobiologia* 734: 201–212, https://doi.org/10.1007/s10750-014-1893-6

Cabanillas-Terán N, Loor-Andrade P, Rodríguez-Barreras R, Cortés J (2016) Trophic ecology of sea urchins in coral-rocky reef systems, Ecuador. *PeerJ* 4: e1578, https://doi.org/10.7717/peerj.1578

Cárdenas-Calle M, Triviño M, Giovanni G, Velásquez M (2018a) Comunidades bentónicas presentes en sitios de buceo en la Reserva Marina El Pelado. *Investigatio Research Review* 11: 67–68, https://doi.org/10.31095/investigatio.2018.11.6

Cárdenas-Calle M, Triviño M, Rubira K, Troccoli, L (2018b) Variación espacial de la diversidad del macrobentos en la Reserva Marina El Pelado. *Revista Lasallista de Investigación* 15: 390–404, https://doi.org/10.22507/rli.v15n2a30

Cárdenas-Calle M, Mora E, Torres G, Pérez-Correa J, Bigatti G, Signorelli J, Coronel J (2020) Marine invertebrate and seaweed biodiversity of continental coastal Ecuador. *Biodiversity Data Journal* 8: e53818, https://doi.org/10.3897/BDJ.8.e53818

Carlton JT (2001) Introduced species in U.S. coastal waters: Environmental Impacts and Management Priorities. Pew Oceans Commission, Arlington, FL, Virginia, 28 pp

Carlton JT, Keith I, Ruiz GM (2019) Assessing marine bioinvasions in the Galápagos Islands: implications for conservation biology and marine protected areas. *Aquatic Invasions* 14: 1–20, https://doi.org/10.3391/ai.2019.14.1.01

Collin R, Diaz M C, Norenburg J L, Rocha RM, Sanchez JA, Schulz A, Schwartz, Megan L, Valdes A (2005) Photographic Identification Guide to Some Common Marine Invertebrates of Bocas Del Toro, Panamá. *Caribbean Journal of Science* 41(3): 638–707

Concepción GT, Crepeau MW, Wagner D, Kahng SE, Toonen RJ (2008) An alternative to ITS, a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus *Carijoa*. *Coral Reefs* 27: 323–336, https://doi.org/10.1007/s00338-007-0323-x

Concepción GT, Kahng SE, Crepeau MW, Franklin EC, Coles SL, Toonen RJ (2010) Resolving natural ranges and marine invasions in a globally distributed octocoral (genus *Carijoa*). *Marine Ecology Progress Series* 401: 113–127, https://doi.org/10.3354/meps08364

Cuesta F, Peralvo M, Merino-Viteri A, Bustamante M, Baquero F, Freile JF, Muriel P, Torres-Carvajal O (2017) Priority areas for biodiversity conservation in mainland Ecuador. *Neotropical Biodiversity* 3: 93–106, https://doi.org/10.1080/23766808.2017.1295705

Doyle JJ, Doyle JL (1990) Isolation of Plant DNA from Fresh Tissue. *Focus* 12: 13–15, https://doi.org/10.2307/2419362

Duchassaing P, Michelotti J (1860) Memoire sur les coralliaires des Antilles. *Memorie della Reale Accademia delle Scienze di Torino* 2: 279–365, https://doi.org/10.5962/bhl.title.11388

Edgar G J, Banks SA, Bessudo S, Cortés J, Guzmán HM, Henderson S, Martinez C, Rivera F, Soler G, Ruiz D (2011) Variation in reef fish and invertebrate communities with level of protection from fishing across the Eastern Tropical Pacific seascape. *Global Ecology and Biogeography* 20: 730–743, https://doi.org/10.1111/j.1466-8238.2010.00642.x

Galván-Villa CM, Ríos-Lara E (2018) First detection of the alien snowflake coral *Carijoa riisei* (Duchassaing and Michelotti, 1860) (Cnidaria: Alcyonacea) in the port of Manzanillo in the Mexican Pacific. *Bioinvasions Records* 7: 1–6, https://doi.org/10.3391/bir.2018.7.1.01

Glynn PW (2003) Coral communities and coral reefs of Ecuador. In: Cortés J (ed), Latin American Coral Reefs. Elsevier, Amsterdam, pp 449–472, https://doi.org/10.1016/B978-044451388-5/50020-5

Glynn PW, Maté JL, Baker AC, Calderón MO (2001) Coral Bleaching and Mortality in Panama and Ecuador during the 1997-1998 El Niño-Southern Oscillation event: Spatial/temporal patterns and comparisons with the 1982-1983 event. *Bulletin of Marine Science* 69(1): 79–109

Gutiérrez S (2012) El invasor del Pacífico. *Hipótesis* 12: 6–7

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18, https://doi.org/10.1111/j.1365-2664.2008.01600.x

Instituto Nazca (2005) Map of the subtidal system. https://instititutonazca.org/downloads/ca_gap_analysis_ecuadorian_coast/mapa_02_sistemas_submareales.pdf (accessed 20 June 2020)

Instituto Nazca (2013) Potencial amenaza de la especie invasora de octocoral *Carijoa riisei* en Áreas Marinas Protegidas de Ecuador. https://institutonazca.org/es/13august2011-potential-threat-of-the-octocoral-species-carijoa-riisei-in-the-galera-san-francisco-marine-reserve/

Kahng SE, Grigg RW (2005) Impact of an alien octocoral, *Carijoa riisei*, on black corals in Hawaii. *Coral Reefs* 24: 556–562, https://doi.org/10.1007/s00338-005-0026-0

Kahng SE, Benayahu Y, Wagner D, Rothe N (2008) Sexual reproduction in the invasive octocoral *Carijoa riisei* in Hawaii. *Bulletin of Marine Science* 82(1): 1–17

Kassambara A, Mundt F (2020) Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. In: R package version 1.0.7. https://cran.r-project.org/package=factoextra

Keith I, Martínez PC (2017) The danger of *Carijoa riisei* invading the Galápagos Marine Reserve. In: Galápagos Report 2015-2016. GNPD, GCREG, CDF and GC. Puerto Ayora, Galápagos, Ecuador, pp 118–124

Kolar CS, Lodge DM (2002) Ecological Predictions and Risk Assessment for Alien Fishes in North America. *Science* 298: 1233–1236, https://doi.org/10.1126/science.1075753

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution 35: 1547–1549, https://doi.org/10.1093/molbev/msy096

Lira AKF, Naud JP, Gomes PB, Santos AM, Perez CD (2009) Trophic ecology of the octocoral *Carijoa riisei* from littoral of Pernambuco, Brazil. I. Composition and spatio-temporal variation of the diet. *Journal of the Marine Biological Association of the United Kingdom* 89: 89–99, https://doi.org/10.1017/S0025315408002385

Lockwood JL, Cassey P, Blackburn TM (2009) The more you introduce the more you get: The role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions* 15: 904–910, https://doi.org/10.1111/j.1472-4642.2009.00594.x

McFadden CS, van Ofwegen LP (2012) Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of *Cornularia* (Cornulariidae). *Invertebrate Systematics* 26: 331–356, https://doi.org/10.1071/IS12035

Mestanza-Ramón C, Capa MS, Saavedra HF, Paredes JR (2019) Integrated Coastal Zone Management in Continental Ecuador and Galapagos Islands: Challenges and Opportunities in a Changing Tourism and Economic Context. *Sustainability* 11: 6386, https://doi.org/10.3390/su11226386

Miloslavich P, Klein E, Díaz J M, Hernández CE, Bigatti G, Campos L, Artigas F, Castillo J, Penchaszadeh PE, Neill PE, Carranza A, Retana MV, Díaz de Astarloa, JM, Lewis M, Yorio P, Piriz, ML, Rodríguez D, Valentin YY, Gamboa L, Martín A (2011) Marine biodiversity in the Atlantic and Pacific coasts of South America: Knowledge and gaps. *PLoS ONE* 6: e14631, https://doi.org/10.1371/journal.pone.0014631

Olsson AA (1961) Mollusks of the tropical eastern Pacific: particularly from the southern half of the Panamic-Pacific faunal province (Panama to Peru); Panamic-Pacific Pelecypoda. Paleontological Research Institution, Ithaca, NY, 574 pp, https://doi.org/10.1002/iroh.19680530114

Padmakumar K, Chandran R, Kumar J, Sornaraj R (2010) *Carijoa riisei* (Cnidaria: Octocorallia: Clavulariidae), a newly observed threat to Gulf of Mannar coral biodiversity? *Current Science* 100(1): 35–37

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rivera F, Martínez P (2011) Guía fotográfica de corales y octocorales: Parque Nacional Machalilla y Reserva de Producción Faunística Marino Costera Puntilla de Santa Elena. Publisher by Instituto Nazca and Conservación Internacional, Ecuador, 78 pp

Sánchez JA (1994) Presencia de *Stylatula diadema* Bayer (Pennatulacea) y *Carijoa riisei* (Duchassaing & Michelotti) (telestacea) en el Caribe Suroccidental, Colombia. *Anales del Instituto de Investigaciones Marinas de Punta de Betín* 23: 137–147, https://doi.org/10.25268/bimc.invemar.1994.23.0.393

Sánchez JA, Ballesteros D (2014) The invasive snowflake coral (*Carijoa riisei*) in the Tropical Eastern Pacific, Colombia. *Revista Biología Tropical* 62: 199–207, https://doi.org/10.15517/rbt.v62i0.16276

Seebens H, Gastner MT, Blasius B (2013) The risk of marine bioinvasion caused by global shipping. *Ecology Letters* 16: 782–790, https://doi.org/10.1111/ele.12111

Sullivan-Sealey K, Bustamante G (1999) Setting geographic priorities for marine conservation in Latin America and the Caribbean. The Nature Conservancy, Arlington, Virginia, 141 pp

Torres GM (2017) Evaluación del fitoplancton como un mecanismo preventivo a la ocurrencia de bloom algal frente a las costas de Esmeraldas, Manta, La Libertad y Puerto Bolívar en Ecuador 2013-2015. Universidad Nacional Mayor de San Marcos, https://hdl.handle.net/20.500.12672/6695 (accessed 9 June 2020)

Venkataraman K, Raghunathan C, Satyanarayana C, Rajkumar R (2016) Invasion of snowflake coral, *Carijoa riisei* (Duchassaing and Michelotti, 1860), in Indian seas: Threats to coral reef ecosystem. *Indian Journal of Geo-Marine Sciences* 45(11): 1403–1408

Wagner D, Kahng SE, Toonen R J (2009) Observations on the life history and feeding ecology of a specialized nudibranch predator (*Phyllodesmium poindimiei*), with implications for biocontrol of an invasive octocoral (*Carijoa riisei*) in Hawaii. *Journal of Experimental Marine Biology and Ecology* 372: 64–74, https://doi.org/10.1016/j.jembe.2009.02.007

Weber L, DeForce E, Apprill A (2017) Optimization of DNA extraction for advancing coral microbiota investigations. *Microbiome* 5: 18, https://doi.org/10.1186/s40168-017-0229-y

Supplementary material

The following supplementary material is available for this article:

Table S1. Sites included in the analysis.
Table S2. Primers used to amplify the *COI* and *NADPH* genes.
Table S3. Distance matrix of ND6 sequence fragments, showing haplotypes from this work and from the work of Concepción et al. 2010.
Table S4. List of environmental variables used for Principal Component Analysis.
Figure S1. Overgrowth of *Carijoa riisei* on invertebrates at Guayaquil Bioregion ("La Pared" on El Pelado Marine Reserve) during April 2015.

This material is available as part of online article from:
http://www.reabic.net/aquaticinvasions/2021/Supplements/AI_2021_Cardenas-Calle_etal_SupplementaryTables.xlsx
http://www.reabic.net/aquaticinvasions/2021/Supplements/AI_2021_Cardenas-Calle_etal_SupplementaryFigure.pdf