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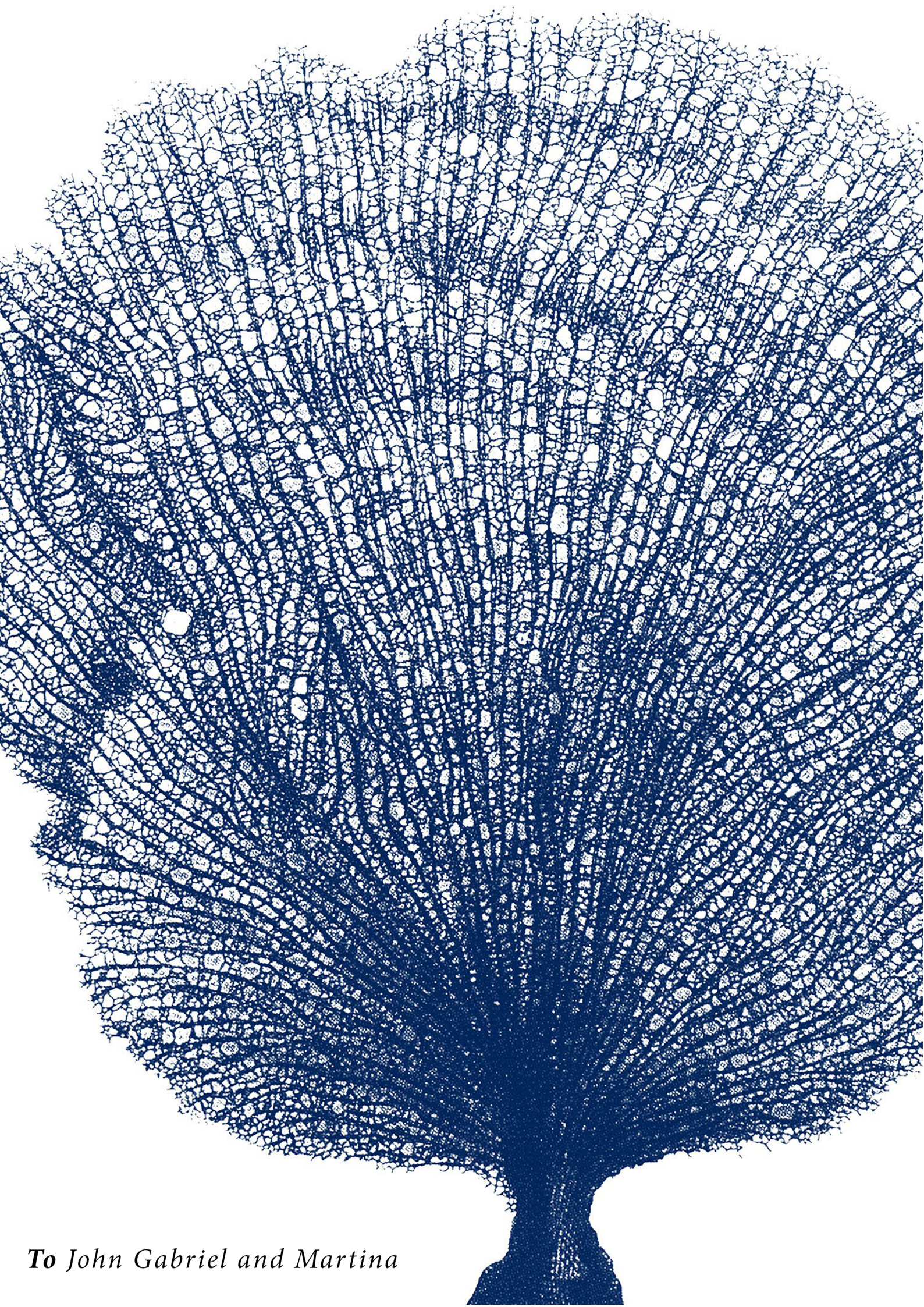
# Effects of Invasive Species, Disease Outbreaks and Climate on Gorgonian Corals in the Tropical Eastern Pacific

Elena Quintanilla Alcaide  
Doctoral Thesis

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Threats on tropical eastern Pacific gorgonian corals

2021



*To John Gabriel and Martina*

JUSTUS LIEBIG UNIVERSITY GIESSEN

**EFFECTS OF INVASIVE SPECIES, DISEASE OUTBREAKS  
AND CLIMATE ON GORGONIAN CORALS IN  
THE TROPICAL EASTERN PACIFIC**

*by*

**ELENA QUINTANILLA ALCAIDE**

**SUPERVISORS**

Prof. Dr. Thomas Wilke

Justus Liebig University Giessen | Germany

Prof. Dr. Juan A. Sánchez

Universidad de los Andes | Colombia

**REVIEWERS**

Prof. Dr. Petra Quillfeldt

Justus Liebig University Giessen | Germany

Prof. Dr. Jean-François Flot

Université libre de Bruxelles | Belgium

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# ABSTRACT



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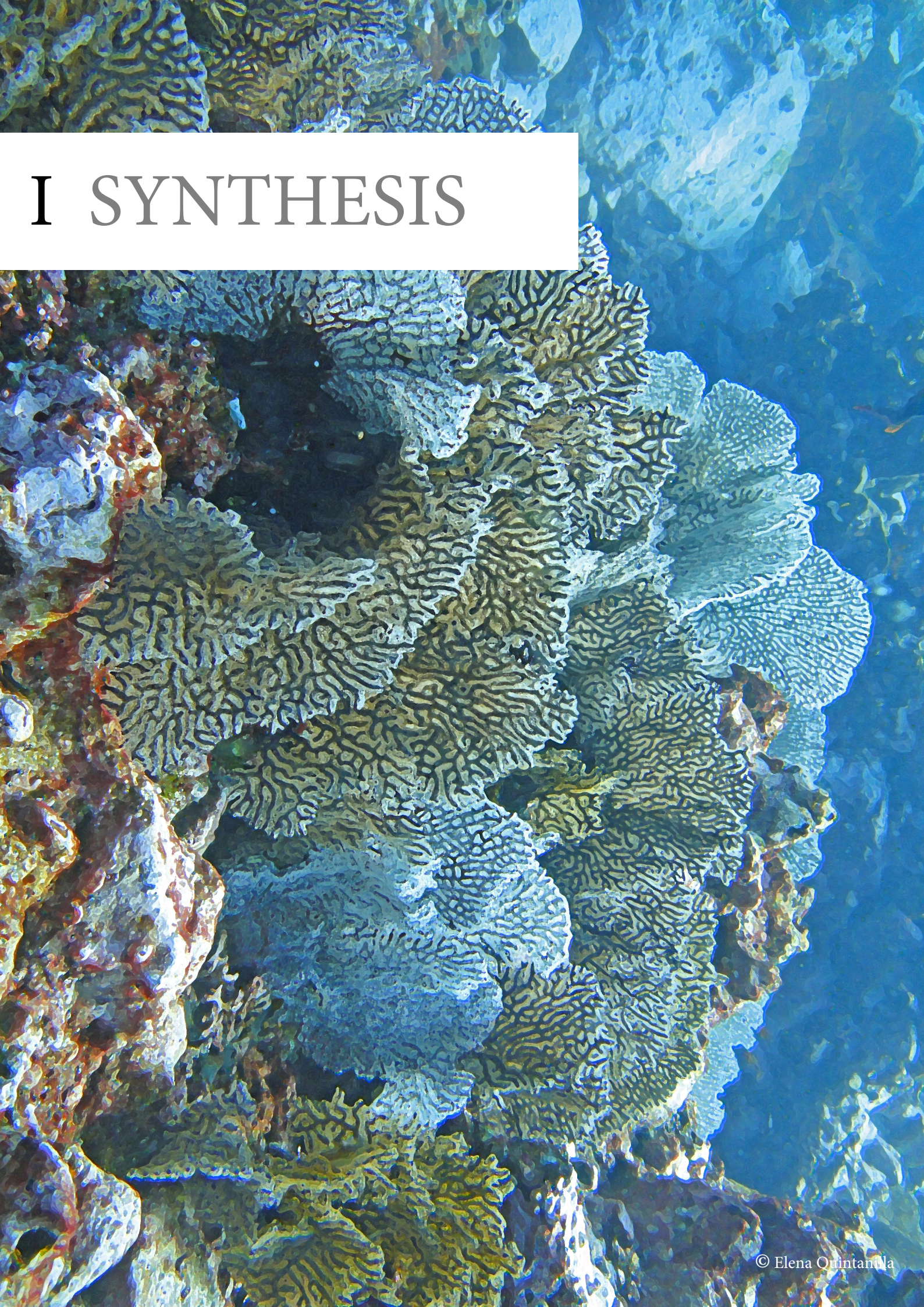
# Abstract

Gorgonian corals (Anthozoa, Octocorallia) play paramount roles in benthic communities. As long-lived and slow-growing species, gorgonians are particularly sensitive to environmental conditions and anthropogenic-related disturbances, being among the most vulnerable reef organisms. However, gorgonian responses to environmental conditions and the effects of abiotic and biotic threats to their populations are poorly understood in undisturbed marine areas. This lack of knowledge challenges the comprehension of gorgonian responses to human-associated disturbances. This doctoral thesis studies the effect of abiotic (i.e. local and global environmental conditions) and biotic (i.e. disease outbreaks and invasive species) factors affecting *Pacifigorgia cairnsi* sea fans, the most abundant coral and a key species surrounding the remote and pristine Malpelo Island, in the Colombian Tropical Eastern Pacific (TEP). In order to assess the health status of *P. cairnsi* and to understand whether these abiotic and biotic threats may represent long-term impacts to their populations, this thesis uses an integrative and multidisciplinary approach that includes phylogeography, metabarcoding, microbial ecology and demography. Major results suggest that, (1) *P. cairnsi* show high densities and their population size structures are driven by local hydrodynamics. Additionally, *P. cairnsi* growth rates are negatively affected by El Niño Southern Oscillation (ENSO) events and the 'Necrotic Patch Disease' (NPD) prevalence is low, while most of the colonies recovered. In addition, (2) the bacterial microbiome associated with the NPD affecting *P. cairnsi* populations behaves opportunistically and is likely in a state of microbial dysbiosis. Moreover, the confinement of the disease-related consortium to symptomatic tissues may facilitate colony recovery by tissue breakage. Finally, (3) the octocoral *Carijoa riisei* constitutes a fully invasive species in the TEP and might have been introduced from the Tropical Atlantic region, probably associated to international marine shipping. Overall, the conclusions include that *P. cairnsi* develop mature and healthy populations at Malpelo Island but threats, such as invasive species and ENSO events, may represent a long-term impact to these key benthic organisms. These valuable data and novel insights help to assess the vulnerability of these gorgonian corals in the absence of direct human-related disturbances. Moreover, this thesis provides crucial baseline data that will serve, in turn, as reference for future research aiming at understanding coral responses to direct anthropogenic pressures and the impact of global climate change on coral communities.

# Zusammenfassung

Gorgonien (Anthozoa, Octocorallia) spielen in benthischen Gemeinschaften eine herausragende Rolle. Als langlebige und langsam wachsende Arten sind Gorgonien besonders empfindlich gegenüber Umweltbedingungen und anthropogen-bedingten Störungen und gehören zu den empfindlichsten Rifforganismen. Allerdings sind die Reaktionen der Gorgonien auf Umweltbedingungen und die Auswirkungen abiotischer und biotischer Faktoren auf ihre Populationen in ungestörten Meeresgebieten nur unzureichend bekannt. Dieser Mangel an Wissen erschwert das Verständnis der Reaktionen der Gorgonien auf durch den Menschen verursachte Störungen. Diese Doktorarbeit untersucht die Auswirkungen abiotischer (d.h. lokaler und globaler Umweltbedingungen) und biotischer (d.h. Krankheitsausbrüche und invasive Arten) Faktoren auf die Art *Pacifigorgia cairnsi*, die am häufigsten vorkommende Koralle und eine Schlüsselart, die die abgelegene und unberührte Insel Malpelo im kolumbianischen tropischen Ostpazifik (TEP) umgibt. Um den Gesundheitszustand von *P. cairnsi* zu beurteilen und zu verstehen, ob diese abiotischen und biotischen Bedrohungen langfristige Auswirkungen auf ihre Populationen haben können, verwendet diese Arbeit einen integrativen und multidisziplinären Ansatz, der Phylogeographie, Metabarcodierung, mikrobielle Ökologie und Demographie umfasst. Die wichtigsten Ergebnisse deuten darauf hin, dass (1) *P. cairnsi* hohe Dichten aufweisen und ihre Populationsgrößenstrukturen von der lokalen Hydrodynamik angetrieben werden. Darüber hinaus werden die Wachstumsraten von *P. cairnsi* durch El Niño Southern Oscillation (ENSO)-Ereignisse negativ beeinträchtigt, und die 'Necrotic Patch Disease' (NPD)-Prävalenz ist gering, während sich die meisten Kolonien erholen. Darüber hinaus (2) verhält sich das bakterielle Mikrobiom, das mit der NPD assoziiert ist, welches die *P. cairnsi*-Populationen beeinflusst, opportunistisch und befindet sich wahrscheinlich im Zustand einer mikrobiellen Dysbiose. Darüber hinaus kann die Beschränkung des krankheitsbedingten symptomatischen Gewebes die Erholung der Kolonie durch Gewebebruch erleichtern. Schließlich (3) stellt die Oktokoralle *Carijoa riisei* eine invasive Art in der TEP dar und könnte aus der tropischen Atlantikregion eingeführt worden sein, wahrscheinlich in Verbindung mit der internationalen Seeschifffahrt. Insgesamt lässt sich schlussfolgern, dass *P. cairnsi* ausgewachsene und gesunde Populationen auf der Insel Malpelo entwickeln, aber Bedrohungen, wie invasive Arten und ENSO-Ereignisse, können langfristige Auswirkungen auf diese benthischen Schlüsselorganismen haben. Diese wertvollen Daten und neuartigen Einsichten helfen dabei, die Anfälligkeit dieser Gorgonien ohne direkte anthropogen-bedingte Störungen einzuschätzen. Darüber hinaus liefert diese Arbeit entscheidende Basisdaten, die wiederum als Grundlage für zukünftige Forschung dienen werden, die darauf abzielt, die Reaktionen der Korallen auf direkten anthropogenen Druck und die Auswirkungen des globalen Klimawandels auf Korallengemeinschaften zu verstehen.

# I SYNTHESIS



# 1 | Introduction

## 1.1 | Corals: Ecosystem engineers

Since decades, the study of corals (Cnidaria, Anthozoa) has aroused great interest among the scientific community due to the paramount role that these organisms play in marine systems. Corals develop reefs and coral-dominated environments worldwide that represent the most productive and diverse marine ecosystems on Earth (Connell 1978; Hoegh-Guldberg 1999). The high productivity of corals is given by the tight nutrient recycling process favoured by their relationship with endosymbionts (dinoflagellates and microbes). As a consequence, coral ecosystems encompass complex food chains and harbour a rich array of world's ocean biodiversity, making them often 'oases' within marine nutrient deserts (Shick and Dykens 1985; Knowlton et al. 2010).

The relevance of coral ecosystems also lays on the goods and services they provide to many millions of people around the world (Moberg and Folke 1999). Reefs represent physical barriers that protect shorelines from currents and strong waves, allowing them in turn to develop ecologically and economically important ecosystems such as seagrass beds and mangroves (Moberg and Folke 1999; Hughes et al. 2017). Additionally, coral systems provide a wide variety of seafood products and harvest natural resources with medical and pharmaceutical interest (Craik et al. 1990; Birkeland 1997). Finally, they also offer aesthetic and cultural benefits including the huge income from tourism and the spiritual values of many local communities (Spurgeon 1992; Moberg and Folke 1999).

## 1.2 | Coral's vulnerability to disturbances

In the last decades coral ecosystems have faced serious decline worldwide due to the effects of natural stressors and anthropogenic disturbances occurring on the global and local scales (Goldberg and Wilkinson 2004; Linares et al. 2008; Sánchez et al. 2019). On one hand, natural hydrodynamic disturbances such as hurricanes, strong storms and wave action comprise of major factors determining species zonation and structuring of reef communities by occasioning coral fragmentation, dislodgement and sedimentation that suffocates all benthic organisms (Lasker 1990; Madin and Connolly 2006; Sánchez 2016). On the other hand, human-related pressures are exercising an unprecedented impact to corals systems around the world, challenging their

recoveries and resilience capacities (Bellwood et al. 2004; Goldberg and Wilkinson 2004; Hughes et al. 2010).

Overall, global-scale anthropogenic disturbances affecting coral systems are related to climate change. The increase in emissions of greenhouse gases has led to global average rises in seawater temperature and, as a consequence, the frequency and severity of global-scale phenomena such as El Niño Southern Oscillation (ENSO) events have risen, affecting corals' essential physiological processes (Raven et al. 2005; Cai et al. 2014). Among the most common consequences of thermal stress in corals is the dissociation of coral-dinoflagellate symbiosis resulting in the expulsion of the photosynthetic symbionts, leading to bleaching events and massive coral die-offs (Hoegh-Guldberg 1999; Ainsworth et al. 2008). Moreover, global warming together with ocean acidification are directly associated with the decrease of coral growth rates, calcification and cover (Erez et al. 2011; Hetzinger et al. 2016).

Besides the impacts derived from anthropogenic global-scale disturbances, numerous local and direct human-related pressures are also threatening corals globally. Sedimentation, pollution and overfishing constitute localized stressors, usually degrading coral reefs along shorelines. These human-derived activities compromise coral growth and recruitment rates, decrease coral calcification and challenge coral photosynthetic performance (Rogers 1990; Birrell et al. 2005; Goatley et al. 2016). In particular, overfishing contributes to the suppression of herbivory, that together with eutrophication promote the increase of algal abundance, suffocating corals and thus affecting the whole community (Smith et al. 2010).

The synergy of different sources of disturbances, including changes in nutrient availability, overfishing, water pollution, sedimentation and warming leads to the increase of coral disease incidence by disrupting corals' microbiomes (Rosenberg and Ben-Haim 2002; Bourne et al. 2009; Vega-Thurber et al. 2009). For instance, the exposure to increased levels of inorganic nutrients (e.g. nitrates and phosphates) can cause the dominance of opportunistic or pathogenic bacterial communities and the loss of coral beneficial symbiotic microbes, thus leading to coral disease occurrence (Vega-Thurber et al. 2012; Zaneveld et al. 2016). In addition, warming has been typically correlated with coral disease outbreaks. Particularly, thermal anomalies in the Caribbean and Pacific Ocean have been immediately followed by outbreaks of white plague, yellow blotch diseases and white syndrome (Miller et al. 2006; Bruno et al. 2007). Even at modest increases of temperature, corals appear to be more vulnerable to opportunistic bacteria. Consequently, diseases act as important modifying factors of reef systems by reducing coral cover, decreasing coral diversity and notably affecting coral life-history traits (Petes et al. 2003; Miller et al. 2009;

Weil and Rogers 2011). These aspects severely compromise the coral resilience and the structure and functioning of the whole ecosystem (Loya et al. 2001; Harvell et al. 2007).

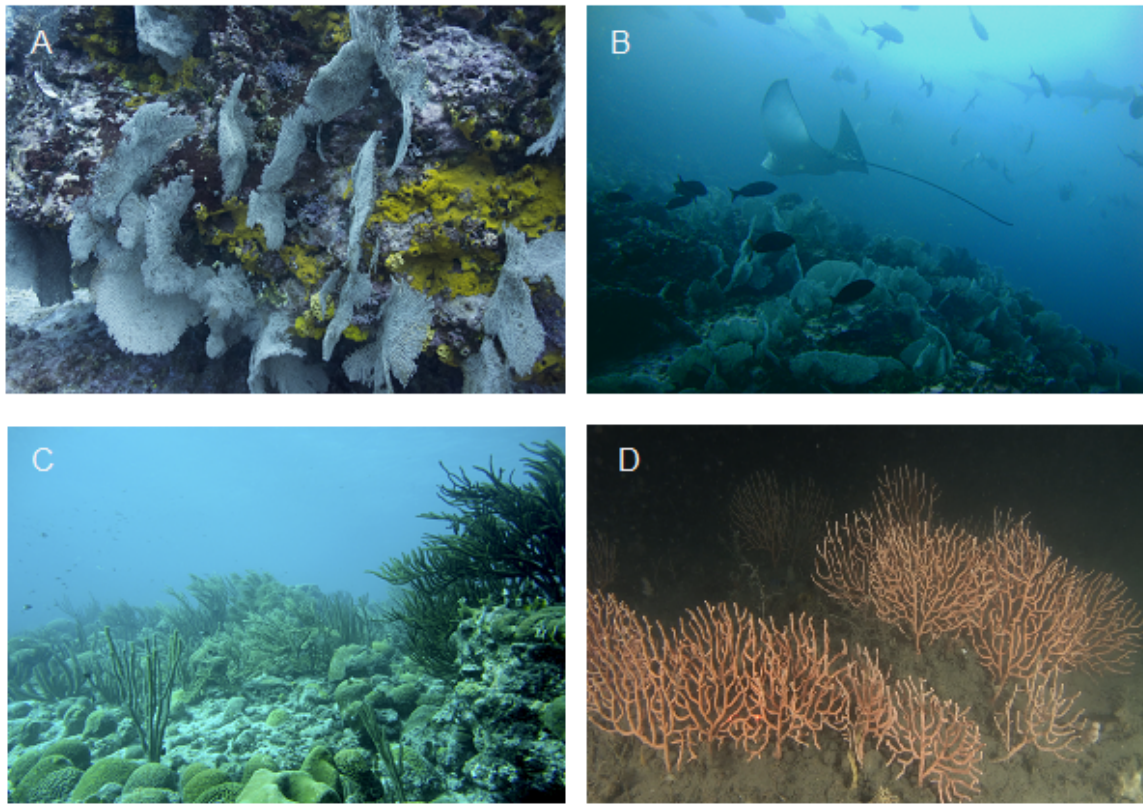
Marine invasive species are also among the most severe threats to coral ecosystems. The increase of shipping activities is an important factor that has promoted the global acceleration of marine invasion rates (Hulme 2009). One of the most harmful marine introductions known to date is the invasion of the lionfish in Western Atlantic and Caribbean coral reefs triggering long-term direct and indirect deleterious effects on native reef communities (Albins and Hixon 2011). Generally, invasive processes cause dramatic impacts into novel ecosystems. For example, the alteration of the community structure, the ecosystem disruption and the displacement and loss of native biodiversity can potentially evoke species extinctions (Loya et al. 2001; Leydet and Hellberg 2015).

As a result of the impact of abiotic and biotic stressors, either acting alone or in synergy, coral systems around the world have faced massive mortalities (Harvell et al. 2007; Maliao et al. 2008). Reductions of coral cover commonly cause a phase-shift from coral-dominated to macroalgal-dominated communities (Maliao et al. 2008). These shifts alter the whole complex ecosystem structure and is not easily reversed (Mumby 2009). Consequently, undesirable and degraded ecosystems states with new configuration of species are increasingly appearing, evidencing the loss of resilience of coral ecosystems (Hughes et al. 2010; Hughes et al. 2017).

### 1.3 | Gorgonian corals: Vulnerable animal forests

Gorgonian corals (Anthozoa, Octocorallia) are broadly distributed, ranging from tropical to polar latitudes. These octocorals play paramount ecological roles in reef-dwelling communities where they are considered 'engineering species' that shape the habitat and increase its complexity by forming three-dimensional structures (Jones et al. 1994; Ballesteros 2006; Sánchez et al. 2016). Due to the structural and functional similarity to the terrestrial forests, gorgonians communities are referred to as 'animal forests' (Rossi et al. 2017) (Figure 1). These corals hold an internal supporting axis made of gorgonin, a proteinaceous material that allows the upright position of the colony and confers it great flexibility facing water motion and hydrodynamic forces (Leversee 1976). Gorgonians usually form dense assemblages providing substrate and habitat for associated biota, thus increasing biomass and biodiversity of benthic communities (Buhl-Mortensen and Mortensen 2005; Sánchez et al. 2016). Moreover, from a strictly functional perspective, gorgonians play key roles in biogeochemical cycles and are responsible of benthic-pelagic

coupling processes via suspension feeding, promoting the flow of matter and energy from pelagic to benthic systems (Gili and Coma 1998; Ribes et al. 1999; Hill et al. 2014).



**Figure 1** | Gorgonian coral assemblages forming animal forests. (A, B) Dense sea fan populations of *Pacifigorgia cairnsi* at Malpelo Island (Colombian TEP). Photos: Elena Quintanilla (A) and John Gabriel Ramírez (B). (C) Gorgonian forests at Curazao reefs. Photo: Lina Gutiérrez (D). *Eunicella cavolinii* forests on the continental shelf in the north of Cap de Creus (Mediterranean Sea, Spain). Photo: IFM-GEOMAR-ICM-CSIC.

The vulnerability of gorgonian corals has become a tangible reality in the last years. Being long-lived, sessile and slow-growing species, gorgonians are particularly sensitive to environmental conditions and anthropogenic disturbances (Solan et al. 2004; Linares et al. 2007). Key aspects of their life-history traits, such as reproduction, recruitment or growth rates, are specifically susceptible to environmental changes and may be severely affected by different source of stress, impairing their population dynamics and recovery capacity (Linares et al. 2007; Santangelo et al. 2015). For instance, extreme storm events and invasive algal overgrowth have negatively affected *Paramuricea clavata*, a paramount structural gorgonian species in coralligenous assemblages from the NW Mediterranean Sea. Specifically, living colonies were fragmented and detached due to the dramatic impact of coastal storms, while the viability of juvenile colonies was found severely compromised as a consequence of the interaction with an invasive algae (Cebrián et al. 2012; Teixidó et al. 2013). Additionally, octocorals, together with

scleractinians, are one of the most disease-impacted cnidarian groups (Weil et al. 2011). The increased incidence of disease outbreaks have been linked to thermal anomalies and nutrient enrichment, leading to mass mortalities of gorgonian species such as *Phyllogorgia dilatata* and *Gorgonia ventalina* in Brazil and in the Caribbean Sea, respectively (Bruno et al. 2003; Weil et al. 2011). In turn, heavy sedimentation and turf algae overgrowth have been associated to low values of abundance and species richness. These stressors have been also related to inhibition of recruitment and mortality of juveniles in temperate and tropical gorgonian species (Linares et al. 2012; Manrique-Rodríguez et al. 2019).

As a consequence, massive mortalities of gorgonian forests have been registered globally, leading to dramatic shifts of understory assemblages. Due to the paramount role that gorgonians play in the structure and functioning of benthic systems, changes in their abundances conduce the decrease of the abundance of vagile benthic flora and fauna, such as calcareous builders, encrusting algae, shrimps, tanaids or mussels, and drive the shift from communities being dominated by erect and encrusting invertebrates to be dominated by algae and turf-forming species (Garrabou and Harmelin 2002; Ponti et al. 2016; Verdura et al. 2019).

#### 1.4 | Gorgonians from the Tropical Eastern Pacific

Gorgonians are often found in reef-dwelling communities dominated by scleractinian corals, as in the case of the Caribbean Sea (Sánchez et al. 1998). However, these soft corals can also dominate rocky reefs, forming dense and complex assemblages (Sánchez et al. 2014). One example is the Tropical Eastern Pacific (TEP), a marine realm ranging from the Sea of Cortez to northern Peru (Robertson and Cramer 2009), that harbours gorgonian corals forming shallow-water populations in seamounts and walls (Sánchez et al. 2011; Sánchez and Ballesteros 2014). These communities often have high levels of diversity, abundance and endemism (Bayer 1953; Van Oppen et al. 2005; Guzman et al. 2008; Sánchez 2016). Gorgonian populations in the TEP are exposed to high-energy environments (i.e. strong currents, waves and swells) (Breedy and Guzman 2002). Additionally, they cope with cold waters and high levels of suspended matter during seasonal upwelling (from December to March), as well as with anomalous increases in seawater temperatures during sporadic ENSO events (Glynn and Colgan 1992; Zapata et al. 2010).

Unlike gorgonian corals in other regions, none of the species found in the TEP are associated with zooxanthallae symbionts, meaning that they are obligate heterotrophs (Van Oppen et al. 2005; Gomez G et al. 2014). The latter is probably related with the fact that the TEP harbours highly productive and turbid waters that provide a wide variety of food sources to be

exploited by suspension feeders (Sánchez 2016). As a consequence, strict photosynthetic organisms such as scleractinian corals are low-developed in the TEP, showing discontinuous distributions and low diversity levels (Bayer 1953; Van Oppen et al. 2005; Guzman et al. 2008; Sánchez 2016).

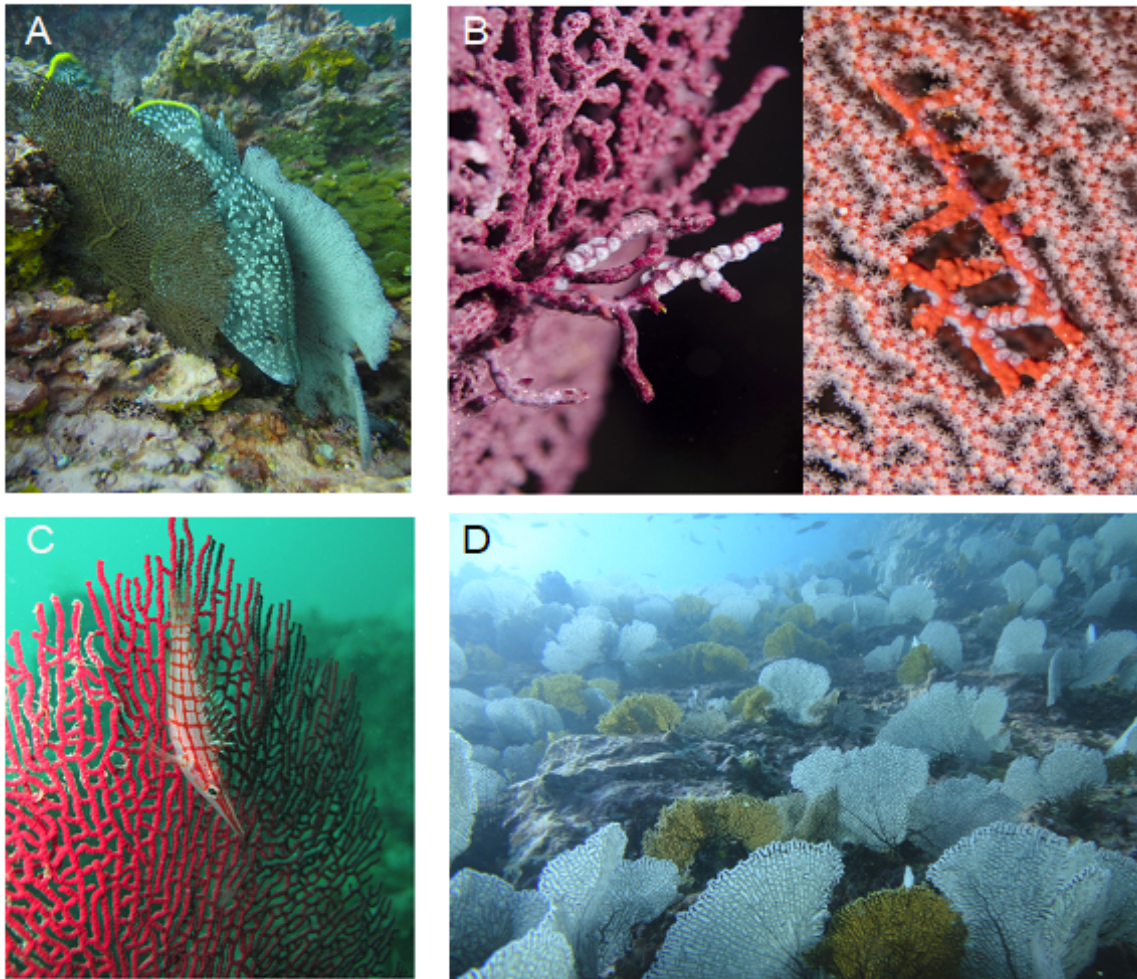
Gorgonian assemblages in the TEP are the most conspicuous invertebrates at rocky infralittoral seascapes down to 30-40 m water depth (Figure 2). Two main features characterize these gorgonian communities: high density (up to 30 colonies/m<sup>2</sup>) and species hyperdominance (i.e. local dominance of few species) (Zapata and Vargas-Ángel 2003; Van Oppen et al. 2005; Sánchez 2016). Typically, sea fans, sea whips and candelabrum corals from *Pacifigorgia*, *Leptogorgia*, *Eugorgia* and *Muricea* genera coexist in the TEP (Gomez G et al. 2014; Sánchez 2016). Particularly, sea fans of the genus *Pacifigorgia* spp. (Octocorallia, Gorgoniidae) are the most abundant and diverse gorgonians in this region with 35 known species (Breedy and Guzman 2002; Vargas et al. 2008). These sea fans are considered keystone species playing important ecological roles due to their strong interactions with invertebrates such as ovulid gastropods, and fishes including *Oxycirrhites typus* and *Dermatolepis dermatolepis* (Sánchez et al. 2016; Sánchez 2016) (Figure 2).

### 1.5 | Threats to gorgonians in the Colombian TEP

Over the recent years, gorgonian declines have specifically been registered in the Colombian TEP (Sánchez et al. 2011). This area belongs to the Chocó Biogeographic Region and is considered a global biodiversity hotspot given its tropical condition and geographical isolation (Gentry 1982; Myers et al. 2000; Palacios-torres et al. 2018). Massive mortalities of native octocorals, particularly of *Pacifigorgia* spp. sea fans have been observed in the Colombian TEP due to the interaction with the octocoral *Carijoa riisei* (Octocorallia, Clavulariidae) (Sánchez and Ballesteros 2014, Figure 3).

Although *C. riisei* was originally described from the US Virgin Islands in the Tropical Atlantic (Duchassaing and Michelotti, 1860), it likely originated in the Indo-Pacific (Concepcion et al. 2010). In recent years, the spread of this tropical stolonifer octocoral has been observed across the Pacific Ocean together with a considerable impact on the local biodiversity in the Indian subcontinent, Hawaii and in the TEP (Kahng and Grigg 2005; Raghunathan et al. 2013; Sánchez and Ballesteros 2014). Over the last 20 years, *C. riisei* has severely harmed Colombian TEP benthic communities, showing a competitive advantage over native octocorals by overgrowing and wiping out colonies of *Muricea* spp., *Leptogorgia* spp. and *Pacifigorgia* spp. at

Malpelo and Gorgona islands and Cabo Corrientes coast (Colombian TEP) (Sánchez et al. 2011; Sánchez et al. 2014; Sánchez and Ballesteros 2014).



**Figure 2** | Gorgonian assemblages in the Tropical Eastern Pacific (TEP). (A) *Pacifigorgia cairnsi* sea fans act as a refuge for *Dermatolepis dermatolepis* at Malpelo Island (Colombian TEP). Photo by Elena Quintanilla. (B) Ovopositions on *Pacifigorgia* cf. *curta* (left) and on *Pacifigorgia cairnsi* at Malpelo Island (Colombia TEP) (right). Photos by Juan A. Sánchez. (C) Longnose hawkfish *Oxyrrhites typus* with a *Pacifigorgia* spp. background camouflage at Cabo Corrientes (Colombian TEP). Photo by Juan A. Sánchez. (D) *Pacifigorgia* spp. sea fans populations at Malpelo Island (Colombian TEP). Photo by Elena Quintanilla.

Additionally, in the last years die-offs related to diseases outbreaks have been observed in the gorgonian sea fans *P. cairnsi*, *P. irene* and *P. eximia* at Malpelo and Cabo Corrientes (Sánchez et al. 2011; Sánchez et al. 2014, Figure 3). Investigations from the coral *Gorgonia ventalina* in the Caribbean indicated that these die-offs in the TEP may be similarly related to an aspergillosis-like fungal disease caused by *Aspergillus sydowii* (Nagelkerken et al. 1997; Toledo-Hernández et al. 2007; Sánchez et al. 2011). The first attempt to diagnose and identify potential fungal pathogens affecting *Pacifigorgia* spp. in the TEP left more questions than answers. In this sense, numerous

fungal species were isolated from both healthy and diseased *P. eximia* and *P. irene* colonies in the Colombian waters (Barrero-Canosa et al. 2013).

## 1.6 | Lack of knowledge

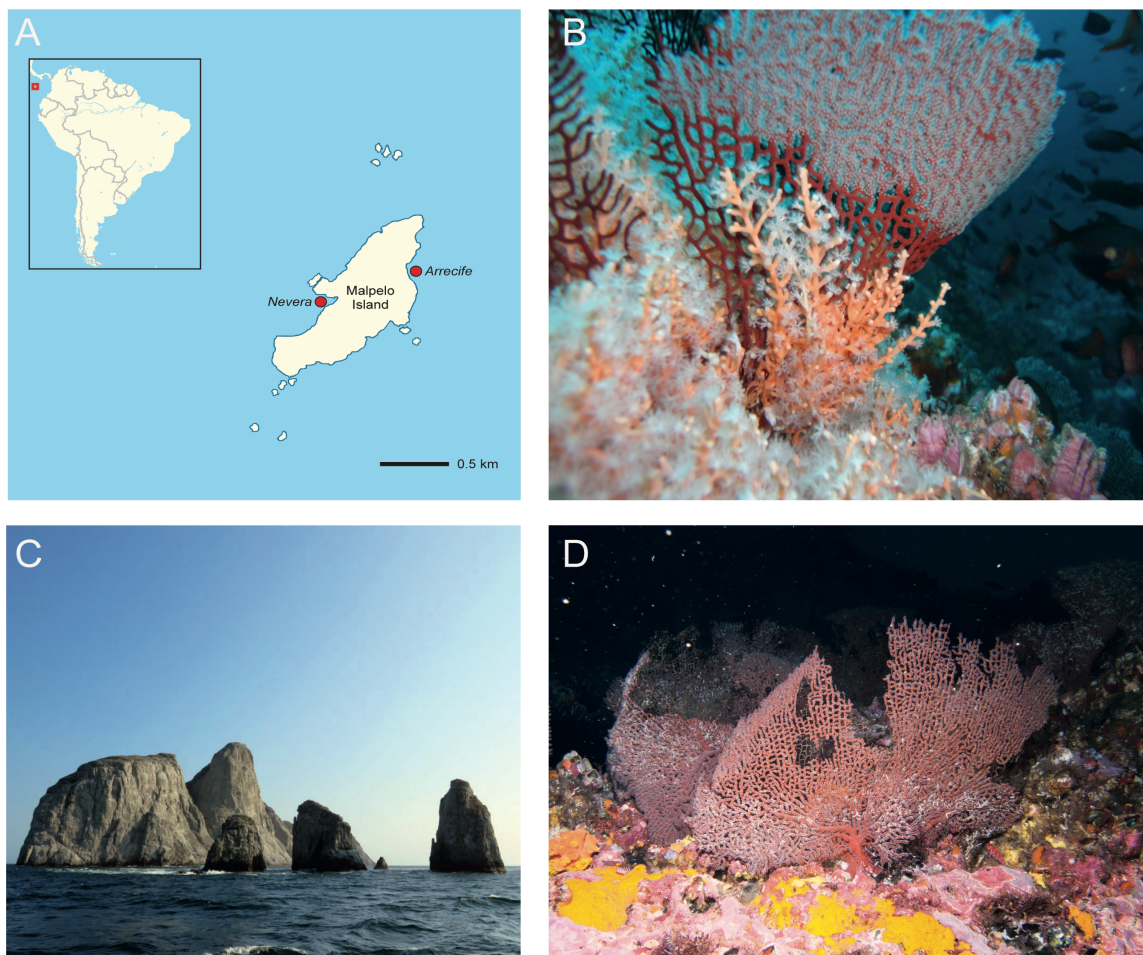
The vulnerability of gorgonian corals to disturbances has been understudied and overlooked in comparison with their scleractinian counterparts. What is more, if we refer to undisturbed gorgonian populations from remote places of difficult access with high-energy environments like those in the Colombian TEP, the number of studies is really scarce (Breedy and Guzman 2002). Particularly, how global and local-scale anthropogenic pressures affect coral systems are indeed difficult to quantify due to the lack of base-line data of unaffected populations. Therefore, information from gorgonian corals largely removed from direct human impacts, such as those found on the offshore Malpelo Island located in the Colombian TEP, is particularly valuable.

Due to its isolation, Malpelo Island lacks information regarding the ecology and demographic parameters of gorgonian corals and how different local and global-scale threats impact their populations. Despite the evident damage of the octocoral *C. riisei* on native Colombian TEP octocorals, the geographical origin of these regional populations remains unknown. In addition, their potential invasive status has not been clearly assessed yet, making the establishment of effective management strategies challenging (*sensu* Stefaniak et al. 2012; Leydet and Hellberg 2015). Moreover, although the bacterial microbiome provides wide benefits to the health status of coral holobionts, there is lack of knowledge of the disease-related changes in the bacterial microbiome of *Pacifigorgia* sea fans, hampering the knowledge of the etiology of these new disease outbreaks observed in the island. Finally, no information exist on the ecology and how local and global environmental parameters impact the demography and the health status of *Pacifigorgia* sea fans around Malpelo.

Understanding the responses to environmental conditions and threats of corals from remote areas, where direct anthropogenic pressures are low or absent, allows obtaining crucial data of the health status and resilience capacity of these key marine systems. The later will serve as a reference to comprehend and predict coral responses and their viability in changing scenarios with the presence of local and global human-related disturbances.

## 1.7 | Target species and study site

*Pacifigorgia cairnsi* sea fans from Malpelo Island (Colombian TEP) are used in this dissertation as a model population from a remote marine area little affected by direct human-related pressures (Figure 3). Rocky reef environments at Malpelo are mainly dominated by species of the genera *Leptogorgia* and *Pacifigorgia* (Sánchez et al. 2011; Sánchez 2016). Of these, *Pacifigorgia cairnsi* is endemic to the TEP and is the most abundant coral around Malpelo where it dominates the infralittoral seascape and forms dense aggregations on rocky outcrops and walls that occur up to 30 m water depth (Sánchez et al. 2011; Sánchez and Ballesteros 2014). *Pacifigorgia cairnsi* is considered a keystone species in this island and plays important ecological roles due to strong interactions with invertebrates and fish (Sánchez et al. 2016; Sánchez 2016). Therefore, the loss or decrease of *P. cairnsi* in Malpelo would dramatically affect the entire ecosystem; point that makes its study and conservation a crucial matter (*sensu* Mills et al. 1993).



**Figure 3 |** Malpelo island in the Colombian TEP and threats to *Pacifigorgia cairnsi* populations. (A) Geographical location map of Malpelo Island. (B) *Carijoa riisei* overgrowing *Pacifigorgia cairnsi* colony in Malpelo. Photo by Juan A. Sánchez. (C) The rocky, isolated and uninhabited Island of Malpelo. Photo by Elena Quintanilla. (D) Necrotic Patch Disease affecting the tissue of *P. cairnsi* colony. Photo by Juan A. Sánchez.

The study site of this thesis, Malpelo, is an oceanic island located about 500 km off the Colombian coast (3°58'30"N, 81°34'48"W, Figure 3). This Fauna and Flora Sanctuary is a marine protected area and a World Heritage Site that belongs to the Marine Conservation Corridor of the TEP together with Gorgona (Colombia), Cocos (Costa Rica), Coiba (Panamá), and Galapagos Islands (Ecuador). It is an uninhabited island, considered a biodiversity oasis to be under pristine conditions (Zapata and Vargas-Ángel 2003; Quimbayo et al. 2016).

## 2 | General and specific objectives

In this doctoral thesis the major goal is to assess the effect of different biotic (i.e. invasive species and disease outbreaks) and abiotic factors (i.e. ENSO events and local hydrodynamic features) on gorgonian corals removed from direct anthropogenic disturbances. This is done in order to identify the coral responses, their health status and to understand whether these threats may represent long-term impacts to these populations.

Specifically, I aim:

- To address the geographical origin of Colombian TEP *Carijoa riisei* populations and to assess whether this octocoral has to be considered an invasive species in this region. To elucidate the area of origin and the potential invasive status of an introduced species is crucial for understanding its success in the newly invaded environment and allows in turn the establishment of effective management strategies.
- To test for disease-related changes in the bacterial microbiome of *Pacifigorgia cairnsi* sea fans from Malpelo Island in the Colombian TEP. This will allow addressing the effect of the disease at the colony level and thus studying the colony recovery potential of gorgonian populations under pristine environmental conditions.
- To assess the effects of global (i.e., ENSO events) and local hydrodynamic parameters on the demography of *P. cairnsi* populations and to evaluate the disease prevalence affecting this species in Malpelo Island. This aim will allow understanding how environmental conditions affect important life-history traits of undisturbed sea fans and will permit addressing the health status of their populations.

## 3 | Publication outlines

### 3.1 | Taking a detour: invasion of an octocoral into the Tropical Eastern Pacific

Quintanilla E., Wilke T., Ramírez-Portilla C., Sarmiento A., Sánchez JA. Taking a detour: invasion of an octocoral into the Tropical Eastern Pacific. *Biological Invasions* 2017, 19(9), 2583-2597. doi:10.1007/s10530-017-1469-2.

The first aim of the thesis can be addressed by the publication Quintanilla et al. 2017 in *Biological Invasions*. In this publication the geographical scenarios for the colonization of the Colombian TEP are evaluated by conducting phylogeographical analyses based on nuclear and mitochondrial sequences of individual specimens from across the species' (native/nonnative) range and by applying hypothesis-specific operational criteria. Additionally, the invasiveness of *C. riisei* is assessed based on the previously proposed 'unified framework for biological invasions'. Major findings showed relatively high genetic differentiation between Colombian TEP populations, on the one side, and Indo-Pacific and Hawaiian populations, on the other side. In contrast, it could not be identified genetic differentiation and significant isolation by distance (IBD) between Colombian TEP and Tropical Atlantic populations. *C. riisei* might have been introduced from the Atlantic into the Colombian TEP, possibly via the Panama Canal. Based on the criteria of the 'unified framework for biological invasions', it is concluded that this octocoral constitutes an invasive species. This paper contributes to the aims of this thesis by revealing the impact of shipping in remote and pristine marine areas by acting as a vector of marine species introductions.

**Author Contributions:** EQ, JAS and TW contributed to the design of the study. EQ, CRP and AS conducted the sampling. EQ generated the data. EQ, CRP analysed the data. EQ, CRP, TW and JAS interpreted data results. EQ wrote the manuscript. EQ, CRP, TW, AS and JAS revised the manuscript.

### 3.2 | Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease

Quintanilla E., Ramírez-Portilla C., Adu-Oppong B., Walljasper. G., Glaeser. SP., Wilke T., Reyes. A., Sánchez. JA. Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease. *Scientific Reports* 2018 8(1), 14636. doi:10.1038/s41598-018-33007-8.

The second aim is addressed by the publication Quintanilla et al. 2018 in *Scientific Reports*. By generating 16S rRNA gene amplicons from tissues of healthy colonies and from symptomatic-asymptomatic tissues of diseased *P. cairnsi* colonies in Malpelo Island, (1) the bacterial community composition of the core microbiome associated with the healthy state is assessed as baseline information for the subsequent analyses. (2) Then tissues from healthy colonies from those affected by the disease are compared to assess disease-related shifts in bacterial community compositions and functional profiles. (3) Finally, disease-related shifts occurring within diseased colonies (i.e. between symptomatic and asymptomatic tissues) are tested in order to identify the relationships between the bacterial microbiome and gorgonian health states at intra-colony level. Overall, major results indicate that potential endosymbionts (mostly *Endozoicomonas* spp.) dominate the core microbiome in healthy colonies. Moreover, healthy tissues differ in community composition and functional profile from those of the symptomatic tissues but do not show differences to asymptomatic tissues of the diseased colonies. A more diverse set of bacteria is observed in symptomatic tissues, together with the decline in abundance of the potential endosymbionts from the healthy core microbiome. Furthermore, according to a comparative taxonomy-based functional profiling, these symptomatic tissues are characterized by the increase in heterotrophic, ammonia oxidizer and dehalogenating bacteria and by the depletion of nitrite and sulphate reducers. Overall, these results suggest that the bacterial microbiome associated with the disease behaves opportunistically and is likely in a state of microbial dysbiosis. It is also concluded that the confinement of the disease-related consortium to symptomatic tissues may facilitate colony recovery. This paper contributes to the aims of this thesis by revealing the effect of disease outbreaks on undisturbed gorgonian corals and their recovery capacity.

**Author Contributions:** EQ, JAS, ARM and TW contributed to the design of the study. EQ and CRP conducted the sampling. EQ, CRP, BAO and GW generated the data. EQ, CRP analysed the data. EQ, CRP, ARM and SPG interpreted data results. EQ wrote the manuscript. EQ, CRP, BAO, GW, SPG, TW, ARM and JAS revised the manuscript.

### 3.3 | Dynamic interplay of ENSO events and local hydrodynamic parameters drives demography and health status of gorgonian sea fan populations on a remote Tropical Eastern Pacific Island

Quintanilla E., Madurell T., Wilke T., Sánchez JA. Dynamic interplay of global and local environmental parameters drives demography and health status of gorgonian sea fan populations

on a remote Tropical Eastern Pacific island. *Frontiers in Marine Science* 2019. 6: 694. doi: 10.3389/fmars.2019.00694

The third aim is addressed in the publication Quintanilla et al. 2019 in *Frontiers in Marine Science*. The impact of global and local environmental parameters is assessed by evaluating demographic parameters and the health status of *Pacifigorgia cairnsi* sea fans in Malpelo Island. Specifically, *P. cairnsi* densities and population size structures are studied under different habitat and local environmental conditions. Moreover, whether ENSO events and local hydrodynamic features including locality, water depth, and upwelling conditions drive *P. cairnsi* growth rates is addressed. Finally, the prevalence of the necrotic patch disease and rates of disease recovery are evaluated. Major findings include that local hydrodynamic parameters shape *P. cairnsi* size structures, that growth rates are affected by thermal anomalies associated to ENSO events and partly by water depth, that overall disease prevalence is low and that it did not correlate with the environmental parameters studied, and that most diseased colonies recover via tissue breakage. This paper contributes to the aims of this thesis by revealing the effect of environmental parameters on undisturbed gorgonian corals and by unveiling their health status.

**Author Contributions:** EQ, TM, TW, and JS contributed to the design of the study, revised the manuscript, and approved the final text. EQ conducted the sampling, generated and analyzed the data, and wrote the manuscript. EQ and TM interpreted and discussed the data results.

This doctoral dissertation contributes to understand the health state gorgonian sea fans from a pristine area and how different abiotic and biotic factors impact their populations. This thesis allows identifying whether threats as invasive species, disease outbreaks and local and global environmental conditions may represent long-term impacts on populations located in a low perturbed marine area. These novel insights serve to evaluate the vulnerability of these gorgonian corals in the absence of direct human-related disturbances and provide crucial baseline data of coral systems that will serve as valuable reference for future research aimed at understanding coral responses to anthropogenic pressures and the impact of global climate change on coral communities.

## 4 | Results and Discussion

This doctoral thesis consists of three studies aiming at assessing the effect of different biotic and abiotic factors to gorgonian corals undisturbed by direct anthropogenic pressures. The lack of knowledge of gorgonians from undisturbed areas challenges the understanding of gorgonian responses in the presence of human-related disturbances. The valuable results of this thesis provided from the application of a multidisciplinary approach including phylogeography, metabarcoding, microbial ecology and demography, allow the understanding of responses of gorgonian populations from pristine conditions.

### 4.1 | *Carijoa riisei* as an invasive species in the Tropical Eastern Pacific: potential role of shipping introductions from the Tropical Atlantic

By conducting phylogeographical analyses and by applying hypothesis-specific operational criteria, this study confirmed the invasive status of *C. riisei* TEP populations based on the proposed ‘unified framework for biological invasions’. Firstly, nuclear and mitochondrial DNA were used from individual specimens occurring across the species’ wide range. With a phylogeographical approach, the genetic diversity of *C. riisei* in the Colombian TEP (Malpelo and Gorgona Islands and Nuqui on the mainland coast) and the genetic relatedness and genetic structure of Colombian TEP and extralimital populations were assessed. Secondly, by using operational criteria, three potential geographical scenarios for the colonization of the Colombian TEP were tested based on the geographical regions of *C. riisei* defined by Concepcion et al. (2010): (I) from the Hawaiian Archipelago region, (II) from the Indo-Pacific region and (III) from the Tropical Atlantic region. Third and finally, the invasiveness of *C. riisei* in the Colombian TEP was empirically assessed, applying the criteria of the ‘unified framework for biological invasions’ according to Blackburn et al. (2011).

The results of this study showed relatively high genetic differentiation between Colombian TEP populations, on the one side, and Indo-Pacific and Hawaiian populations, on the other side. In contrast, it could not be identified genetic differentiation and significant isolation by distance (IBD) between Colombian TEP and Tropical Atlantic populations. Therefore, this study revealed the Tropical Atlantic as the likely source region for *C. riisei* populations in the Colombian TEP, possibly via shipping through the Panama Canal. Based on the criteria of the ‘unified framework for biological invasions’, it is also concluded that this octocoral constitutes an invasive species. This is the first study proving the origin and invasiveness of *C. riisei* in the TEP. Herein, the

results are crucial to address proper management strategies in order to protect native communities, particularly gorgonian populations that are especially vulnerable to the interaction with this invasive species.

#### 4.2 | Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease

The second study described the disease affecting gorgonian corals (*P. cairnsi*) around Malpelo Island as the 'necrotic patch disease' (NPD) and tested for disease-related changes in the bacterial microbiome of this species. By analysing 16S rRNA gene amplicons, the core bacterial microbiome associated with healthy colonies was found to be identical in basal and peripheral tissues. Additionally, bacterial microbiome revealed shifts in diseased tissues driven by the decrease of potential endosymbionts composing the healthy core microbiome and the appearance of an opportunistic consortium. The results suggested that the bacterial microbiome associated with the disease behaves opportunistically, likely being in a state of microbial dysbiosis. Moreover, the NPD-related consortium was found confined to the symptomatic tissues of the affected colonies. This is suggested to facilitate colony recovery by natural breaking offs of gorgonian tissues due to the highly-energy environment. The latter may contribute to colony recovery from NPD and ultimately to the resilience of these gorgonian populations.

#### 4.3 | Dynamic interplay of ENSO events and local hydrodynamic parameters drives demography and health status of gorgonian sea fan populations'

Finally, the third study of this thesis evaluated demographic parameters and the health status of *P. cairnsi* populations in Malpelo Island to assess the impact of global and local environmental parameters on these gorgonian populations. *Pacifigorgia cairnsi* densities and population size structures were studied under different habitat and local environmental conditions. In addition, whether ENSO events and local hydrodynamic features including locality, water depth, and upwelling conditions drive *P. cairnsi* growth rates was assessed. Moreover, the prevalence of the NPD and rates of disease recovery were evaluated. Major findings revealed that local hydrodynamic parameters shaped *P. cairnsi* size structures, that growth rates were affected by thermal anomalies associated to ENSO events (and partly by water depth), that overall disease prevalence was low (6%), that NPD did not correlate with the environmental parameters studied and that most diseased colonies (57%) recovered via tissue breakage. Nonetheless, the low level of disease prevalence found indicates that the island's pristine conditions might facilitate disease

resistance. Moreover, the findings suggest an interesting trade-off between growth rates and colony recovery in shallow waters related to tissue breakage.

## 5 | Conclusions

Overall, the results of this thesis suggest that *P. cairnsi* develop mature populations that are in good health state in areas with limited human-related disturbances like Malpelo Island in the Colombian TEP. The latter is evidenced by the high colony density found, as well as, by the low disease prevalence and high colony recovery registered. However, these findings demonstrate that gorgonian corals from pristine conditions are highly vulnerable to the interaction with biotic and abiotic pressures such as invasive species and thermal anomalies associated to ENSO events, challenging their long-term viability if the impact of these factors are prolonged and intensified through time.

The octocoral *Carijoa riisei* represents a fully invasive species in the Colombian TEP. Due to its invasiveness, association to marine shipping, ability to survive and reproduce and fast-spreading potential in the newly invaded area (Coles and Eldredge 2002; Lira et al. 2009; Sánchez and Ballesteros 2014; Barbosa et al. 2014), this octocoral definitely represents a severe threat to gorgonians around Malpelo. Given all these features and the inherent damage caused to gorgonian colonies (Kahng and Grigg 2005; Raghunathan et al. 2013; Sánchez et al. 2014; Sánchez and Ballesteros 2014), *C. riisei* may pose a major long-term impact to *P. cairnsi* populations in Malpelo, especially if this invasive species reaches the densest gorgonians' areas of the island. The fact that the geographical origin of *C. riisei* populations in the TEP has been revealed and its invasiveness confirmed makes this study crucial to establish strategies to protect native species from one of the very few invasive coral species worldwide.

Additionally, global thermal conditions such as ENSO events impact growth rates of *P. cairnsi* populations in Malpelo. It is widely known that thermal stress severely affects different aspects of corals' physiology. For instance, zooxanthellate corals show special vulnerability to high temperatures by losing their association with the photosynthetic symbiont (*Symbiodinium*) leading to coral bleaching and colony death (Glynn 1984; Podestá and Glynn 2001). However, this thesis evidenced that undisturbed gorgonians that lack zooxanthelle are also vulnerable to abnormal temperatures and to thermal stress associated with ENSO episodes. The fact that *P. cairnsi* growth rates, a crucial demographic parameter, decrease with positive abnormal

temperatures in ENSO events remains of concerns since this is a keystone species within the regional benthic food web of the Malpelo Island marine ecosystem (Sánchez et al. 2016; Sánchez 2016; Quintanilla et al. 2019). Therefore, the loss or decrease of *P. cairnsi* populations in Malpelo would dramatically affect the entire ecosystem (*sensu* Garrabou and Harmelin 2002). This makes the conservation of this species a crucial matter and relevant since climatic predictions for the frequency and severity of ENSO events expected to increase over the next decades due to global climate change (Santoso et al. 2013; Cai et al. 2014).

Finally, this thesis contributed to the description and the study of the new disease outbreaks affecting *P. cairnsi* sea fans in Malpelo- the NPD. This disease consists in a dysbiosis (i.e. microbial imbalance) potentially driven by environmental pressures such as anomalous seawater temperatures. These findings contribute to open a debate in coral disease research that many marine diseases may be the consequence of microbial dysbiosis and the rise of opportunistic or polymicrobial infections rather than being driven by single pathogens. Nonetheless, the low prevalence of NPD might thus be related to the pristine conditions of Malpelo Island and the high recovery levels of *P. cairnsi* sea fans through natural breaking offs of affected tissues may contribute to convalescence and ultimately to the resilience of these gorgonian populations. Given these evidences I conclude that NPD does not represent a long-term impact on *P. cairnsi* populations so far, and I can state that coral diseases are also present in pristine marine areas, being part of natural population dynamics.

This doctoral thesis has greatly contributed to the understanding of the ecology and threats of a less studied group of key marine organisms from pristine areas in the TEP. These crucial insights here revealed will serve as base-line data for the study of coral resilience capacity facing direct human-related pressures, as well as will offer a starting point to predict coral responses under scenarios of changing environmental conditions.

## 6 | Outlook

Future studies may focus on monitoring ecological dynamics and community impact of *C. riisei* populations in the Colombian TEP and neighbouring marine regions. Additionally, I encourage conservation geneticists identifying selection signals of this invasive species for local adaptation by using genome-wide markers. Given the pivotal role that endosymbionts play in coral health status, future studies should make emphasis on elucidating their specific functions within the holobiont by examining, for instance, transcriptomic profiles. Moreover, I encourage coral microbiome researchers to explore how environmental disturbances affect microbiome disruptions triggering disease outbreaks. This would help to understand coral immune responses to predicted climate change conditions. Finally, I suggest that in future works comparative studies between coastal vs. off-shore coral species in order to better understand the anthropogenic-derived impacts and the long-term resilience capacity of these key benthic organisms under a globally changing scenario.

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# II PUBLICATIONS



# Taking a detour: invasion of an octocoral into the Tropical Eastern Pacific

Elena Quintanilla  · Thomas Wilke · Catalina Ramírez-Portilla ·  
Adriana Sarmiento · Juan A. Sánchez

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**Abstract** The tropical snowflake octocoral *Carijoa riisei*, which is thought to be native to the Indo-Pacific biogeographical region, has been increasingly reported from the Colombian Tropical Eastern Pacific (TEP) over the past two decades. Massive mortalities of native octocorals, particularly in *Pacificorgia* spp. and *Muricea* spp., were observed due to *C. riisei* overgrowth. However, the area of origin of TEP *C. riisei* remains unknown and its potential invasive status has not been addressed yet. We evaluated geographical scenarios for the colonization of the Colombian TEP by conducting phylogeographical analyses based on nuclear and mitochondrial sequences of 306 individual specimens from across the species' (native/non-native) range and applying

hypothesis-specific operational criteria. Additionally, we assessed whether *C. riisei* has to be considered an invasive species based on the previously proposed 'unified framework for biological invasions'. Our results showed relatively high genetic differentiation between Colombian TEP populations, on the one side, and Indo-Pacific and Hawaiian populations, on the other side. In contrast, we could not identify genetic differentiation and significant isolation by distance (IBD) between Colombian TEP and Tropical Atlantic populations. *C. riisei* might have been introduced from the Atlantic into the Colombian TEP, possibly via the Panama Canal. Based on the criteria of the 'unified framework for biological invasions', we also conclude that this octocoral constitutes an invasive species. Our study may serve as a basis for establishing strategies to protect native species from one of the very few invasive coral species worldwide.

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E. Quintanilla · C. Ramírez-Portilla ·  
A. Sarmiento · J. A. Sánchez  
Laboratorio de Biología Molecular Marina (BIOMMAR),  
Departamento de Ciencias Biológicas-Facultad de  
Ciencias, Universidad de los Andes, Carrera 1 #18A–10,  
Bogotá, Colombia

E. Quintanilla (✉) · T. Wilke · C. Ramírez-Portilla ·  
J. A. Sánchez  
Department of Animal Ecology and Systematics, Justus  
Liebig University Giessen, Heinrich-Buff-Ring 26-32  
IFZ, 35392 Giessen, Germany  
e-mail: e.quintanilla10@uniandes.edu.co

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origin · Operational criteria

## Introduction

Although coral reefs and coral-dominated subtidal ecosystems are highly productive and diverse, they are facing a severe worldwide decline (Moberg and Folke 1999; Ferreira et al. 2001; Pandolfi et al. 2003).

Marine invasive species are among the most serious threats to these ecosystems, in addition to disease outbreaks and other threats derived from natural and anthropogenic disturbances (Coles and Eldredge 2002; Goldberg and Wilkinson 2004; Hoegh-Guldberg et al. 2007). The increase of shipping activities is one important factor that has promoted the acceleration of marine invasion rates (Hulme 2009). In consequence, hundreds of marine invertebrate introductions have led to the transformation of marine communities worldwide (Carlton 1987; Carlton and Geller 1993; Carlton et al. 2011).

Among the wide diversity of marine invasive invertebrates, octocorals and corals in general, are not a group typically associated with invasiveness. The snowflake octocoral *Carijoa riisei* (Duchassaing and Michelotti 1860) likely originated in the Indo-Pacific (Concepcion et al. 2010), though it was originally described from the US Virgin Islands in the Tropical Atlantic. In recent years, the spread of this tropical stolonifer octocoral has been observed across the Pacific Ocean together with a considerable impact on the local biodiversity, because massive coral die-offs have been reported as a result of *C. riisei* overgrowth (Kahng and Grigg 2005; Raghunathan et al. 2013; Sánchez and Ballesteros 2014).

Following the first observation of *C. riisei* in coral reefs in the southern part of India (Padmakumar et al. 2011), it quickly spread along the Indian subcontinent (Raghunathan et al. 2013; Prabhukumar et al. 2015). In the Hawaiian Archipelago, *C. riisei* was first reported in 1972 (Evans et al. 1974; Thomas 1979) and its occurrence was linked to the death of more than 50% of black corals below 70 m depth (Grigg 2003; Kahng and Grigg 2005). Furthermore, in the Tropical Eastern Pacific (TEP), the spread of *C. riisei* is evident from records in Panamá and Colombia (Gomez et al. 2014; Sánchez and Ballesteros 2014), and recent observations in Ecuador (Baez Espinosa 2015; Zeas Valarezo 2015).

Over the last 20 years, *C. riisei* has severely harmed Colombian TEP benthic communities in subtidal rocky ecosystems that harbor highly diverse and endemic octocoral faunas (Sánchez et al. 2014). This species shows a competitive advantage over native octocorals by overgrowing and it has wiped out colonies of *Muricea* spp., *Leptogorgia* spp. and *Pacificorgia* sea fans at Malpelo Island, Gorgona Island and Cabo Corrientes (Sánchez et al. 2011;

Sánchez and Ballesteros 2014). For instance, owing to the adverse interaction with *C. riisei*, sea fan mortalities exceeding 87% were observed in Gorgona Island between 2011 and 2013 (Sánchez and Ballesteros 2014).

Features that potentially promote *C. riisei* spreading are a stolon morphology, a generalist filter feeding behavior, fast growth rates, few natural predators and the symbiotic interaction with the sponge *Desmaysamma anchorata*, which seems to confer *C. riisei* cytotoxic compounds (Calcinai et al. 2004; Wagner et al. 2009; Lira et al. 2009; Gomez et al. 2014; Sánchez and Ballesteros 2014). Indeed, its spread, competitive advantage of substrate colonization and aggressive overgrowth on octocorals in the Colombian TEP have been associated to its fast growth rate through stolons. This allows *C. riisei* forming dense and continued patches along the benthic substrate and walls in the TEP (Sánchez and Ballesteros 2014; pers. obs.).

Despite the evident impact of this species on native Colombian TEP octocorals, the geographical origin of these regional *C. riisei* populations remains unknown. Elucidating the area of origin of an introduced species, however, is crucial for understanding its success in the newly invaded environment (Ficetola et al. 2008; Xu et al. 2014). Moreover, the potential invasive status of *C. riisei* in the TEP has not been clearly assessed, making the establishment of effective management strategies challenging (sensu Stefaniak et al. 2012; Leydet and Hellberg 2015).

This study therefore aims at identifying the geographical origin of Colombian TEP *C. riisei* populations and assessing whether *C. riisei* has to be considered an invasive species in this region:

1. We used nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) from individual specimens occurring across the species' wide range together with a phylogeographical approach to assess the genetic diversity of *C. riisei* in the Colombian TEP, and to identify the genetic relatedness and genetic structure of Colombian TEP and extralimital populations.
2. We then used operational criteria to test three potential geographical scenarios for the colonization of the Colombian TEP based on the geographical regions of *C. riisei* defined by Concepcion et al. (2010): (I) from the Hawaiian

- Archipelago region, (II) from the Indo-Pacific region and (III) from the Tropical Atlantic region.
3. Finally, we empirically assessed the invasiveness of *C. riisei* in the Colombian TEP, applying the criteria of the ‘unified framework for biological invasions’ according to Blackburn et al. (2011).

## Materials and methods

### Study area

The target area of this study is the Colombian TEP, which is influenced by the Intertropical Convergence Zone. Strong seasonal upwelling causes the decrease of sea surface temperature (SST) at the beginning of the year (Vargas-Angel 1996; Zapata et al. 2010), and anomalous increases in SST occur during El Niño–Southern Oscillation (ENSO) events (Glynn and Colgan 1992; Zapata and Vargas-Ángel 2003). The study sites included mainland (Nuquí) and oceanic sites (Gorgona and Malpelo islands).

Nuquí is situated in the Chocó region at the northern Colombian Pacific coast. It hosts rocky reefs 100–500 m off the coast, with a predominance of octocorals (Sánchez and Ballesteros 2014). Due to high levels of precipitation, these reefs constantly receive siliciclastic sediments that decrease water visibility (Barrero-Canosa et al. 2013). Gorgona is a continental island located 35 km off the coast and part of the Eastern Tropical Pacific Marine Corridor together with Galápagos, Cocos, Coiba and Malpelo islands. Gorgona Island provides a significant fresh-water input, which decreases salinity and water clarity in its marine surroundings (Zapata and Vargas-Ángel 2003). Malpelo is an oceanic island situated about 500 km off the coast. Highly diverse rocky reefs dominate the subtidal zone (Birkeland et al. 1975) and gorgonians represent the most abundant coral group (Sánchez et al. 2011). Clear oceanic waters surround the island, supporting the occurrence of corals down to 30 m water depth (Zapata and Vargas-Ángel 2003).

### Sample collection

Samples of *C. riisei* were collected in the target area by SCUBA diving. Each of the three sites included several sampling localities. In all localities, *C. riisei*

was growing in continuous, big patches on benthic substrates. Moreover, we observed *C. riisei* overgrowing gorgonians such as *Leptogorgia* spp. and *Pacifigorgia* spp. The sampling was conducted along 15 m transects, ranging between 10 and 20 m water depth according to the orographic characteristics of each site. *Carijoa riisei* branches were taken for DNA work at least two meters apart along transects to avoid collecting individuals from the same colony.

We also sampled *C. riisei* colonies from coral reefs in the Colombian Caribbean. Samples were taken from separated colonies in Santa Marta (Punta Aguja) and Cartagena (Imelda). There, *C. riisei* was found to be highly patchy, confined to small colonies that formed parts of local coral reefs as reported by Sánchez (1994). However, there was no evidence that *C. riisei* harms corals or other benthic organisms.

After collection, all samples were preserved in 96% ethanol and kept at  $-80^{\circ}\text{C}$  until further analyses. Collections were possible with research permit No. 105 (2013), issued by Autoridad Nacional de Licencias Ambientales-ANLA, Ministerio de Ambiente y Desarrollo Sostenible, Colombia and Contrato de Acceso a Recursos Genéticos para Investigación Científica Sin Interés Comercial No. 106, 20 (2014) RGE0114.

### DNA isolation and sequencing

DNA from 73 *C. riisei* individuals was extracted using a CTAB protocol (Coffroth et al. 1992), and DNA concentrations were measured spectrophotometrically. The nuclear gene encoding the 54 kDa subunit of the signal recognition particle (SRP54) was amplified using the *Carijoa*-specific primers CrSRP54f (5'-CGAACTAAAATTAGAAGAAAACGAAG-3') and CrSRP54r (5'-TCATACATGTCTCTCAGCGTAAAC-3') (Concepcion et al. 2008). PCR reactions were carried out in 15  $\mu\text{l}$  total volumes containing final concentrations of 5 ng/ $\mu\text{l}$  DNA template, 0.2 mM dNTPs, 3.5 mM  $\text{MgCl}_2$ , 1 mg/ml Bovine Serum Albumin (BSA), 1.5 U hot-start Taq DNA polymerase, 0.4  $\mu\text{M}$  each primer and 1X of reaction buffer. The PCR cycling profile consisted of one cycle at  $95^{\circ}\text{C}$  for 10 min to activate the polymerase, followed by 39 cycles at  $95^{\circ}\text{C}$  for 30 s,  $58.1^{\circ}\text{C}$  for 30 s and  $72^{\circ}\text{C}$  for 30 s. The final extension period comprised  $72^{\circ}\text{C}$  for 5 min.

Additionally, encoding mitochondrial regions for the NADH dehydrogenase subunits 2 and 6 (ND2 and ND6) were amplified. Primers 16S-647F (5'-ACA CAGCTCGGTTTCTATCTACAA-3'), ND2-1418R (5'-ACATCGGGAGCCACATA-3') were used for ND2, and ND6-1487F (5'-TTTGGTTAGT TATTGCCTTT-3'), ND3-2126R (5'-CACAT TCA TAGACCGACTT-3') for ND6 (Sánchez et al. 2003). A total volume of 15 µl was used in PCR reactions containing the same final concentrations as described above, except for 1 U hot-start Taq DNA polymerase. The PCR profile was as follows: one cycle of 95 °C for 2 min to activate the hot-start DNA polymerase, 35 cycles of 94 °C for 1.5 min, 57 °C for 1.5 min, 72 °C for 1 min and a final extension of 72 °C for 5 min.

PCR success was checked using a 1% agarose gel. PCR purification was done by adding 0.5 µl of exonuclease (10 U) and 1 µl of alkaline phosphatase (1 U) to 5 µl of PCR product. The solutions were mixed and incubated in a thermal cycler 15 min at 37 °C followed by 15 min at 85 °C. All samples were subsequently sequenced (forward/reverse) using the BigDye Terminator Kit v3.1 (Life Technologies) with the capillary electrophoresis automated sequencer ABI-3500 (Life Technologies).

Consensus sequences were assembled using Geneious 9.0 (Biomatters Ltd.) and multiple sequence alignments were generated with AliView (Larsson 2014) using MUSCLE with default parameters (Edgar et al. 2004). We identified alleles in heterozygous individuals using PHASE v2.1 (Stephens et al. 2001, Stephens and Donnelly 2003) as implemented in DnaSP v5.0 (Librado and Rozas 2009). The mitochondrial regions (ND2-ND6) were concatenated according to Concepcion et al. (2008).

#### Global dataset and regions

In order to conduct phylogeographical analyses, our new sequences from Colombia (TEP and Caribbean) were complemented with published sequences (Concepcion et al. 2008, 2010), resulting in a total dataset of 306 individual specimens (see Online Resource 1 for GenBank accession numbers and Table 1 for geographic coordinates of study sites and respective biogeographical regions). We did not include mitochondrial haplotypes that contained IUPAC ambiguity

codes published by Concepcion et al. (2008), as they cannot be considered distinct haplotypes.

#### Genetic diversity and demographic indices

Allele/haplotype ( $h$ ) and nucleotide ( $\pi$ ) diversity indices were calculated for each region using Arlequin 3.5 (Excoffier et al. 2005). Demographic parameters were assessed in Arlequin by calculating Tajima's  $D$  (Tajima 1989) and Fu's  $F$  (Fu 1997) to test for selective neutrality and population equilibrium. Significantly negative values for both indices suggest departure from neutrality, likely indicating demographic expansion. The significance of Tajima's  $D$  and Fu's  $F$  ( $\alpha = 0.05$ ) was determined with 10,000 permutations. All analyses were performed separately for the nuDNA and mtDNA datasets.

#### Phylogeographical analyses

In order to reconstruct phylogeographic relationships, we constructed maximum parsimony networks from the nuclear and mitochondrial *C. riisei* datasets with TCS 1.21 (Clement et al. 2000), applying a default connection limit of 95%. Analyses of molecular variance (AMOVA) for both genetic datasets were carried out with Arlequin in order to understand how genetic variation is partitioned among populations within the Colombian TEP region ( $F_{ST}$ ). Additionally, AMOVA groupings (I) Colombian TEP and Hawaii regions, (II) Colombian TEP and Indo-Pacific regions and (III) Colombian TEP and Tropical Atlantic regions ( $F_{CT}$ ) were evaluated. Significance was assessed with 10,000 permutations. To estimate the genetic differentiation between populations within sites, pairwise differences based on allele/haplotype frequencies were calculated in Arlequin from both datasets (mtDNA and nuDNA), using the fixation index  $F_{ST}$ . Significance levels were assessed with 10,000 permutations under the null hypothesis of no genetic differentiation. Sequential Bonferroni corrections (Rice 1989) and the B-Y method for a false discovery rate (FDR) (Benjamini and Yekutieli 2001) were applied to adjust significance levels for multiple comparisons.

Additionally, we performed Principal coordinate analyses (PCoA) using the package 'ape' v4.1 (Paradis et al. 2004) for the R statistical environment v3.3.2 (R Core Team, 2016) to provide a spatial representation

**Table 1** Sample information, genetic diversity and demographic indices of *Carijoa riisei* populations

Regions	Sites	Geographic Coordinates	nuDNA				mtDNA								
			N	n	h (± SD)	π (± SD)	Fu's Fs (p value)	Tajima's D (p-value)	N	n	h (± SD)	π (± SD)	Fu's Fs (p-value)	Tajima's D (p-value)	
Colombian TEP	Nuquí	5°32'17"N 77°33'38"W	62	4						30	1				
	Gorgona	2°58'14"N 78°9'14"W	42	3	0.517 (0.042)	0.010 (0.007)	4.128 (0.937)	-0.323 (0.430)		21	1	0.000 (0.000)	0.000 (0.000)	NA	0.000 (1.000)
Tropical Atlantic	Malpelo	3°58'30"N 81°34'48"W	24	4						12	1				
	Colombian Caribbean	11°15'48"N 74°12'3"W	18	2						7	1				
	East Atlantic	0°43'13"N 7°6'11"E	12	5						6	2				
Florida		24°54'36"N 80°42'32"W	16	3	0.633 (0.050)	0.010 (0.007)	1.877 (0.830)	-0.755 (0.258)		23	1	0.331 (0.065)	0.0003 (0.0003)	0.041 (0.421)	-1.153 (0.111)
	Puerto Rico/US Virgin Islands	17°55'28"N 66°29'31"W	22	5						18	2				
Hawaii	Brazil	22°32'23"S 41°35'12"W	2	1						1	1				
	Panamá	8°51'42"N 81°10'23"W	-	-						5	1				
	Big Island	19°52'18"N 155°3'48"W	16	5						26	2				
	Kaua'i	21°51'16"N 159°29'16"W	16	5	0.757 (0.025)	0.015 (0.009)	3.154 (0.903)	1.484 (0.930)		10	2	0.327 (0.056)	0.001 (0.0008)	4.184 (0.947)	0.139 (0.614)
	Maui	20°52'9"N 156°42'25"W	36	5						26	2				
Indo-Pacific	O'ahu	21°17'52"N 157°59'12"W	44	6						30	3				
	Australia	25°16'28"S 133°46'30"E	8	7						6	4				
	Indonesia	08°19'38"S 107°09'32"E	30	11						18	5				
	Mauritius	20°35'10"S 57°29'32"E	6	2						4	3				
	Palau	7°26'46"N 134°30'19"E	42	9	0.925 (0.011)	0.033 (0.018)	-5.240 (0.085)	-0.253 (0.474)		52	6	0.8462 (0.022)	0.002 (0.001)	-1.713 (0.290)	-0.711 (0.266)
Fiji/Tonga		18°40'30"S 178°59'8"E	6	3						3	3				
	Mariana/Caroline Islands	16°36'53"N 145°37'0"E	6	6						3	2				
	Papua New G./Salomon Islands	11° 4'2"S 155°15'2"E	6	4						1	1				

Sample size (N), unique alleles and haplotypes (n), gene diversity (h), nucleotide diversity (π), Fu's neutrality test (Fu's Fs) and Tajima's D

NA not applicable

of the pairwise  $F_{ST}$  values among the populations to evaluate the assignment of the geographical regions (Hawaii, Indo-Pacific, Tropical Atlantic and TEP).

In order to assess the potential effect of geographic distance in restricting dispersal of *C. riisei* populations, a stratified IBD model was implemented. Because the standard IBD model does not take into account hierarchical (geographic) structures and thus may introduce false-positive results (Meirmans 2012), a stratified Mantel test was applied to evaluate the correlation between matrices (log-transformed and non-transformed) of pairwise  $F_{ST}$  values and geographic distances (km) using the R package ‘vegan’ v2.4 (Oksanen et al. 2017). Nuclear and mitochondrial datasets were analyzed separately to evaluate congruence using 30,000 randomizations, and significance levels for multiple comparisons were adjusted by applying the B-Y method FDR (Benjamini and Yekutieli 2001). Different subsets were analyzed: all regions, Indo-Pacific, Hawaii, Tropical Atlantic and TEP. In addition, the stratified IBD was assessed between the TEP region and the remaining regions in order to test the explanatory power of geographic distance in the observed genetic differentiation.

The minimum number of alleles/haplotypes per site included in the analyses was five. Accordingly, we excluded the populations from Brazil, Papua New Guinea/Salomon islands, Fiji, Mariana/Caroline islands and Mauritius from the mtDNA dataset, and Brazil from the nuDNA dataset for the AMOVA, the pairwise  $F_{ST}$  differences, and the IBD analyses.

#### Operational criteria for geographical scenarios

In order to evaluate the three potential geographical scenarios for the colonization of the Colombian TEP, we used a set of operational criteria involving shared alleles and haplotypes, genetic diversity, phylogeographical relationships and isolation by distance. This information was obtained from the results of the present study. Additionally, we included a set of operational criteria from published references comprising first presence reported (Duchassaing and Michelotti 1860; Evans et al. 1974; Thomas 1979), oceanic currents (Dana 1975; Cortes 1997; Wood et al. 2016), geographic barriers (Ekman 1953; Budd et al. 1996; Glynn and Ault 2000; Robertson et al. 2004; Baums et al. 2012) and potential shipping routes (Carlton 1987; Cohen 2006, Concepcion et al. 2010).

We assessed and summed up the qualitative levels (high, medium and low) of these criteria supporting each geographical scenario.

#### Assessment of the potential invasiveness of *C. riisei*

The invasiveness of *C. riisei* in the Colombian TEP was assessed by applying the criteria of ‘the unified framework for biological invasions’ suggested by Blackburn et al. (2011). This approach considers that the invasion process can be divided into several stages. Barriers separate these stages that populations need to overcome in order to pass into the next stage. Consequently, the specific terminology, categorization and management interventions used depend on the stage reached in the invasion process (Blackburn et al. 2011).

## Results

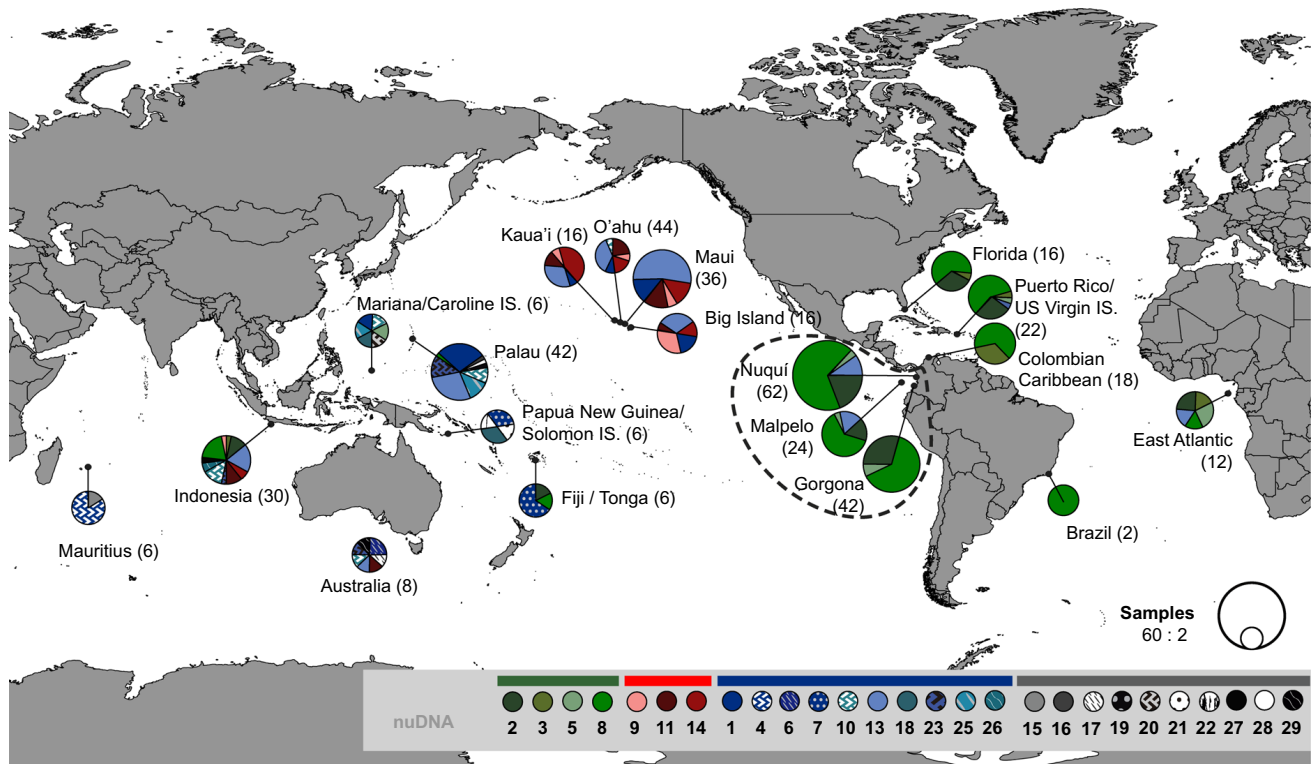
#### Allele and haplotype identification

A trimmed alignment of 129 bp was obtained for the nuclear dataset (SRP54). Five alleles were identified in the Colombian samples, namely A2, A3, A8, A5 and A13 (Fig. 1). In the three TEP sites, A8 was found to be the most abundant allele (65.63%), followed by A2 (21.88%), A13 (7.81%) and A5 (4.69%). A13 was present in the Malpelo and Nuquí samples, but not in Gorgona. Colombian Caribbean samples mainly exhibited A8 (66.67%) but also A3 (33.33%), which is absent in the Colombian TEP but it is shared with other sites in the Tropical Atlantic (Online Resource 1 and Fig. 1).

For the concatenated mitochondrial dataset, we obtained a trimmed alignment of 1154 bp and only one haplotype (H18) was identified in all Colombian samples (Fig. 2).

#### Genetic diversity and demographic indices

Allele/haplotype diversities ( $h$ ) in the Colombian TEP region were 0.517 ( $\pm 0.042$  SD) for the nuDNA dataset and 0 for the mtDNA dataset (see Table 1). For the nuDNA dataset, populations from the Colombian TEP were more similar to those from the Tropical Atlantic than to Hawaiian and Indo-Pacific sites. For all



**Fig. 1** Map of allele (SRP54) frequencies of worldwide *Carijoa riisei* populations (numbers in parenthesis indicate the number of samples per site). Alleles are colour-coded according

regions, our neutrality tests resulted in non-significant values, thus rejecting assumptions of population size changes.

#### Phylogeographical analyses

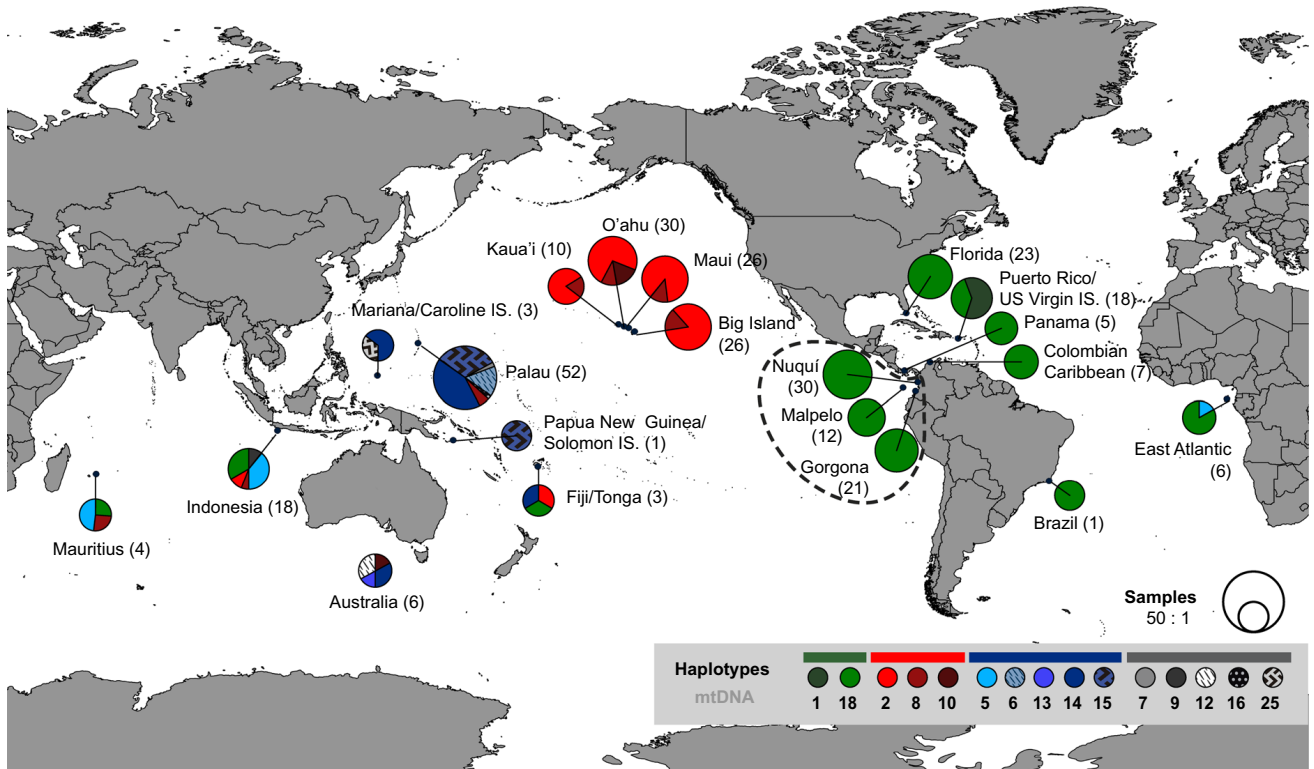
The TCS analysis for the nuDNA dataset revealed two unconnected networks (Fig. 3a). The first consisted of all alleles observed in Hawaiian and Indo-Pacific sites. Additionally, this network contained A13, the only allele shared among the four regions. The second network included the alleles found in the Colombian TEP, the Tropical Atlantic and the Indo-Pacific. A8 and A2 were the most abundant alleles found in the Colombian TEP and the Tropical Atlantic. They formed a cluster together with A3, indicating a close relationship between these alleles. All alleles found in the Colombian TEP were shared with individuals from the Tropical Atlantic region but also with some Indo-Pacific sites (e.g., with Indonesia). In contrast, the TCS analysis conducted with the mtDNA data yielded a single network. There, the only haplotype found in the TEP (H18) is mainly shared with populations from the Tropical Atlantic. It is also shared with Indonesia and,

to the region where they have the highest abundance. A dashed line highlights Colombian TEP populations. For a list of alleles (*bold numbers*) see Online Resource 1

to a lesser extent, with other Indo-Pacific sites (Fig. 3b).

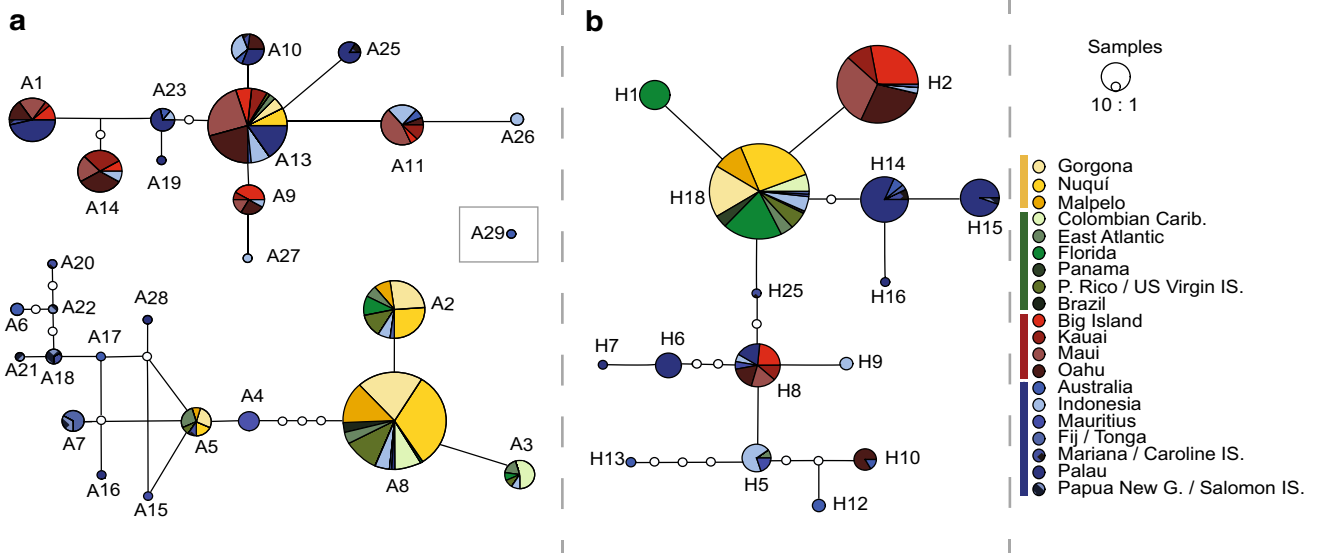
The AMOVA did not reveal significant genetic structure across Colombian TEP sites, not even for the nuDNA dataset ( $F_{ST} = 0.017$ ,  $P > 0.05$ ; Table 2). Between the Colombian TEP and the Tropical Atlantic, no significant genetic variance was found for the nuDNA data ( $F_{CT} = 0.007$ ,  $P > 0.05$ ) or for the mtDNA data ( $F_{CT} = 0.01$ ,  $P > 0.05$ ). Colombian TEP and Hawaiian populations showed the highest differentiation variation, i.e., 34.23% ( $F_{CT} = 0.342$ ,  $P < 0.05$ ) and 80.95% ( $F_{CT} = 0.809$ ,  $P < 0.05$ ) for nuclear and mitochondrial data, respectively. The AMOVA also revealed significant genetic variance between the Colombian TEP and Indo-Pacific regions for the nuDNA data (20.32%;  $F_{CT} = 0.203$ ,  $P < 0.05$ ) (Table 2; Fig. 4).

Pairwise  $F_{ST}$  values showed similar patterns of genetic differentiation observed in the AMOVA. Populations from the Colombian TEP did not show significant genetic differentiation compared to those from the Tropical Atlantic, except for Nuquí versus East Atlantic for nuDNA, and Nuquí, Gorgona and Malpelo versus Puerto Rico for mtDNA data. Genetic



**Fig. 2** Map of haplotype (ND2-ND6) frequencies of worldwide *Carijoa riisei* populations (numbers in parenthesis indicate the number of samples per site). Haplotypes are colour-coded

according to the region where they have the highest abundance. A dashed line highlights Colombian TEP populations. For a list of haplotypes (**bold numbers**) see Online Resource 1



**Fig. 3** Statistical parsimony network of alleles (**a**; SRP54) and haplotypes (**b**; ND2-ND6) of *Carijoa riisei* (95% connection limit). Colours represent sampling sites. Colour ranges refer to biogeographical regions: yellow (TEP), green (Tropical

Atlantic), red (Hawaii) and blue (Indo-Pacific). Areas of circles are proportional to the number of individuals sharing the respective haplotypes or alleles. White dots indicate missing haplotypes or alleles

differentiation was significant between all Colombian TEP and Hawaiian populations and between Colombian TEP and Indo-Pacific populations for both

nuDNA and mtDNA datasets (Online Resource 2). Additionally, the PCoA based on pairwise  $F_{ST}$  values supported the regional grouping of populations

**Table 2** Results of the analysis of molecular variance (AMOVA) for the nuDNA and mtDNA datasets for *Carijoa riisei*

Source of variation	nuDNA		mtDNA	
	Percentage of variation (variance)	<i>F</i> value	Percentage of variation (variance)	<i>F</i> value
<i>Malpelo vs Nuquí vs Gorgona</i>				
Among populations	1.69 (0.01134 Va)	0.017 $F_{ST}$	NA	NA
Within populations	98.31 (0.66085 Vb)		NA	
Total	(0.6722)		NA	
<i>TEP vs Tropical Atlantic</i>				
Among groups	0.71 (0.002 Va)	0.007 $F_{CT}$	1.08 (0.001 Va)	0.01 $F_{CT}$
Among populations within groups	4.18 (0.012 Vb)	0.042 $F_{SC}^*$	53.67 (0.053 Vb)	0.547 $F_{SC}^*$
Within populations	95.12 (0.272 Vc)	0.048 $F_{ST}^*$	45.25 (0.044 Vc)	0.547 $F_{ST}^*$
Total	(0.286)		0.009	
<i>TEP vs Hawaii</i>				
Among groups	34.23 (0.164 Va)	0.342 $F_{CT}^*$	80.95 (0.417 Va)	0.809 $F_{CT}^*$
Among populations within groups	0.87 (0.004 Vb)	0.013 $F_{SC}$	0 (0 Vb)	0 $F_{SC}$
Within populations	64.91 (0.312 Vc)	0.351 $F_{ST}^*$	19.10 (0.098 Vc)	0.809 $F_{ST}^*$
Total	(0.480)		0.515	
<i>TEP vs Indo-Pacific</i>				
Among groups	20.32 (0.092 Va)	0.203 $F_{CT}^*$	47.77 (0.229 Va)	0.477 $F_{CT}$
Among populations within groups	7.51 (0.034 Vb)	0.094 $F_{SC}^*$	10.88 (0.052 Vb)	0.208 $F_{SC}^*$
Within populations	72.16 (0.327 Vc)	0.278 $F_{ST}^*$	41.35 (0.198 Vc)	0.586 $F_{ST}^*$
Total	(0.453)		0.480	

NA not applicable

\*  $P < 0.05$ 

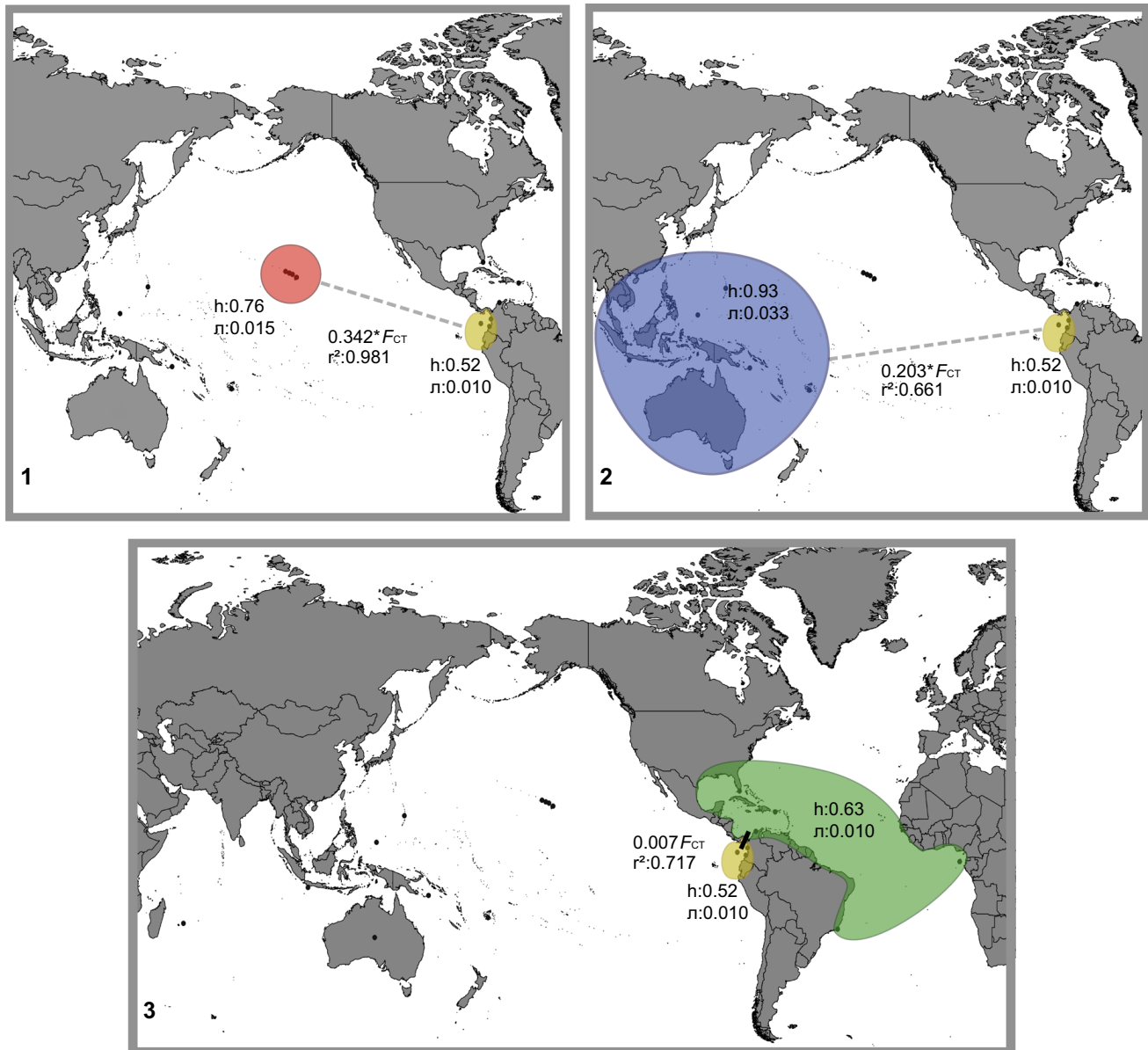
(Hawaii, Indo-Pacific, Tropical Atlantic and TEP) for both nuclear and mitochondrial data (Online Resource 3). This is consistent with the marine bioregionalization published by Spalding et al. (2007), which considers these geographical regions as being different biogeographic marine realms.

The IBD analyses indicated that log-transformed and non-transformed matrices yield similar results (Online Resource 4). According to the non-transformed matrix, non-significant stratified IBD was detected, for the mtDNA data, within the overall dataset ( $r^2 = 0.46$ ,  $P > 0.009$ ,  $n = 105$ ) as well as between Colombian TEP versus Tropical Atlantic ( $r^2 = 0.176$ ,  $P > 0.012$ ,  $n = 28$ ), Colombian TEP versus Hawaiian populations ( $r^2 = 0.993$ ,  $P > 0.013$ ,  $n = 21$ ) and Colombian TEP versus Indo-Pacific populations ( $r^2 = 0.849$ ,  $P > 0.013$ ,  $n = 21$ ). Similar results were obtained by the IBD analysis of the nuDNA dataset, revealing that the overall dataset ( $r^2 = 0.499$ ,  $P > 0.0089$ ,  $n = 153$ ) as well as

Colombian TEP versus Tropical Atlantic ( $r^2 = 0.717$ ,  $P > 0.013$ ,  $n = 21$ ), Colombian TEP versus Hawaiian populations ( $r^2 = 0.98$ ,  $P > 0.013$ ,  $n = 21$ ) and Colombian TEP versus Indo-Pacific populations ( $r^2 = 0.661$ ,  $P > 0.011$ ,  $n = 45$ ) showed non-significant isolation by distance (see also Fig. 4).

#### Operational criteria for geographical scenarios

Applying the operational criteria for inferring the geographical origin of Colombian TEP populations of *C. riisei* (see Table 3), scenarios 1 (Hawaii to Colombian TEP) and 2 (Indo-Pacific to Colombian TEP) have to be rejected. Instead, the most likely geographical scenario for the colonization of the Colombian TEP is scenario 3 (i.e., Tropical Atlantic to Colombian TEP). In fact, all criteria, except for oceanic currents (which is not applicable), supported the latter assumption (Table 3).



**Fig. 4** Geographical scenarios and genetic indices for the colonization of *Carijoa riisei* into the TEP: 1. From Hawaii to TEP, 2. From Indo-Pacific to TEP, 3. From Tropical Atlantic to TEP. Genetic diversity as well as AMOVA and IBD results are

derived from nuDNA. Allele diversity ( $h$ ), nucleotide diversity ( $\pi$ ), AMOVA F-statistics and correlation between genetic and geographic distances ( $r^2$ ). \* $P < 0.05$

#### Assessment of the invasiveness of *C. riisei* in the Colombian TEP

The application of ‘the unified framework for biological invasions’ introduced by (Blackburn et al. 2011) puts *C. riisei* populations from the Colombian TEP into the category E (fully invasive species). Accordingly, *C. riisei* has left the ‘transport’, ‘introduction’ and ‘establishment’ stages, and is now in the ‘spread’

stage, making it a ‘fully invasive species’ in this region.

#### Discussion

The major goals of the present study were to identify the geographical origin of *Carijoa riisei* in the Colombian TEP and to clarify its invasive status.

**Table 3** Assessment of the operational criteria for three potential geographical scenarios for the colonization of the Colombian TEP by *Carijoa riisei*

Geographical scenarios	Haplotypes and alleles shared	Genetic diversity	Genetic structure	Phylogeographical relationships	Isolation by distance	First presence report	Oceanic currents	Geographic barriers	Marine shipping routes
1. From Hawaii to TEP	-	-	-	-	+	+	-	-	-
2. From Indo-Pacific to TEP	+	-	O	+	+	NA	+	-	-
3. From Tropical Atlantic to TEP	+	+	+	+	+	+	NA	+	+

+, high support; O, medium support; -, low or no support; NA, not applicable

Our genetic analyses together with an assessment of operational criteria for three biogeographical scenarios (i.e., from the Hawaiian Archipelago to the Colombian TEP, from the Indo-Pacific to the Colombian TEP and from the Tropical Atlantic region to the Colombian TEP) revealed the Tropical Atlantic to be the likely source region for *C. riisei* populations in the Colombian TEP, based on the current data. Furthermore, applying the ‘unified framework for biological invasions’, we confirmed the invasive status of TEP populations.

#### Geographical origin of *C. riisei* in the TEP

The first hypothesis (i.e., from the Hawaiian Archipelago to the Colombian TEP) has to be rejected as we clearly evidenced a lack of close genetic relationships between *C. riisei* populations from Hawaii and the Colombian TEP. Moreover, the lack of shared alleles and haplotypes suggest that the respected populations have been genetically isolated for some time. According to the IBD model, geographic distance does not explain this genetic isolation. Since the model considers linear distances and assume landscape homogeneity (Noguerales et al. 2016), some geographic barrier might explain the observed pattern. In fact, the TEP is considered the most isolated tropical marine bioregion in the world due to the Eastern Pacific Barrier (EPB) that separates this region from the central and western Pacific (Robertson et al. 2004). EPB is the largest deep-water expanse in the world, representing a strong barrier for organisms with larval dispersal, such as corals (Ekman 1953; Baums et al. 2012).

Applying a 95% connection limit, the TCS network analysis of the nuDNA dataset (Fig. 3a) resulted in unconnected networks. Some authors suggests that such a pattern potentially indicates the existence of cryptic species (Hart and Sunday 2007; Chen et al. 2010; De Barro and Ahmed 2011; Neusser et al. 2011). This premise also receives support from biological data. Specimens from Colombia (both TEP and Caribbean), for example, lack the bioluminescence (Juan A. Sánchez, unpublished data) that has been reported for *C. riisei* in Hawaii (Kahng 2005).

Regarding the second hypothesis of geographical origin (i.e., from the Indo-Pacific to the Colombian TEP), significant genetic differentiation was observed between *C. riisei* from the Colombian TEP and the

Indo-Pacific regions, although some of the respective populations share haplotypes and alleles. The latter may explain why the respective genetic structure was not as high as between Hawaii and Colombian TEP. In theory, the presence of the North Equatorial Counter-current (NECC), which flows from west to east, could have supported coral establishment in the TEP from the western Pacific (Dana 1975; Cortes 1997). However, the existence of the EPB separates and isolates eastern Pacific corals from other Pacific regions (Combosch et al. 2008; Baums et al. 2012) even during ENSO events, when the flow of the NECC is enhanced (Wood et al. 2016). Therefore, it is very unlikely to expect natural dispersion of *C. riisei* from the Indo-Pacific to the TEP. Moreover, marine shipping activities between the TEP and other Pacific regions have been minor in the last century (Carlton 1987; Concepcion et al. 2010). Therefore, the TEP has not been considered a major receiver area for introduced species by trans-Pacific shipping (Carlton 1987).

As for the third hypothesis of geographical origin (i.e., from the Tropical Atlantic to the Colombian TEP), *C. riisei* populations from the Colombian TEP are genetically very similar to populations from the Tropical Atlantic region. However, taking the closure of the Central American corridor into account and the subsequent independent evolution of transisthmian faunas during the Plio-Pleistocene (Budd et al. 1996; Glynn and Ault 2000), we would expect strong genetic differentiation between *C. riisei* populations from these regions if the introduction were ancient (sensu Grapputo et al. 2005; Xavier et al. 2009).

Thus, the observed absence of genetic differences suggests a contemporary and possibly human-mediated exchange between these two regions. In fact, the present results strongly support the hypothesis of a recent introduction of *C. riisei* from the Atlantic into the Colombian TEP, most likely through the Panama Canal. Since its opening in 1914, the Panama Canal has been enabling species introductions commonly associated with vessel-borne transport either as hull fouling or in ballast tanks (Cohen 2006). This may also apply to the introduction of *C. riisei* into the TEP from the Tropical Atlantic, as vessel-borne transport is supported by the fouling lifestyle of the species. Moreover, ramets and stolons could have survived in ballast waters as *C. riisei* is commonly associated with commercial harbours (Coles and Eldredge 2002).

In conclusion, the hypotheses of an introduction of Colombian TEP *C. riisei* from the Indo-Pacific, the native range of the species, or the Hawaiian Islands are rejected. Instead, individuals in the Colombian TEP probably originated from the Tropical Atlantic. Thus, *C. riisei* likely took a large detour into the TEP and two main dispersal events might be evidenced—an old dispersion from the Indo-Pacific, possibly Indonesia, into the Tropical Atlantic and a secondary spread from there into the TEP.

#### Invasive status of *C. riisei* in the TEP

According to the most likely geographical scenario of origin inferred, *C. riisei* was unintentionally introduced into the Colombian TEP from the Tropical Atlantic. Based on the ‘unified framework for biological invasions’ proposed by Blackburn et al. (2011), the first (geographical) barrier (in this case the Isthmus of Panama) has been overcome. This species also has succeeded in surviving and reproducing in the new environment since Colombian TEP *C. riisei* shows competitive traits that allow for a rapid expansion along the substrate through stolons, and new colonies grow after periodically senescence of old ramets (Sánchez and Ballesteros 2014; pers. obs.). In addition, there are traits that might have favoured the spread of the species into other Colombian TEP locations such as vegetative reproduction, continuous gamete release, fast growth rate, generalist feeding and a high dispersal capacity due to its fouling biology and ramet fragmentation (Thomas 1979; Lira et al. 2009; Gomes et al. 2012; Barbosa et al. 2014; Sánchez and Ballesteros 2014). Moreover, the genetic similarity found among Nuquí, Gorgona and Malpelo populations might suggest the dominance of asexual propagation of *C. riisei* in the Colombian TEP (sensu Roman and Darling 2007). Clonal reproduction has been frequently associated with invasive species, as it may stimulate rapid spread and higher invasion success in novel environments (Sakai et al. 2001; Okada et al. 2009; Zhang et al. 2010). This potential dominance of asexual propagation of *C. riisei* might explain its high ubiquity in the Colombian TEP. Additionally, *C. riisei* shows tolerance to the environmental heterogeneity observed among the three sites in the Colombian TEP (Zapata and Vargas-Ángel 2003; Barrero-Canosa et al. 2013),

which might also contribute to its invasive success (Madariaga et al. 2014).

In conclusion, we found that *C. riisei* represents an invasive species in the Colombian TEP. Accordingly, individuals of this species are able to survive, reproduce and disperse in a spectrum of habitats in the newly invaded area (Blackburn et al. 2011). Considering its threat to native benthic communities, mainly gorgonians such as *Pacificorgia* spp., the classification of being an invasive species is crucial to address management strategies in order to protect native communities (Sakai et al. 2001; Blackburn et al. 2011). Finally, this study highlights the invasive capacity of a group of organisms rarely associated with invasiveness–octocorals.

## Outlook

As our study highlights the high dispersal potential of *C. riisei* via oceanic shipping routes and given that the species has recently been reported from Ecuador (Baez Espinosa 2015; Zeas Valarezo 2015), future studies may focus on the ecological dynamics and community impact of *C. riisei* in the Colombian TEP and neighbouring. As the invasive status of the species has been confirmed, we also encourage broader population studies using genome-wide markers. This will allow to study population expansions in a higher resolution and to identify selection signals for local adaptation. The latter may help depicting the traits promoting the species' persistence and invasiveness. Finally, our data point to the existence of a cryptic *Carijoa* species complex, which might warrant further attention.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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# SCIENTIFIC REPORTS

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## Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease

Elena Quintanilla<sup>1,2</sup>, Catalina Ramírez-Portilla<sup>1,3</sup>, Boahemaa Adu-Oppong<sup>4</sup>, Gretchen Walljasper<sup>4</sup>, Stefanie P. Glaeser<sup>5</sup>, Thomas Wilke<sup>1</sup>, Alejandro Reyes Muñoz<sup>3,4,6</sup> & Juan A. Sánchez<sup>1,2</sup>

Microbiome disruptions triggering disease outbreaks are increasingly threatening corals worldwide. In the Tropical Eastern Pacific, a necrotic-patch disease affecting gorgonian corals (sea fans, *Pacifigorgia* spp.) has been observed in recent years. However, the composition of the microbiome and its disease-related disruptions remain unknown in these gorgonian corals. Therefore, we analysed 16S rRNA gene amplicons from tissues of healthy colonies (n = 19) and from symptomatic-asymptomatic tissues of diseased colonies (n = 19) of *Pacifigorgia cairnsi* (Gorgoniidae: Octocorallia) in order to test for disease-related changes in the bacterial microbiome. We found that potential endosymbionts (mostly *Endozoicomonas* spp.) dominate the core microbiome in healthy colonies. Moreover, healthy tissues differed in community composition and functional profile from those of the symptomatic tissues but did not show differences to asymptomatic tissues of the diseased colonies. A more diverse set of bacteria was observed in symptomatic tissues, together with the decline in abundance of the potential endosymbionts from the healthy core microbiome. Furthermore, according to a comparative taxonomy-based functional profiling, these symptomatic tissues were characterized by the increase in heterotrophic, ammonia oxidizer and dehalogenating bacteria and by the depletion of nitrite and sulphate reducers. Overall, our results suggest that the bacterial microbiome associated with the disease behaves opportunistically and is likely in a state of microbial dysbiosis. We also conclude that the confinement of the disease-related consortium to symptomatic tissues may facilitate colony recovery.

Corals worldwide are severely threatened by the increased incidence of diseases over recent decades<sup>1,2</sup>. As an important modifying factor of reef systems, diseases may reduce coral cover, decrease diversity and affect coral life-history traits<sup>3</sup>. As a consequence, changes in coral communities and massive coral die-offs have occurred globally, challenging the resilience of coral ecosystems<sup>4-6</sup>.

Corals are meta-organisms comprising the coral animal itself and a microbial community (the 'microbiome') such as protists, bacteria, archaea, viruses and fungi, which collectively constitute the coral holobiont<sup>7,8</sup>. The microbiome confers benefits to the holobiont including nutrient acquisition and disease resistance through the production of antibiotic compounds<sup>9,10</sup>. Indeed, metabolic complementation (i.e. production of metabolites by each partner that are crucial for the survival of other's holobiont partners) highlights reciprocal relationships existing between microbiome and coral host<sup>11</sup>. Identifying the stable and consistent components occurring within

<sup>1</sup>Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Giessen, Germany. <sup>2</sup>Department of Biological Sciences, Laboratory of Marine Molecular Biology (BIOMMAR), Universidad de los Andes, Bogotá, Colombia. <sup>3</sup>Department of Biological Sciences, Research Group on Computational Biology and Microbial Ecology (BCEM), Universidad de los Andes, Bogotá, Colombia. <sup>4</sup>Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St Louis, Missouri, USA. <sup>5</sup>Institute of Applied Microbiology, Justus Liebig University Giessen, Giessen, Germany. <sup>6</sup>Max Planck Tandem Group in Computational Biology, Universidad de los Andes, Bogotá, Colombia. Correspondence and requests for materials should be addressed to E.Q. (email: [e.quintanilla10@uniandes.edu.co](mailto:e.quintanilla10@uniandes.edu.co))

the coral microbiome, the ‘core microbiome’, provides insights into the functions that these assemblages offer to the holobiont and enables the understanding of critical microbiome changes associated with disturbances<sup>12,13</sup>. Such changes in the composition and function of the microbiome affect holobiont fitness and disease susceptibility, potentially leading to disease outbreaks<sup>1,14</sup>.

Understanding coral disease causation remains challenging as complex interactions exist between causative agents, environment and host<sup>1,15</sup>. External factors such as infectious or opportunistic pathogens and environmental stressors may affect a compromised coral holobiont, potentially triggering diseases<sup>15</sup>. In particular, complex interactions have been identified within coral microbial communities driving disease processes<sup>16</sup>. The latter may explain why the etiology of most coral diseases cannot be attributed to single causative agents and hence, terms such as ‘opportunistic pathogens’, ‘polymicrobial diseases’, ‘pathobiome’ and ‘dysbiosis’ (i.e. microbial imbalance) have been associated with coral diseases<sup>16–18</sup>.

Healthy and disease-related coral microbiomes are poorly understood at an intra-colony level<sup>19</sup>. Different bacterial assemblages may occur within diseased colonies, revealing intermediate health states<sup>20</sup>. Therefore, studies of tissues differentially affected within diseased colonies may promote a better understanding of the spatial effects of the disease-associated microbiome and disease progression, which in turn may allow for the identification of different coral health states.

Die-offs related to disease outbreaks have been observed in the gorgonian sea fan *Pacifigorgia cairnsi* (Gorgoniidae: Octocorallia)<sup>21,22</sup>. This species is native to the TEP and dominates the infralittoral seascape at Malpelo Island (Colombian TEP), forming dense aggregations on rocky outcrops and walls that occur up to 30 m water depth<sup>21,23</sup>. In this study we tested for disease-related changes in the bacterial microbiome of *P. cairnsi* sea fans by generating 16S rRNA gene amplicons from healthy ( $n = 19$ ) and diseased ( $n = 19$ ) colonies. In order to achieve this goal, we identified the bacterial community composition of the core microbiome associated with the healthy state as a baseline for our subsequent analyses. We then compared tissues from healthy colonies and tissues affected by the disease to assess disease-related shifts in bacterial community compositions and functional profiles. Finally, we tested for disease-related shifts occurring within diseased colonies (i.e. between symptomatic and asymptomatic tissues) in order to identify the relationships between the bacterial microbiome and gorgonian health states at an intra-colony level.

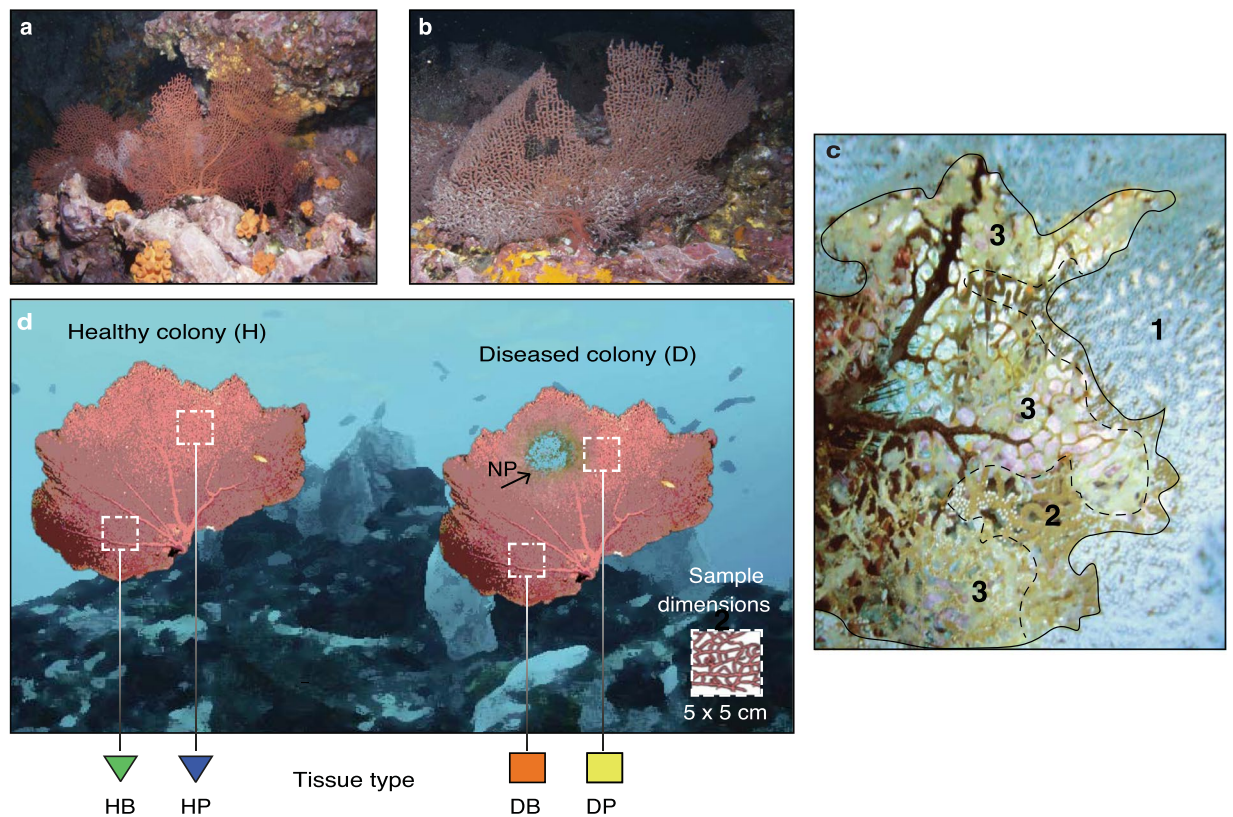
## Results

The total count of quality-filtered reads obtained was 3,726,194, with a minimum of 13,117 and a maximum of 103,590 of counts per sample. We decided to normalize at 29,000 reads per sample in order to remove a minimum number of samples from the study and considering the fact that rarefaction curves reached near saturation well before 29,000 reads for the three alpha diversity metrics evaluated (Supplementary Fig. S1). This trend was observed in 73 of the total 80 samples. The alpha diversity metrics of samples from each tissue type at a subsampling of 29,000 reads per sample are summarized in Supplementary Table S1. See Fig. 1 for sampling design.

**Core bacterial microbiome composition of healthy colonies.** Bacterial community compositions associated with tissue samples from healthy colonies (HB and HP) did not differ. Non-significant differences were observed in species richness Chao 1, in number of OTUs (operational taxonomic units) and in Shannon diversity between HB and HP samples ( $n = 37$ ) (Supplementary Fig. S1). Beta diversity analysis, visualized using the first two coordinates of the PCoA plot did not show clusters of samples corresponding to types of tissues (Fig. 2a). Moreover, non-significant differences in community compositions and in the homogeneity of multivariate dispersions were inferred between these two types of tissues (PERMANOVA,  $P > 0.05$ , Permutational Analyses of Multivariate Dispersions, PERMDISP,  $P > 0.05$  Table 1a). Accordingly, the Similarity Percentage (SIMPER) analysis revealed that the same set of OTUs contributed to the homogeneity within each type of tissue (Supplementary Table S2).

Given the similarity of the alpha diversity metrics and community compositions of healthy colonies, the core bacterial microbiome of *P. cairnsi* was defined as all OTUs present in all healthy samples. Eighteen OTUs accounted for the cumulative 95% of total abundance. From those, seven OTUs comprised the core microbiome, with *Mycoplasma* and *Endozoicomonas* being the prevalent components (Fig. 3). *Mycoplasma* (OTU1) was the most abundant core member ( $44.87 \pm 13.43$  average relative abundance given in %), while *Endozoicomonas* was the most diverse bacterial group among the core microbiome with four OTUs: OTU3 ( $19.69 \pm 10.10$ ), OTU2 ( $17.94 \pm 9.72$ ), OTU755 ( $8 \pm 3.80$ ) and OTU175 ( $0.44 \pm 0.20$ ). OTUs assigned to the family Oceanospirillaceae (OTU6,  $2.12 \pm 1.75$ ) and unclassified bacteria (OTU8,  $1.08 \pm 0.71$ ) also comprised the core bacterial microbiome. Additionally, some OTUs were present in the majority of the samples (>86%), displaying low relative abundances: Bacteroidales (OTU4,  $1.22 \pm 1.64$ ), Alteromonadales (OTU7,  $0.67 \pm 1.30$ ), *Synechococcus* (OTU22,  $0.34 \pm 0.95$ ), Spirochaetes (OTU23,  $0.18 \pm 0.26$ ) and unclassified bacteria (OTU13,  $0.68 \pm 1.27$ ) (Fig. 3).

**Comparison of bacterial community compositions and functional profiles between tissue samples from healthy and diseased colonies.** Symptomatic samples (DP) harboured bacterial assemblages different from those present in the asymptomatic samples (HB, HP and DB). The number of observed OTUs, bacterial richness and Shannon diversity within samples were significantly higher in DP compared to HB, HP and DB samples (Supplementary Fig. S1). In the ordination analysis, DP samples clustered apart from HB, HP and DB samples, suggesting different bacterial community composition (Fig. 2b). Moreover, PERMANOVA analyses revealed significant differences in community compositions in pair-wise comparisons between DP samples and samples from each type of the asymptomatic tissues ( $P < 0.0083$ , Table 1b). The homogeneity observed in multivariate dispersions among the four sampling groups (PERMDISP,  $P > 0.0083$ , Table 1b) confirmed that the significant differences obtained with PERMANOVA were due to differences in bacterial community compositions<sup>24</sup>.

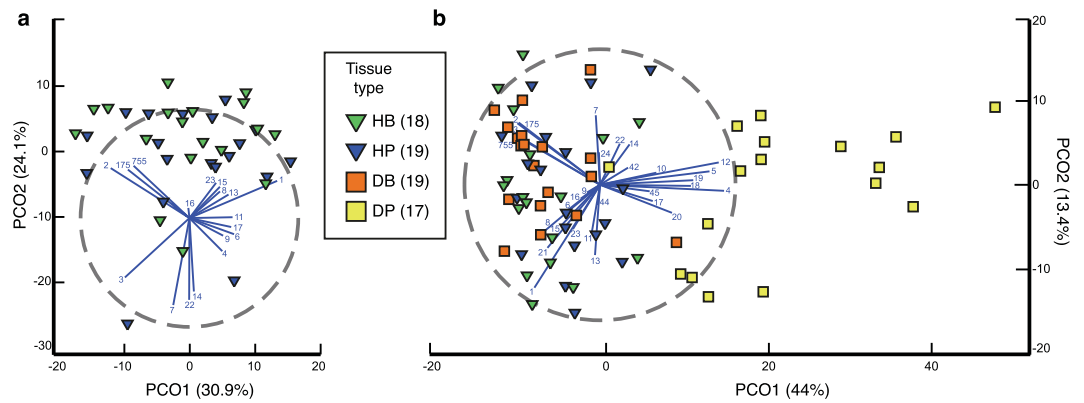


**Figure 1.** Sampling design of healthy and diseased colonies of *Pacifigorgia cairnsi*. (a) Healthy colony and (b) diseased colony affected by NPD at Malpelo Island. (c) Detail of *P. cairnsi* lesion showing tissue with extended polyps (1), tissue with retracted polyps (2) and necrotic areas lacking polyps and coenenchyme (3). (d) Diagram showing sampling design. Samples (5 × 5 cm) were taken from peripheral tissue of healthy colonies (HP), basal tissue of healthy colonies (HB), peripheral (symptomatic) tissue of diseased colonies (DP) and basal (but asymptomatic) tissue of diseased colonies (DB). Necrotic patches (NP) were not included in DP samples.

Considering all samples ( $n = 73$ ), twenty-nine OTUs accounted for the cumulative 95% of total abundance. Eleven from these twenty-nine OTUs contributed to a greater extent to the differentiation between symptomatic (DP) and asymptomatic samples (DB, HP and HB), exhibiting two opposite trends: enrichment and depletion (Fig. 4). In terms of enrichment, a member from the order Bacteroidales (OTU4) was more abundant in DP samples than in the rest of asymptomatic samples and contributed the highest proportion to this differentiation (about 20%) (Fig. 4, Supplementary Table S3 and S4). In addition, OTUs from the genus *Aquimarina* (Flavobacteriaceae, OTU5) and the genus *Loktanelia* (Rhodobacteraceae, OTU12) also displayed higher abundances in DP samples, contributing over 10% and about 4% to the differentiation, respectively. Relative abundances of representatives from the family Flavobacteriaceae (OTU19), the genus *Polaribacter* (Flavobacteriaceae, OTU20) and the order Oceanospirillales (OTU18) increased in DP samples, but contributed to a lesser extent to the dissimilarity (<3%). In contrast, the relative abundances of the *Endozoicomonas* and *Mycoplasma* OTUs, previously described as part of the core microbiome of the healthy colonies, decreased in DP samples and contributed between 1.70% and 8.80% to the dissimilarity with the rest of asymptomatic samples (Fig. 4, Supplementary Tables S3 and S4).

In addition to shifts in bacterial community compositions, we also observed a different taxonomy-based functional profile in DP samples in comparison to the asymptomatic samples (HB, HP and DB). DP samples clustered in two groups enriched in bacterial taxa related to the physiological categories ‘anaerobic’, ‘heterotroph’, and the metabolic categories ‘dehalogenation’ and ‘ammonia oxidizer’. In contrast, these symptomatic samples displayed depletion in bacterial taxa linked to ‘nitrite reducer’ and ‘sulphate reducer’ metabolisms (Fig. 5, Supplementary Fig. S2). Interestingly, we observed functional diversity within healthy samples, although the bacterial community compositions did not differ significantly. Contrasting patterns regarding nitrogen fixers and sulphate and nitrite reducers were observed between clusters of mainly asymptomatic samples (Fig. 5).

**Characterisation of *Pacifigorgia cairnsi* disease lesions.** Based on field observations, diseased colonies are characterized by necrotic patches (visible gorgonian axis without coenenchyme). Lesions are commonly located at the periphery of the colony and multifocally distributed. The shape of lesions is circular to irregular with undulating margins (*sensu*<sup>25</sup>). Moreover, lesions are typically surrounded by tissue with retracted polyps (Fig. 1). Based on these observations, we here refer to this newly reported disease as ‘necrotic patch disease’ (NPD). According to the microscopic pathology, no differences were observed among the four types of tissue. The coenenchyme from the surface and from longitudinal and transversal sections revealed the same microscopic



**Figure 2.** PCoA plots based on a Bray-Curtis dissimilarity matrix of bacterial community compositions in healthy and diseased *P. cairnsi* samples. (a) Community compositions from samples of healthy colonies (HB and HP) and (b) from samples of healthy and diseased colonies (HB, HP, DB and DP) were compared using Bray-Curtis dissimilarity metric on the square-root transformed relative abundances. Note that disease basal (DB) samples showed no symptoms of disease. Principal Coordinate Analysis was used for visualization purposes, and the first two components (explaining over 50% of the variation) are displayed. The number of samples from each type of tissue is indicated within parenthesis. Vectors' numbers correspond to taxa assigned at OTUs level: (1) genus *Mycoplasma*, (2) genus *Endozoicomonas*, (3) genus *Endozoicomonas*, (4) order Bacteroidales, (5) genus *Aquimarina*, (6) family Oceanospirillaceae, (7) order Alteromonadales, (8) domain Bacteria, (9) order Kiloniellales, (10) class Alphaproteobacteria, (11) class Alphaproteobacteria, (12) genus *Loktanella*, (13) domain Bacteria, (14) family Pirellulaceae, (15) class Alphaproteobacteria, (16) order 34P16, (17) order Kiloniellales, (18) order Oceanospirillales, (19) family Rhodobacteraceae, (20) genus *Polaribacter*, (21) domain Bacteria, (22) genus *Synechococcus*, (23) class Spirochaetes, (24) order CAB-I, (42) genus *Nitrosopumilus*, (44) genus *Vibrio*, (45) species *Polymorphum gilvum*, (175) genus *Endozoicomonas*, (755) genus *Endozoicomonas*.

	Source	df	PERMANOVA				PERMANOVA Pair-wise tests			PERMDISP		
			SS	MS	Pseudo-F	P (perm)	Groups	t	P (perm)	Groups	t	P (perm)
a	Tissue types	1	220	220.260	0.869	0.519				HB, HP	0.932	0.523
	Res	35	8872	253.470			—	—	—			
	Total	36	9092									
b	Tissue types	3	11499	3833	12.368	0.0001*	DB, DB	4.969	0.0001*	DB, DB	2.628	0.024
	Res	69	21384	309.910			DB, HB	4.659	0.0001*	DB, HB	1.754	0.126
	Total	72	32883				DB, HP	4.317	0.0001*	DB, HP	1.229	0.295
							DB, HB	0.982	0.462	DB, HB	0.763	0.486
							DB, HP	1.455	0.026	DB, HP	1.638	0.159
							HB, HP	0.933	0.539	HB, HP	0.709	0.526

**Table 1.** PERMANOVA and PERMDISP analyses. (a) Samples from healthy colonies (HB and HP) and (b) samples from healthy and diseased colonies (HB, HP, DB and DP) \* $P < 0.0083$ , Bonferroni corrected.

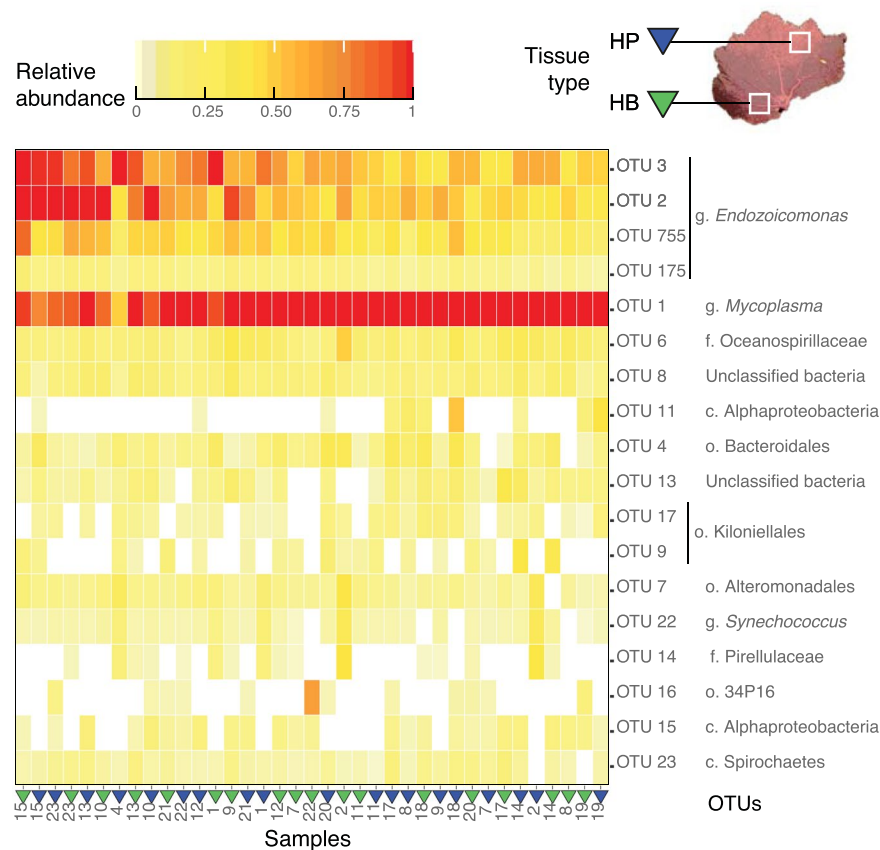
irregular morphology in HB, HP, DP and DB. Additionally, no signs of fungal presence (i.e. hyphae and conidiphores) were observed in healthy or diseased tissues (Supplementary Fig. S3).

## Discussion

In this study, we tested NPD-related changes in the bacterial microbiome of *P. cairnsi* sea fans. Our major findings are (i) the core microbiome composition associated with healthy colonies is similar in basal and peripheral tissues, (ii) bacterial microbiome shifts in diseased tissues are driven by the decrease of potential endosymbionts composing the healthy core microbiome and the appearance of an opportunistic consortium, and (iii) the NPD-related consortium is confined to the symptomatic tissues of the affected colonies.

Bacterial communities constituting the core microbiome associated with healthy colonies did not differ significantly between basal and peripheral tissues. In terms of photosynthetic endosymbionts, different spatial arrangements have been observed within branching scleractinian corals as a consequence of small-scale environmental variability (e.g. different light intensity and water flow)<sup>26</sup>. Correlation between bacterial communities and microhabitats within the colony is not well known yet. We hypothesize that the flexible fan-shape, which allows uniform water flow through the mesh in order to maximize the filter-feeding efficiency<sup>27</sup>, may lead to the similarity of bacterial communities observed between peripheral and basal parts.

The bacterial core microbiome of the sea fans was dominated by members assigned to the order Oceanospirillales and the genus *Mycoplasma*. Four OTUs from Oceanospirillales belonged to the genus



**Figure 3.** Heatmap of relative abundances of OTUs in tissue samples from healthy colonies. Eighteen OTUs that accounted for 95% of the cumulative abundance in healthy colonies are displayed, with the top seven OTUs corresponding to the core microbiome (g: genus, f: family, c: class, o: order). Samples (columns) were ordered according to their spatial position displayed along the first PCoA component (explaining over 30% of the variation, Fig. 2a).

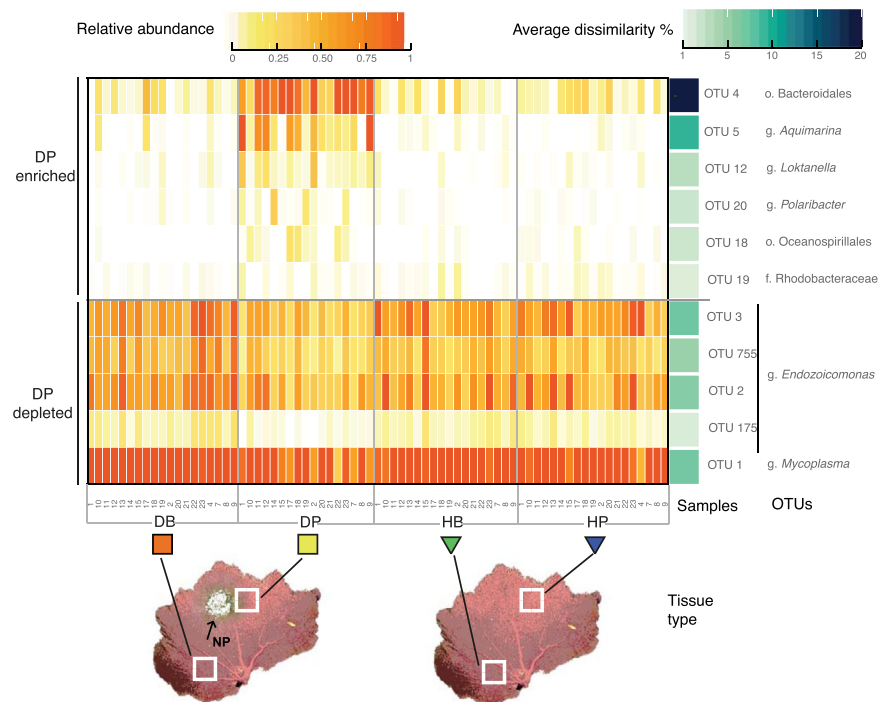
*Endozoicomonas*, being the most diverse taxonomic group of the core. *Endozoicomonas* are commonly found as dominant endosymbionts in scleractinian and gorgonian core microbiomes<sup>28–31</sup>. Multiple metabolic functions are attributed to this taxon within healthy coral holobionts such as gluconeogenesis, transport of molecules and synthesis of amino acids<sup>32,33</sup>. Additionally, *Endozoicomonas* spp. may play an important role in coral health by providing antimicrobial activity<sup>34</sup>. Particularly, *Endozoicomonas* and *Pseudovibrio* isolates from coral and sponges showed antagonistic effects against different bacterial groups including known coral pathogens as *Vibrio coralliilyticus*<sup>10,34,35</sup>.

The most abundant OTU in all samples was assigned to the genus *Mycoplasma*. This taxon is a common microbiome member in gorgonians<sup>36,37</sup> and cold-water scleractinians<sup>38,39</sup>. Even though *Mycoplasma* spp. have been suggested to be harmless commensals or endosymbionts in corals and in some cnidarians<sup>38–40</sup>, their specific role within the coral holobiont remains unclear.

Overall, our data revealed that the taxa constituting the sea fan bacterial core microbiome are also abundant in other healthy gorgonians, such as the Mediterranean *Leptogorgia sarmentosa* and *Eunicella* spp.<sup>14</sup>, the Caribbean *Antillologorgia elisabethae*<sup>41</sup> and the eastern Pacific *Muricea* spp.<sup>37</sup>. This indicates that these bacterial groups play similar roles in maintaining the holobiont's health status. However, specific strains of *Mycoplasma* and *Endozoicomonas* have been associated with particular gorgonian species, potentially suggesting coevolutionary bacteria-host associations<sup>28,37,42,43</sup>. Hence, identifying the specific role that each of these potential endosymbionts play within their hosts might provide valuable insights to understand the relationships between gorgonians and their microbiomes.

Significant shifts in bacterial community compositions and functional profiles were observed between healthy-colony and NPD-affected tissues. The relative abundances of the main core members (i.e. *Endozoicomonas* and *Mycoplasma*) decreased in diseased tissues, while a different array of more diverse bacteria arose. The depletion of healthy-state associated taxa in conjunction with the increase of bacterial diversity, is not only common in corals affected by Yellow Band Disease (YBD)<sup>20</sup>, White Plague Disease (WPD)<sup>44,45</sup> and Black Band Disease (BBD)<sup>18</sup>, but is also common in necrotic and unusual coral lesions<sup>46,47</sup>.

An OTU assigned to the order Bacteroidales contributed the most to the differentiation between healthy-colony and symptomatic tissues. Interestingly, this OTU corresponded to a low-abundant member of the *P. cairnsi* bacterial microbiome in the healthy tissues (Figs 3 and 4). Physiological or competitive constraints acting in healthy tissues might be eliminated during the disease, allowing native bacteria to increase



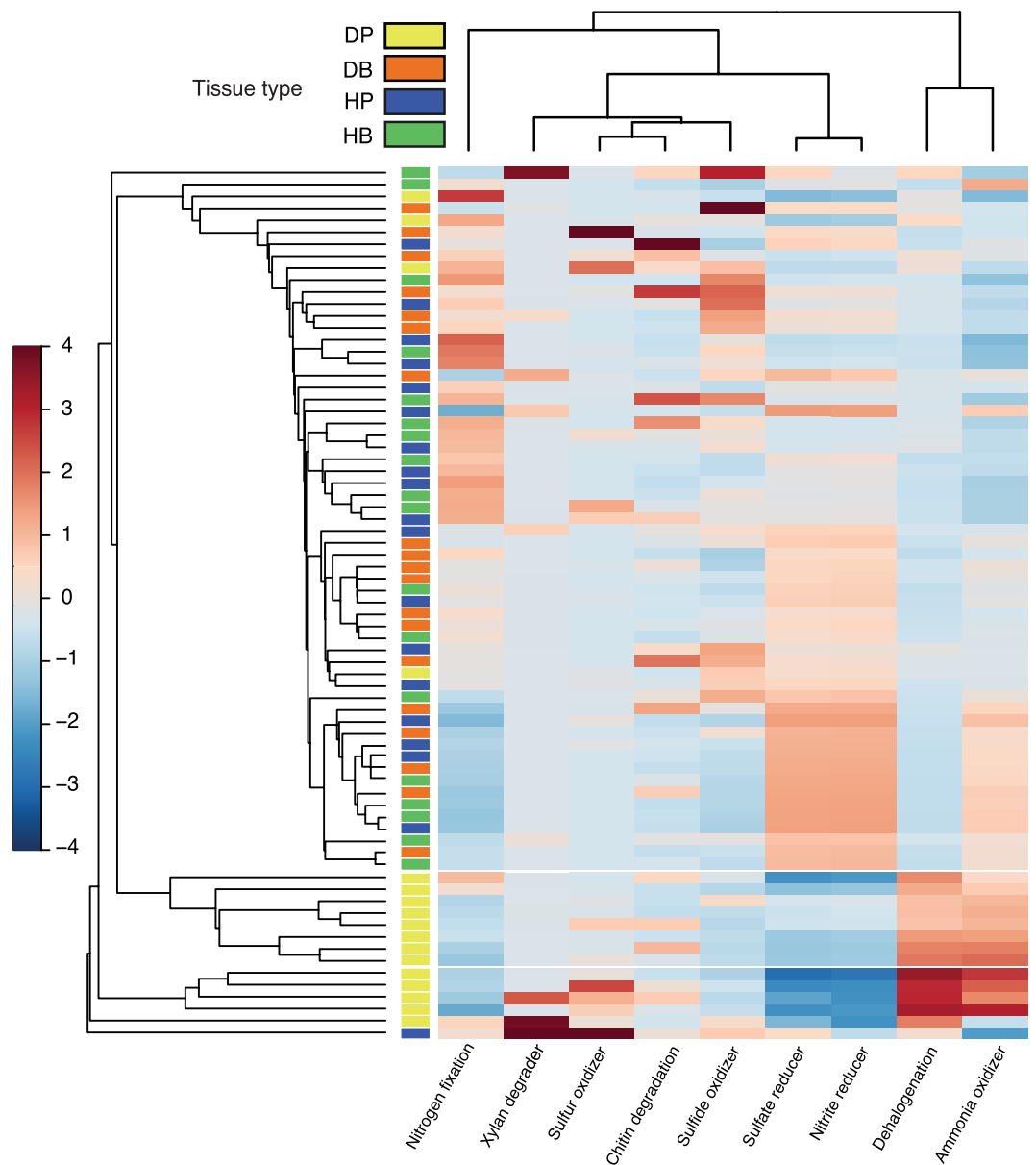
**Figure 4.** Heatmap of the relative abundances of OTUs in tissue samples from healthy and diseased colonies. According to the SIMPER analyses, the eleven OTUs depicted mainly contributed to the differentiation between symptomatic (DP) and asymptomatic samples (HB, HP, DB), (g: genus, f: family, c: class, o: order). Average dissimilarity (%) corresponds to OTU's contribution to the dissimilarity between DP and the rest of samples (DB, HP and HB).

opportunistically<sup>46</sup>. Additionally, the diseased tissues exhibited relatively high abundances of members of the families Flavobacteriaceae and Rhodobacteraceae, which have been associated with polymicrobial consortiums in BBD and WPD<sup>18,45</sup>. These taxa are considered opportunistic commensals capable of degrading the coral host tissue<sup>18,44</sup>. Specifically, *Aquimarina* spp. and *Polaribacter* spp. have been found in bleached marine macroalgae<sup>48</sup> and degrading polymers in lobsters' lesions<sup>49</sup>. Overall, the NPD-related consortium was comprised of opportunistic bacteria represented by i) native microbiome members at increased abundances and by ii) a group of bacteria with the potential ability for degrading the host tissue, persisting in a broad host-range.

Shifts in the bacterial community compositions were consistent with changes in the functional profiles between tissues from healthy colonies and symptomatic tissues. The most noteworthy switch was the decrease of sulphate and nitrite reducing taxa in diseased tissues. This shift may be related to the reduction of potential endosymbionts in symptomatic tissues since these metabolic functions have previously been identified in bacterial symbionts associated with healthy corals<sup>50–52</sup>. Although increased abundances of ammonia oxidizing bacteria were observed in some healthy samples, this metabolic function was remarkably increased in diseased tissues. The latter may respond to the higher availability of nitrogen compounds (particularly  $\text{NH}_3$ ) in symptomatic tissues, derived from organic matter decomposition<sup>53</sup>, which in turn may be related to the increase of anaerobic, heterotrophic, and dehalogenating taxa<sup>54,55</sup>. These findings, suggest that the NPD-related consortium likely uses diverse sources for energy, carbon and nutrient acquisition, suggesting that it opportunistically exploits available niches that may emerge as a consequence of the decrease of resident bacteria and the decay of coral tissue.

Additionally, we did not observe any evidence of fungal presence in HP, HB, DP and DB in our morphological assessment of tissues. Fungal species, such as *Aspergillus sydowii*, have previously been reported as causative agent of aspergillosis, a disease causing mass mortalities of *Gorgonia ventalina* sea fans in the Caribbean<sup>56</sup>. However, the role of *A. sydowii* as pathogen has been questioned<sup>57,58</sup>, as it has been found in healthy and diseased gorgonian octocorals in TEP (Tropical Eastern Pacific)<sup>59,60</sup>. Whether NPD affecting *P. cairnsi* is the same disease observed in other *Pacifigorgia* species in the TEP or whether it is aspergillosis, should be further addressed. However, we did not observe tissue purpling (i.e. host-produced melanin) as a response against fungal presence in any *P. cairnsi* sea fan, which has been considered to be a characteristic symptom of aspergillosis<sup>61,62</sup>. Therefore, the diagnoses of the disease here described (i.e. necrotic patches surrounded by tissue with retracted polyps and no microscopic evidence of fungal presence) lead us to consider NPD as a novel disease affecting gorgonian sea fans in the TEP.

Overall, our results suggest that disruptions in the natural bacterial community, depicted as a decrease of potential endosymbionts (e.g. *Endozoicomonas*), may favour the appearance of opportunistic taxa in diseased gorgonians. Correlation exists between coral health and the presence of endosymbionts, such as *Endozoicomonas* that are involved in nutrient acquisition and production of antimicrobial compounds<sup>34,41,63</sup>. Decreasing abundances of these endosymbionts are characteristic of diseased and anthropogenically impacted corals<sup>64,65</sup>, suggesting that imbalance in resident coral microbiota may have dramatic effects on coral health. The NPD-related



**Figure 5.** Taxonomy-based functional profiling of bacterial communities in samples from healthy and diseased colonies. Shifts in potential functional differences are represented by a relative abundance scale showing the enrichment (red colour) and depletion (blue colour) in different metabolic profiles mapped to the corresponding taxonomic information by METAGENassist. Hierarchical clustering of samples and functions was performed by a single linkage algorithm using Euclidean distance measurements.

consortium is likely in a state of microbial imbalance (i.e. dysbiosis)<sup>66</sup>, potentially driven by environmental pressures such as anomalous sea water temperatures<sup>22</sup>. In fact, it has been argued that many marine diseases may be the consequence of microbial dysbiosis and the rise of opportunistic or polymicrobial infections<sup>18,46,67</sup> rather than being caused by single pathogens<sup>67–69</sup>.

The lack of differences in bacterial community compositions and functional profiles between tissues from healthy colonies and the asymptomatic tissues from diseased colonies suggests that the latter may be healthy. Hence, two health states could be identified within diseased colonies: (i) the diseased state of tissues affected by the NPD-related consortium and (ii) the healthy state of asymptomatic tissues colonized by a natural microbiome community. Our data thus suggest that the disease-related consortium is locally confined in the symptomatic tissues of *P. cairnsi*. This contrasts with findings in *Orbicella faveolata* affected by YBD, where asymptomatic tissues hosted microbial communities that differed both from symptomatic and healthy tissues, thus suggesting intermediate health states<sup>20</sup>.

The confinement of the disease-related consortium in *P. cairnsi* may also facilitate colony recovery. Corals showing localized BBD showed reduction of mortality rates as well as the halt of disease progression after disease treatment (i.e. removal of the affected area and sealing it with marine epoxy)<sup>70</sup>. However, diseased *P. cairnsi* sea

fans have been seen recovering naturally after NPD incidences<sup>21,22</sup>. Breakages of both fragile NPD-affected areas and healthy tissue were observed in *P. cairnsi* due to the effect of strong currents (EQ pers. obs.). Thus, natural breaking offs of affected tissues may contribute to convalescence and ultimately to the resilience of these gorgonian populations.

Our study reveals shifts in the bacterial microbiome associated with a newly reported disease affecting tropical gorgonian corals. It recognizes the strong relationship between coral disease and shifts in the microbiome, and reveals the potential link between the spatial effects of the disease-related consortium at intra-colony level and disease recovery. Given the pivotal role that endosymbionts play in coral health status, future studies should focus on elucidating their specific functions within the holobiont, in order to better understand host-microbiome associations. Additionally, we encourage exploring the effect of environmental disturbances in microbiome disruptions triggering disease outbreaks, by implementing long-term studies and examining transcriptomic host profiles.

## Methods

**Sample collection.** A total of 40 colonies (20 healthy and 20 diseased) of *Pacifigorgia cairnsi* sea fans were sampled around Malpelo, an oceanic remote island about 500 km off the Colombian coast in the Tropical Eastern Pacific (3°58'30"N, 81°34'48"W). All samples were collected on the same day between 10 and 15 m depth by Scuba diving at the 'El Arrecife' site. Sampled sea fans were adult colonies of approximately the same size in order to avoid age-related variations in microbial communities<sup>71</sup>. Healthy and diseased colonies were chosen based on the absence or presence of damaged tissues, respectively. From each colony two samples (5 × 5 cm) were taken, one from the periphery and one from the base, separated by at least 20 cm. Accordingly, 20 samples were obtained from each of the four types of tissues: peripheral tissue from healthy colonies (HP), basal tissue from healthy colonies (HB), peripheral (symptomatic) tissue from diseased colonies (DP) and basal (but asymptomatic) tissue from diseased colonies (DB) (Fig. 1). The actual necrotic parts consisting of dead gorgonian axis (without coenenchyme) were not included in the symptomatic tissue samples but only the surrounding area of the wounds (Fig. 1). In order to prevent sample contamination, all wearing gloves and tools used to collect or manipulate samples were either disposed or sterilized after single use. Furthermore, each sample was gently rinsed with 100 ml filtered fresh water in order to remove exogenous or transient microorganisms loosely associated with the coral tissue. Samples were stored in RNAlater (Thermo Fisher Scientific, Waltham, USA) and preserved at -80 °C until subsequent DNA extraction.

Collections were made possible with research permit No.105 (2013), issued by the Autoridad Nacional de Licencias Ambientales-ANLA, Ministerio de Ambiente y Desarrollo Sostenible, Colombia and Contrato de Acceso a Recursos Genéticos para Investigación Científica Sin Interés Comercial No. 106, 20 (2014) RGE0114.

**DNA extraction and 16S rRNA gene sequencing.** DNA was extracted from approximately 100 mg of sea fan tissue using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, USA) after macerating the sample in liquid nitrogen. DNA was initially quantified using a Nanodrop 2000 UV-Bis Spectrophotometer (Thermo Fisher Scientific) and corroborated prior to sequencing with a Qubit fluorometer HS assay kit (Life Technologies, Carlsbad, USA).

The variable V4 region of the 16S rRNA gene was sequenced using the 515 F/806 R PCR primers and Illumina flowcell adapter sequences according to the earth microbiome protocol<sup>72</sup> (<http://www.earthmicrobiome.org/emp-standard-protocols/16s/>). The Takara Taq DNA polymerase premix was used for PCR amplifications as described by Pehrsson *et al.*<sup>73</sup>. Barcoded amplicons were pooled and sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA), implementing 2 × 250 bp paired-end read libraries.

**Data pre-processing and OTU picking.** Sequenced reads were pre-processed and Operational Taxonomic Units (OTUs) were generated following the UPARSE pipeline<sup>74</sup> with the modifications suggested by Gibson *et al.*<sup>75</sup> and Pehrsson *et al.*<sup>73</sup>. In brief, reads were de-multiplexed using *split\_libraries\_fastq.py* script included in QIIME v1.9.1<sup>76</sup>. Then, paired-end reads were processed by USEARCH v8.1.1861<sup>77</sup> as follows: merged (*usearch-fastq-mergepairs*) requiring a final length of 253 bp ± 5 bp, quality filtered (*usearch-fastq-filter*) allowing a maximum expected error of 0.5, dereplicated (*usearch-derep*), sorted excluding singletons (*usearch-sortbysize*), clustered in OTUs (*usearch-cluster\_otus*) and checked for chimeras (*usearch-uchime\_ref*) using the ChimeraSlayer gold database (v. microbiomeutil-r20110519, downloaded in May 2016). Reads were later mapped to OTUs and their assignment was performed at 97% identity threshold (*usearch-usearch-global*). OTUs were aligned using PyNAST<sup>78</sup> and taxonomy was assigned by RDP classifier<sup>79</sup> against the GreenGenes database<sup>80</sup> as implemented by QIIME scripts (*align\_seqs.py*, *assign\_taxonomy*, *filter\_alignment.py*). Assigned taxonomy was synchronized with OTUs in Biom format tables<sup>81</sup>. The complete dataset has been deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA403829.

In order to obtain a comprehensive description of the within-sample bacterial community, alpha diversity metrics (total number of observed OTUs, species richness Chao1 and Shannon diversity) and rarefaction plots with 29,000 sequences per sample were generated through QIIME (*alpha\_rarefaction.py*, *single\_rarefaction.py*). Differences in alpha diversity metrics were tested between all pairs of tissue types with Kruskal-Wallis tests as implemented in the software PAST v3.12<sup>82</sup>. The significance level was adjusted for the number of comparisons tested by Bonferroni correction.

**Bacterial community analyses.** Multivariate analyses were conducted to assess differences in bacterial community compositions between samples (beta diversity): (i) from healthy colonies (HD and HP) and (ii) from healthy and diseased colonies (HP, HD, DP and DB). In both cases we considered taxa accounting for the cumulative 95% of total abundance. In order to visualize differences in bacterial community compositions between

samples from tissue types we performed principal coordinate analyses (PCoA), applying a square-root transformation to relative abundances and calculating Bray-Curtis dissimilarity matrices. Permutational Analyses of Variance (PERMANOVA<sup>83</sup>) were conducted to test differences in bacterial community compositions between tissue types (9999 permutations). Additionally, Permutational Analyses of Multivariate Dispersions (PERMDISP<sup>24</sup>) were used to test for homogeneity of multivariate dispersions (9999 permutations) between sampling groups. The significance level was adjusted for the number of comparisons tested by Bonferroni correction. Similarity Percentage (SIMPER) analyses were used to identify the taxa contributing to the greatest extent to the observed patterns. All multivariate analyses were performed at family and OTU level using PRIMER 6 & PERMANOVA+ software<sup>84</sup>. Additionally, heatmaps were generated with the R package ggplot2<sup>85,86</sup> through the RStudio suite<sup>87</sup> to visualize patterns of similarity in OTUs' abundances between types of tissues. As all samples were taken from the same reef and at the same day, we defined the core bacterial microbiome of *P. cairnsi* as those OTUs present in 100% of HB and HP sample tissues<sup>14</sup>.

Putative functional differences associated with differences in bacterial community compositions among tissue types were assessed by using METAGENassist<sup>88</sup>. OTUs filtering and normalization parameters were used as described by Hadaidi *et al.*<sup>89</sup>. Euclidean distance measure (single linkage algorithm) was used to visualize functional profiles (i.e. metabolism, oxygen requirements, carbon and energy source) in heatmaps mapped to the microbial communities.

**Characterisation of *Pacifigorgia cairnsi* disease lesions.** In order to provide a detailed description of disease lesions, we conducted field investigations based on *in situ* underwater photographs from healthy and affected colonies.

This information was used subsequently to characterize and name the *P. cairnsi* disease as 'necrotic patch disease' according to a framework systematically describing gross lesions in corals<sup>25</sup>.

Moreover, the microscopic pathology of lesions was addressed using scanning electron microscopy (SEM, JSM 6490-LV)<sup>90</sup>. Accordingly, HP, HB, DP and DB tissues from five healthy and five diseased colonies were fixed in 2% glutaraldehyde, washed in distilled sterile water, dehydrated in a series of ethanol solutions (30, 50, 70, 80, 90, 95 and 100%) and finally carbon coated. SEM images were obtained from the surface and from longitudinal and transversal sections of all types of tissues at 45, 250 and 1000 x magnifications. Finally, detailed observations of polyps, coenenchymes and gorgonian axes were done using SEM at up to 2000x magnification.

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## Author Contributions

E.Q., J.A.S., A.R.M. and T.W. contributed to the design of the study. E.Q. and C.R.P. conducted the sampling. E.Q., C.R.P., B.A.O. and G.W. generated the data. E.Q., C.R.P. analysed the data. E.Q., C.R.P., A.R.M. and S.P.G. interpreted data results. E.Q. wrote the manuscript. E.Q., C.R.P., B.A.O., G.W., S.P.G., T.W., A.R.M. and J.A.S. revised the manuscript.

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# Dynamic Interplay of ENSO Events and Local Hydrodynamic Parameters Drives Demography and Health Status of Gorgonian Sea Fan Populations on a Remote Tropical Eastern Pacific Island

Elena Quintanilla<sup>1,2\*</sup>, Teresa Madurell<sup>3</sup>, Thomas Wilke<sup>1</sup> and Juan A. Sánchez<sup>1,2</sup>

<sup>1</sup> Department of Animal Ecology and Systematics, Faculty of Biology and Chemistry, Justus Liebig University Giessen, Giessen, Germany, <sup>2</sup> Departamento Ciencias Biológicas, Laboratorio de Biología Molecular Marina (BIOMMAR), Universidad de los Andes, Bogotá, Colombia, <sup>3</sup> Institute of Marine Sciences, Consejo Superior de Investigaciones Científicas, Barcelona, Spain

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### \*Correspondence:

Elena Quintanilla  
e.quintanilla10@uniandes.edu.co

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Gorgonian corals occurring in shallow waters are vulnerable to changing environmental conditions and human-related pressures such as pollution, overfishing, and diseases. However, anthropogenic effects on coral systems are difficult to quantify due to the lack of base-line data of unaffected populations. In order to assess the impact of global and local environmental parameters on gorgonian populations removed from direct anthropogenic impact, we evaluated demographic parameters and the health status of *Pacificorgia cairnsi* (Gorgoniidae: Octocorallia) populations in Malpelo Island, a remote and pristine marine area in the Tropical Eastern Pacific of Colombia. Specifically, we studied *P. cairnsi* densities and population size structures under different habitat and local environmental conditions. We also studied whether ENSO events and local hydrodynamic features including locality, water depth, and upwelling conditions drive *P. cairnsi* growth rates. Finally, we evaluated the prevalence of the necrotic patch disease and rates of disease recovery. Major findings were that local hydrodynamic parameters shaped *P. cairnsi* size structures, that growth rates were affected by thermal anomalies associated to ENSO events and partly by water depth, that overall disease prevalence was low (6%) and that it did not correlate with the environmental parameters studied, and that most diseased colonies (57%) recovered via tissue breakage. The fact that *P. cairnsi*, a keystone species within the regional benthic food web, is affected by thermal anomalies remains of concern because these global events are predicted to increase in frequencies and severity in the future. Nonetheless, the low level of disease prevalence found indicates that the island's pristine conditions might facilitate disease resistance. Moreover, the findings suggest an interesting trade-off between growth rates and colony recovery in shallow waters related to tissue breakage. This study provides crucial base-line data for future investigations aiming at understanding coral responses to anthropogenic pressures and the impact of global climate change on coral communities.

**Keywords:** gorgonian corals, Tropical Eastern Pacific, disease prevalence, growth rates, ENSO

## INTRODUCTION

Gorgonian corals are among the most vulnerable reef organisms (Goldberg and Wilkinson, 2004; Sánchez et al., 2019). Being long-lived and slow-growing species, they are particularly sensitive to natural disturbances (Linares et al., 2008). Moreover, gorgonian corals are increasingly affected by disease outbreaks and abundances of many species have declined worldwide (Bourne et al., 2009; Sánchez et al., 2011, 2014). This is of great concern given that these organisms play a key ecological role in benthic communities and are considered “engineering species,” shaping the habitat and increasing its complexity by forming three-dimensional structures (Jones et al., 1994; Ballesteros, 2006; Sánchez et al., 2016). Additionally, they provide substrate and habitat for associated biota, thus increasing biomass and biodiversity of benthic communities (Buhl-Mortensen and Mortensen, 2005; Sánchez et al., 2016). Therefore, changes in gorgonian abundances in response to natural and anthropogenic disturbances may have dramatic effects on entire communities (Garrabou and Harmelin, 2002).

Anthropogenic effects on coral systems, however, are often difficult to quantify due to the lack of base-line data for unaffected populations. Thus, information from populations largely removed from human impact, such as those on the offshore Island Malpelo located in the Tropical Eastern Pacific (TEP), is particularly valuable. This uninhabited island is considered to be under pristine conditions (Zapata and Vargas-Ángel, 2003; Quimbayo et al., 2016). While gorgonians are typically diverse in the TEP (Bayer, 1953; Van Oppen et al., 2005; Sánchez, 2016), rocky reef environments of Malpelo Island are mainly dominated by species of the genera *Leptogorgia* and *Pacifigorgia* (Sánchez et al., 2011; Sánchez, 2016). Of these, *Pacifigorgia cairnsi*, which is endemic to the TEP, is considered a keystone species in Malpelo. It dominates the infralittoral seascape by forming gorgonian forests on rocky outcrops and walls up to 30 m depth (Sánchez et al., 2011; Sánchez and Ballesteros, 2014). This species also plays an important ecological role due to, for instance, strong interactions with invertebrates such as ovulid gastropods and fishes including *Oxycirrhites typus* and *Dermatolepis dermatolepis* (Sánchez et al., 2016; Sánchez, 2016). Therefore, the loss or decrease of *P. cairnsi* in Malpelo would dramatically affect the entire ecosystem; a fact that makes its study and conservation a crucial matter (*sensu* Mills et al., 1993).

These gorgonian populations cope with cold waters and high levels of suspended matter during seasonal upwelling (from December to March), as well as anomalous increases in seawater temperature during sporadic El Niño-Southern Oscillation (ENSO) events (Glynn and Colgan, 1992; Zapata et al., 2010). Moreover, in recent years, *P. cairnsi* die-offs in Malpelo have been associated to disease outbreaks (Sánchez et al., 2011; Barrero-Canosa et al., 2013), namely the “necrotic-patch disease” (NPD) (Quintanilla et al., 2018).

However, the impact of natural environmental stressors on *P. cairnsi* communities in the pristine island of Malpelo remains poorly understood, calling for coral response studies involving demographic traits such as growth rates, density, size

structure, and disease susceptibility (*sensu* Bruno et al., 2011; Rico-Esenaro et al., 2019).

In this study, we therefore assessed the impact of ENSO events and local-scale environmental parameters on the demography and health status of gorgonian populations in the absence of direct anthropogenic pressures. First, we compared *P. cairnsi* densities and population size structures under different habitat and hydrodynamic settings to understand how local conditions drive these population parameters. We then identified the respective environmental features affecting growth rates. Finally, we evaluated NPD prevalence and colony recovery to assess their health status.

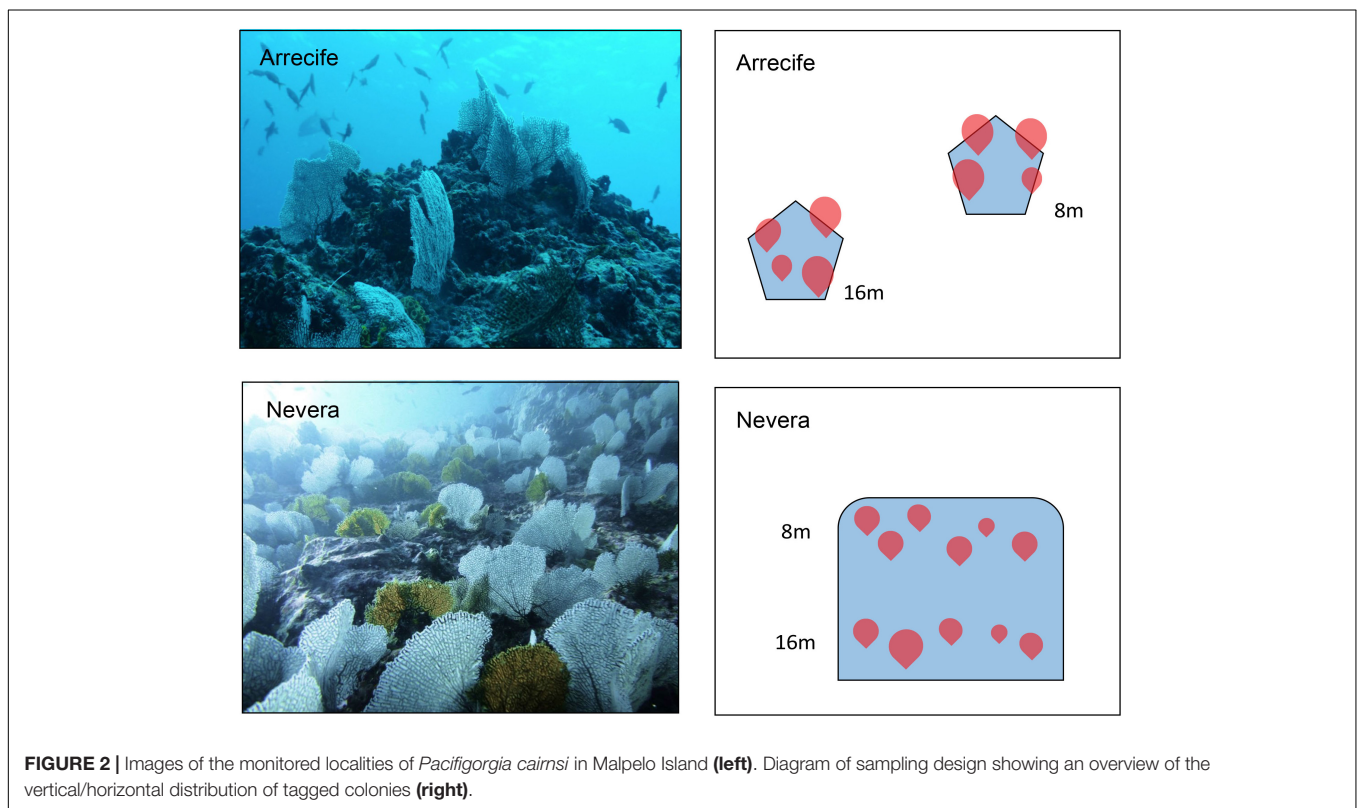
## MATERIALS AND METHODS

### Study Site and Data Collection

The study was conducted in the TEP island Malpelo, which is located about 500 km off the Colombian coast (3°58′30″N, 81°34′48″W) (Figure 1). This Fauna and Flora Sanctuary is a marine protected area and a World Heritage Site that belongs to the Marine Conservation Corridor of the TEP together with Gorgona (Colombia), Cocos (Costa Rica), Coiba (Panamá), and Galapagos Islands (Ecuador). A total of 80 *P. cairnsi* colonies of different sizes were studied in two localities exposed to leeward or windward conditions depending on dry/wet seasons and wind patterns (Velásquez-Jiménez et al., 2016). The first locality, Arrecife, is located in the northeastern part of the island, which is constantly impacted by strong waves. *P. cairnsi* colonies are patchy distributed on large stones along the rocky reef. The second locality, Nevera, is located in a sheltered



**FIGURE 1 |** Geographical location map of Malpelo Island and the studied localities (Arrecife and Nevera).



bay in the southwestern part of the island. *P. cairnsi* sea fans are continuously distributed along a wall up to depths of approximately 18–20 m (Figure 2). However, hydrodynamic conditions change considerably with water depth. Accordingly, depths of up to 10 m are influenced by strong waves and tidal movements (Velásquez-Jiménez et al., 2016). To reflect these different conditions, we studied in both sites populations in “shallow” (8 m) and “deep” (16 m) waters.

To assess the size structure, 10 quadrants each (50 × 50 cm) were randomly placed at the two depths (8 and 16 m) in the Arrecife and Nevera localities (40 quadrants in total) in June 2016. All colonies within each quadrant were counted and their total areas were registered. Underwater pictures (PowerShotG12, Canon) were taken together with an acrylic board containing a defined scale. Most underwater photographs had to be corrected for skewedness by using a standardized grid as they were taken with a deviation from the vertical plane of the colony. Total colony area was obtained by contouring the colony perimeter of each corrected photograph using Image J® software (Research Services Branch – NIMH, Bethesda, MD, United States). Colonies measuring <5 cm<sup>2</sup> were considered recruits as defined by Gomez et al. (2014) and six size classes were delimited: <5, 5–100, 101–200, 201–300, 301–400, and >400 cm<sup>2</sup>.

To study gorgonian growth rates, the total area of each tagged colony was registered by underwater pictures during eight consecutive monitoring times, covering a 3-year study period: July 2013, December 2013, March 2014, July 2014, March 2015, September 2015, March 2016, and June 2016. Growth rates for each colony were obtained by calculating the difference

in area (cm<sup>2</sup>) between two consecutive monitoring times and expressed as cm<sup>2</sup> month<sup>-1</sup>. Additionally, tissue breakage, colony recovery, and disease prevalence (% of diseased colonies) were reported over the study period.

According to the National Oceanic and Administration (NOAA)<sup>1</sup>, abnormal positive seawater temperatures associated to ENSO events ranged from +0.5 to +2.3°C during the monitoring period. As the periods July 2014–March 2015 and March 2016–June 2016 included more months with abnormal positive temperatures than with normal ones, they were considered as ENSO periods (Table 1). “La Niña” events (the cool phase of ENSO) did not occur during the study period.

<sup>1</sup><http://www.cpc.ncep.noaa.gov>

**TABLE 1 |** Upwelling conditions and ENSO events during monitoring periods.

Monitoring period	Upwelling condition	ENSO event
July 2013–December 2013	Absent	−0.4/−0.2 (ENSO absent)
December 2013–March 2014	Present	−0.2/−0.4 (ENSO absent)
March 2014–July 2014	Absent	−0.4/+0.2 (ENSO absent)
July 2014–March 2015	Present	+0.2/+0.6 (ENSO present)
March 2015–September 2015	Absent	+0.6/+1.8 (ENSO present)
September 2015–March 2016	Present	+1.8/+2.2 (ENSO present)
March 2016–June 2016	Absent	+2.2/+0.5 (ENSO present)

For each monitoring period, the oscillation range of temperatures is shown. See **Supplementary Material** for sea surface water temperature (SST) data at Malpelo Island reflecting upwelling conditions during the study period.

**TABLE 2** | Number of *Pacifigorgia cairnsi* colonies and average densities in Malpelo Island in June 2016.

Sampling site	Total number of colonies	Average density ( $\pm$ SD)
Arrecife shallow (8 m)	216	24 ( $\pm$ 6.93)
Arrecife deep (16 m)	140	14 ( $\pm$ 8.89)
Nevera shallow (8 m)	188	18.8 ( $\pm$ 6.54)
Nevera deep (16 m)	172	17.2 ( $\pm$ 6.81)
Total	716	8.5 ( $\pm$ 7.92)

Average density is expressed as number of colonies/m<sup>2</sup> ( $\pm$ SD).

### Data Analyses

Differences in size structures and densities between localities and water depths were assessed using Kruskal–Wallis and Mann–Whitney tests as implemented in PAST v3.12 (Hammer et al., 2001). The significant level was adjusted for the number of comparisons tested by Bonferroni correction. To evaluate the effect of five factors [locality, water depth, upwelling conditions (local thermal conditions), ENSO events (global thermal conditions), and colony area] on growth rates and disease prevalence, linear mixed effect (LME) models were constructed using the R v.3.1.2 (R Studio Team, 2015) package nlme (Pinheiro et al., 2009). Colony and periods were treated as random effects; locality, water depth, local and global thermic conditions, and colony area as fixed effects. Full and reduced models were fitted using a restricted maximum likelihood (REML) approach and models were compared using Akaike’s Information Criterion (AIC).

To understand site-specific effects, LME models were also applied individually to the Arrecife and Nevera datasets.

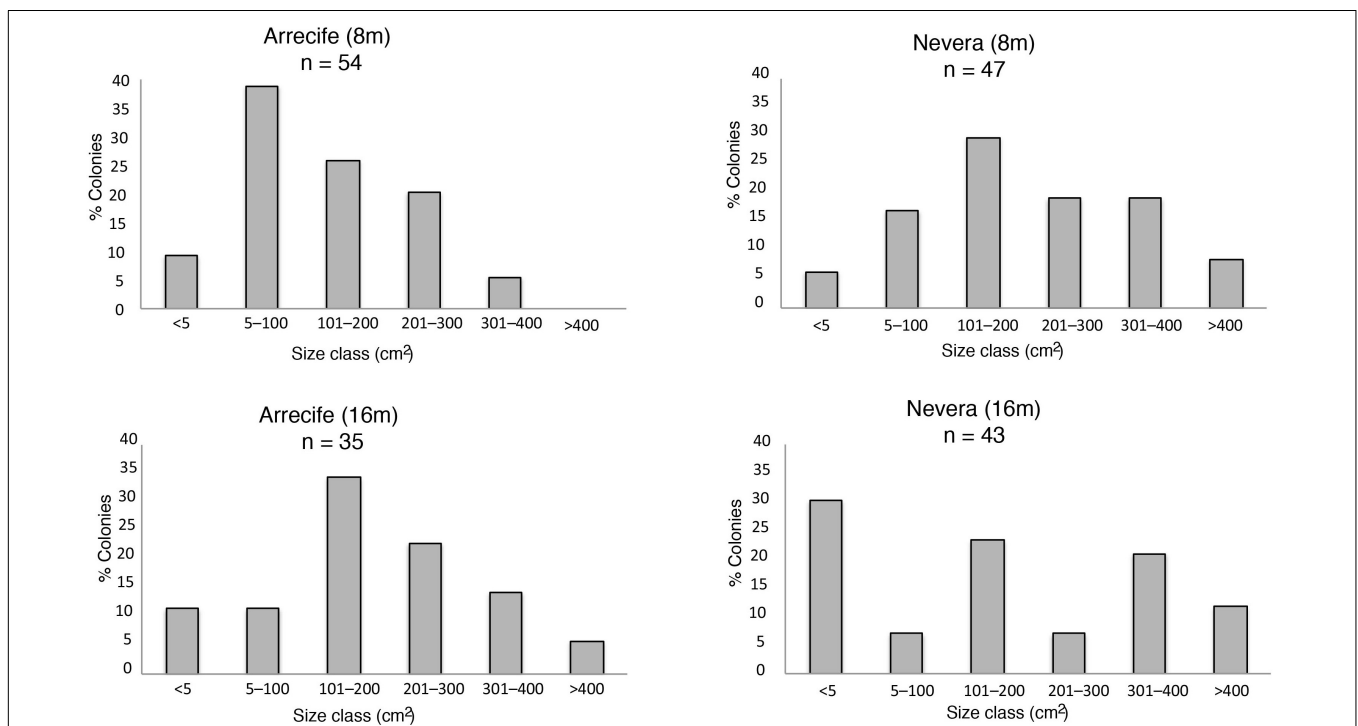
## RESULTS

### Density and Size Structure

The average *P. cairnsi* density observed was 18.5 ( $\pm$ 7.92) col m<sup>-2</sup> and did not differ between localities (Mann–Whitney test,  $P > 0.05$ ,  $P = 0.96$ ) or water depths (Mann–Whitney pairwise test,  $P > 0.008$ ,  $P = 0.93$ ) (Table 2). Images from a total of 179 *P. cairnsi* colonies were analyzed to study the size structure in June 2016. Overall, mean colony areas did not show significant differences between Arrecife and Nevera (Mann–Whitney test,  $P > 0.05$ ), but displayed significant differences between shallow sites (Mann–Whitney pairwise test,  $P < 0.008$ ). In particular, the Arrecife shallow site showed lower proportions of large colonies and higher percentage of young colonies (<100 cm<sup>2</sup>) than Nevera shallow. Additionally, the percentage of recruits (<5 cm<sup>2</sup>) and big colonies (>300 cm<sup>2</sup>) were higher in Nevera deep than in any other site (Figure 3).

### Growth Rates

The average growth rate measured for *P. cairnsi* was 6.16 cm<sup>2</sup> month<sup>-1</sup>. Visual examination of residuals of the best-fit LME models indicated that the assumptions of normality, homogeneity, and independence were all met. Global thermal conditions (ENSO events) and water depth had a significant effect on Nevera *P. cairnsi* growth rates ( $P = 0.001$  and  $P = 0.045$ ,



**FIGURE 3** | Size-frequency distribution of *Pacifigorgia cairnsi* colonies in shallow and deep water sites at Arrecife and Nevera localities in Malpelo Island in June 2016 ( $n$  = number of colonies).

**TABLE 3** | Results of the best-fitted linear mixed effect model for parameters driving *Pacifigorgia cairnsi* growth rates in Malpelo Island.

Locality/parameter	Value	Std. error	DF	t-value	p-value
<b>Arrecife</b>					
Area	0.028	0.015	18	1.843	0.082
Depth	-0.226	0.447	35	-0.505	0.617
Thermal upwelling	-3.684	2.724	18	-1.352	0.193
ENSO positive	0.082	3.197	18	0.025	0.979
<b>Nevera</b>					
Area	0.006	0.009	3	0.625	0.576
Depth	-0.945	0.453	32	-2.086	0.045*
Thermal upwelling	4.409	3.493	42	1.262	0.213
ENSO positive	-12.557	3.699	42	-3.394	0.001**
<b>Arrecife + Nevera</b>					
Area	0.01	0.007	3	1.295	0.285
Depth	-0.546	0.307	68	-1.775	0.08
Thermal upwelling	2.631	2.552	63	1.03	0.306
ENSO positive	-6.934	2.593	63	-2.674	0.009**

\* $p < 0.05$ , \*\* $p < 0.01$ .

respectively). Growth rates of colonies from Arrecife were not significantly affected by the studied parameters. Considering both localities together, only ENSO events had an effect on growth rates (Table 3).

## Disease Prevalence, Tissue Breakage, and Colony Recovery

None of the studied parameters had a significant effect on NPD prevalence. Overall, mean disease prevalence registered during the eight monitoring times was 6.02% ( $\pm 9.49\%$ ) (Table 4). Overall, 37.80% of the healthy colonies showed tissue breakage at some point during the study period. In particular, the Arrecife shallow and deep sites displayed 33.34 and 15.78% of healthy-broken colonies, respectively, and Nevera shallow and Nevera deep sites 61.12 and 42.85%, respectively. 57% of diseased colonies recovered from NPD; all of them showed signs of tissue breakage (Figure 4).

## DISCUSSION

In this study, we assessed the effects of global (i.e., ENSO events) and local hydrodynamic parameters on the demography of *P. cairnsi* populations and evaluated their health status

in a remote marine area, currently not directly affected by anthropogenic processes. Our major findings were: (i) local hydrodynamics defined *P. cairnsi* size structures, (ii) growth rates were driven by thermal anomalies and partly by water depth, (iii) disease prevalence was low and did not correlate with any of the studied environmental parameters, and (iv) most diseased colonies recovered via tissue breakage.

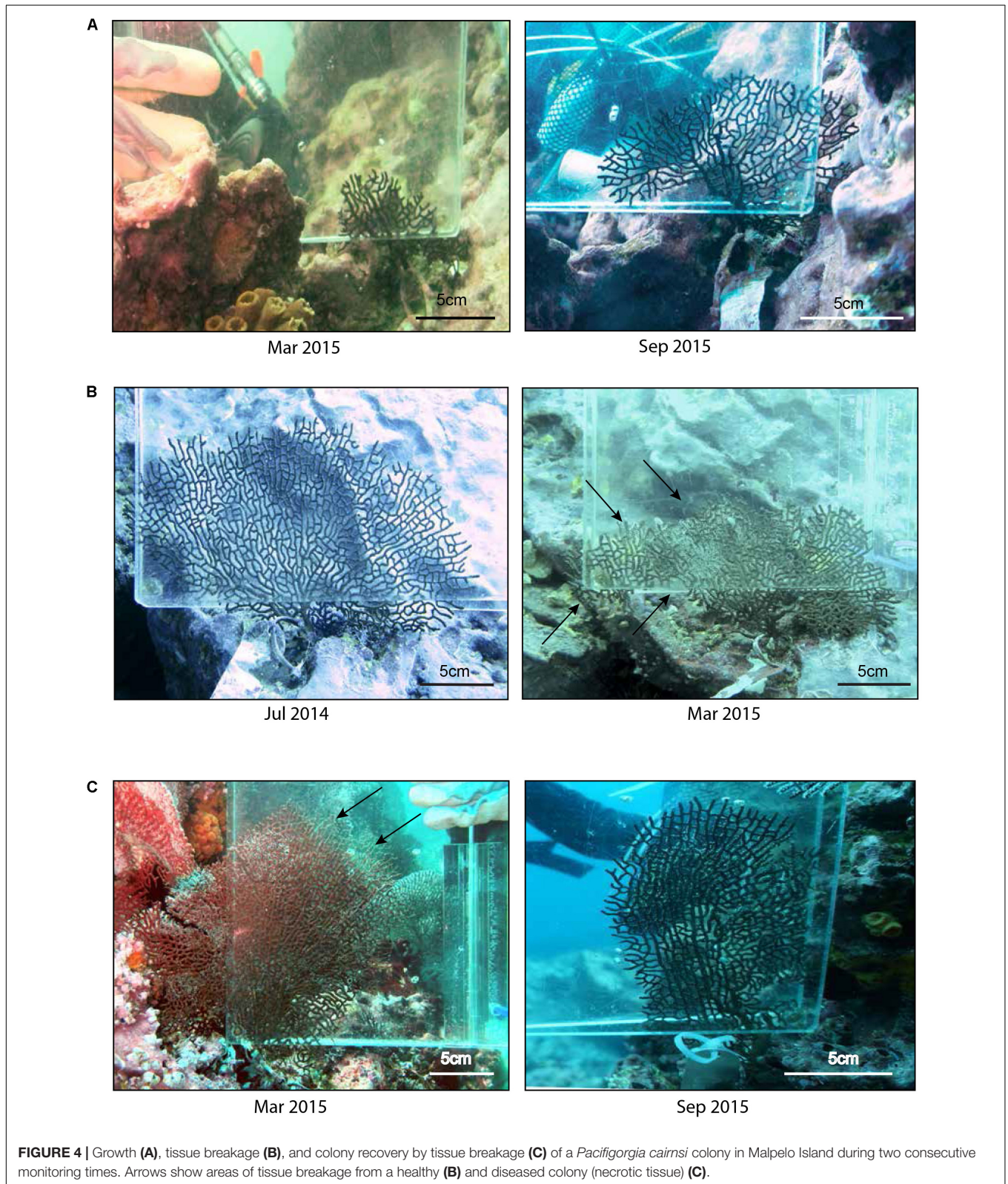
*Pacifigorgia cairnsi* densities did not differ between the localities and sites (water depth) studied. Although this pattern is consistent with those from Coiba Island (Panamanian TEP), the average density of *P. cairnsi* in Malpelo Island (18 col  $m^{-2}$ ) was considerably higher than in Coiba Island (1.2 col  $m^{-2}$ ) (Gomez et al., 2014). Patchy species distribution of gorgonians within TEP areas is attributed to variable oceanographic and environmental conditions (e.g., upwelling, freshwater and runoff sediment inputs, suspended matter, etc.) (Zapata et al., 2010; Barrero-Canosa et al., 2013; Gomez et al., 2014).

However, size structures of *P. cairnsi* colonies in Malpelo Island differed among localities and sites. Arrecife shallow showed both the highest proportion of small colonies and the lowest percentage of large colonies. As hydrodynamic forces may cause dislodgement and tissue breakage (Lasker, 1990; Chang et al., 2007), the permanent exposition of this locality to strong water motions might make local *P. cairnsi* colonies particular vulnerable. Additionally, the Nevera deep site exhibited the greatest percentage of both recruits and large colonies. Abiotic factors including light intensity, temperature, and water movement may considerably change with water depth (Garrabou et al., 2002; Mass et al., 2007) and thus modulate demographic parameters of benthic organisms such as gorgonian corals. Sheltered environments associated with deep sublittoral habitats in the Mediterranean Sea, for example, allow *Eunicella singularis* gorgonian populations to reach larger colony sizes than in shallow waters (Gori et al., 2011). Similarly, larger *Gorgonia ventalina* colonies were found at deeper environments in the Gulf of Mexico, where water motion decreases significantly with water depth (Jordán-Dahlgren, 1989). Therefore, local perturbations such as high hydrodynamic forces in shallow waters may explain the higher percentage of broken colonies both in Nevera and Arrecife shallow sites compared to Nevera deep site. Additionally, the latter site showed the highest recruitment rate, suggesting that reproductive success increases with colony size (Coma et al., 1995).

We also found that growth rates of *P. cairnsi* were affected by thermal anomalies associated with ENSO events. Average

**TABLE 4** | NPD prevalence of *Pacifigorgia cairnsi* (% diseased colonies) in Malpelo Island during eight monitoring times (July 2013, December 2013, March 2014, July 2014, March 2015, September 2015, March 2016, and June 2016).

Site	July 2013	December 2013	March 2014	July 2014	March 2015	September 2015	March 2016	June 2016
Arrecife shallow	0	3.57	0	0	11.11	8	6.67	0
Arrecife deep	0	13.33	50	7.69	0	6.25	6.67	0
Nevera shallow	0	2.7	16.67	0	9.09	8.33	0	-
Nevera deep	0	0	8	7.14	6.67	5	3.7	-
Average	0	4.9	18.67	3.71	6.72	6.90	4.26	0
(SD)	(0)	(5.82)	(21.97)	(4.29)	(4.83)	(1.56)	(3.17)	(0)



temperatures are a major factor controlling coral growth rates (Chindapol et al., 2013). Specifically, elevated seawater temperatures related to ENSO episodes exercise stress on corals

as there is a thermal optimum for coral growth (Lough and Cooper, 2011). Coral responses to thermal stress are well studied in zooxanthellate reef-building corals and include changes in

coral physiology, reduction of growth rates, up-regulation of heat-shock proteins, coral bleaching, and ultimately die-offs of entire coral reefs (Ainsworth et al., 2008; Woolstra et al., 2009; Lough and Cooper, 2011). In particular, recurrent ENSO episodes have increased thermal stress in Caribbean and Eastern Pacific reef-building corals, leading to bleaching and growth declines (Glynn et al., 2001; Manzello, 2010; Hetzinger et al., 2016). However, while thermal stress response (e.g., up-regulation of stress proteins and bleaching; Prada et al., 2009; Dias and Gondim, 2015; Löhelaid et al., 2015) has also been observed in zooxanthellate octocorals, the latter organisms seem to be more resilient to environmental stressors and show higher recovery rates after ENSO episodes than reef-building corals (Schleyer and Benayahu, 2010; Tsounis and Edmunds, 2017).

Nevertheless, responses of azooxanthellate octocorals, such as *P. cairnsi*, to thermal stress remain largely elusive. Despite the assumption that octocorals represent the most resistant coral organisms in regard to thermal stress (Tsounis and Edmunds, 2017), our findings indicate that azooxanthellate gorgonians are also vulnerable (as zooxanthellate ones) to abnormal temperatures and changes in productivity and nutrient levels associated with ENSO events (Harvell et al., 2001; Turk et al., 2011). This is of particular relevance as these global-scale events are predicted to increase in frequency and severity over the next decades (Santoso et al., 2013; Cai et al., 2014). It has also been shown that the coral recovery capacity declines with increasing frequency of ENSO episodes; exacerbated by the fact that ENSO-associated effects on coral communities may last for months or years following initial impact (Cai et al., 2014; Glynn et al., 2017). This is of great concern for benthic communities in Malpelo Island as *P. cairnsi* is a keystone species there, and its decline might have severe adverse effects on the entire benthic food web.

Apart from temperature, water motion and light are also main environmental parameters affecting coral growth (Todd, 2008; Chindapol et al., 2013). Colonies in deeper waters, for example, display decreased growth rates, minimizing metabolic demand under low-light conditions (Grigg, 2006; Kahng et al., 2010). However, *P. cairnsi* lacks zooxanthellae and light may thus not be a major limiting factor. In fact, our results suggest that hydrodynamic forces might be the main parameter affecting growth rates. Due to wave-induced tissue breakage, shallow-water colonies have to allocate considerable energy resources to tissue repair (Lartaud et al., 2016). Accordingly, the fact that depth did not contribute significantly to decreased growth rates in Arrecife deep suggests that these colonies, as the shallow ones, are probably allocating energy in tissue repair due to wave-induced tissue breakage. In contrast, deeper (and larger) colonies from Nevera deep might be able to allocate more energy to recruitment rather than to tissue repair and growth due to the sheltered environment as noted in other gorgonian corals (Coma et al., 1995; Heino and Kaitala, 1999).

Our results also showed that the prevalence of NPD affecting *P. cairnsi* in Malpelo Island was low (i.e., 6%) compared to coral diseases in other areas. For example, aspergillosis affects 6–39% of *G. ventalina* colonies throughout the Caribbean Sea

(Weil, 2004; Toledo-Hernández et al., 2007). These high levels of disease prevalence in the Caribbean Sea, compared to the TEP, have been attributed to higher levels of long-term thermal and anthropogenic stress in the former region (Barton and Casey, 2005; Ruiz-Moreno et al., 2012).

The low level of NPD prevalence observed in our study sites might thus be related to the pristine conditions of Malpelo Island. Moreover, the fact that we did not find correlations between local/global environmental parameters and NPD prevalence suggests a random and occasional behavior of the disease in *P. cairnsi*. This assumption is also supported by the fact that NPD affecting *P. cairnsi* in Malpelo Island behaves opportunistically and is likely in a state of microbial dysbiosis (Quintanilla et al., 2018). Interestingly, while tissue breakage in shallow, wave-exposed waters is energetically costly and affects growth rates, it also facilitates recovery from NPD via physical loss of affected, peripheral tissues. Overall, the NPD patterns observed in *P. cairnsi* colonies in Malpelo Island indicate that coral diseases are also present in pristine marine areas, being part of a natural population dynamic.

## CONCLUSION

Our work provides crucial base-line data on demographic and health parameters of gorgonian populations in a remote and pristine marine area removed from direct anthropogenic impact. Overall, colonies developed into mature gorgonian populations, showing high-density levels and size structures driven mostly by local hydrodynamics. However, global thermal conditions such as ENSO events negatively affected *P. cairnsi* growth rates. This is of great concern as the taxon represents a key stone species within the benthic food web and as these global-scale events are predicted to increase in frequencies and severity under conditions of climate change. Nonetheless, the low levels of disease prevalence found suggest that the island's pristine conditions might facilitate disease resistance. Moreover, our results indicate an interesting trade-off between growth rates and colony recovery in shallow waters related to tissue breakage. Finally, this study facilitates future research aimed at understanding coral responses to anthropogenic pressures and the impact of global climate change on coral communities.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

EQ, TM, TW, and JS contributed to the design of the study, revised the manuscript, and approved the final text. EQ conducted the sampling, generated and analyzed the data, and wrote the manuscript. EQ and TM interpreted and discussed the data results.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00694/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# III APPENDICES

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# Declaration

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