

Black Coral Assemblages from Machalilla National Park (Ecuador)

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Abstract

Little is known about the density and structure of black coral populations of the continental Pacific coasts of Central and South America. Species diversity and the ecology of the antipatharian fauna of Machalilla National Park (Province of Manabí, Ecuador) were surveyed using SCUBA and two species, *Myriopathes panamensis* and *Antipathes galapagensis*, were identified. New information on the two species and their associated fauna were obtained through both underwater observations and laboratory analyses. Specific associations with stalked barnacles and parasitic zoanthids were described. An underwater visual census indicated that the black coral assemblage had a maximal density between depths of 15-30 m. *M. panamensis* commonly occurred below 20 m depth while *A. galapagensis* was mainly recorded from deeper than 25 m depth. The surveyed sites were characterized by sparse rocks mixed with sandy patches, and the occurrence of black corals was mainly related to the availability of rocky substrate. With an average density of 0.5 colonies m⁻², the shallow black coral community of the Machalilla National Park is one of the densest in the world. The data from this study represent a clear baseline for the monitoring of the population dynamics of benthic organisms in an area subjected to periodic El Niño and La Niña events, which may greatly affect the composition and abundance of the marine communities.

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Introduction

Black corals (Anthozoa, Antipatharia) represent a small order of colonial hexacorallians mainly occurring below 100 m depth in both tropical and sub-tropical regions (Pax et al. 1987). In some areas, however, abundant and diverse communities have been recorded within 50 m depth (Tazioli et al. 2007), where antipatharians contribute to the three-dimensionality of the coral reefs and host a rich associated fauna.

In South America the most studied shallow water populations of black corals (surveyed within the diving depth range) are confined to the Caribbean Sea (Warner 1981, Sanchez 1999), where 16 species have been recorded (Echeverria 2002, Opresko and Sanchez 2005). The most important Caribbean assemblages have been described between depths of 5-40 m in Colombia (Opresko and Sanchez 1997, Sanchez et al. 1998, Sanchez 1999), Trinidad and Jamaica (Warner 1981, Warner and Opresko 2004, Warner 2005), Panama (Guzman and Guevara 1999, Sanchez and Collin 2003), Cayman, Cuba and Mexico (Castorena and Metaca 1979, Guitart et al. 1997, Padilla and Lara 2003, respectively).

The existing literature concerning the Pacific coasts of Mexico, Panama, Colombia and Ecuador reports two black coral species, *Myriopathes panamensis* (Verrill, 1869) and *Antipathes galapagensis* Deichmann, 1941 (Verrill 1869, Opresko 1976, Opresko and Grigg 1977; Martinez and Robinson 1983, Kerstitch 1989, Opresko 2001, Opresko 2003, Hickman 2008). Both species are described as occurring only in limited areas: *M. panamensis* in the shallow waters of Panama Bay (9-17 m depth) (Opresko 1976) and Ecuador, mainly the Galapagos Archipelago (up to 50 m depth) (Martinez and Robinson 1983, Romero 1997, Hickman 2008), while *A. galapagensis* was originally reported from deep waters (90 m) off the Galapagos (Deichmann 1941, Opresko 2003). Successively the latter species was reported also from the Gulf of California to Ecuador coasts (3-76 m depth) (Kerstitch 1989, Hickman 2008). More recently, Reimer and Fujii (2010)

reported this species also from the shallow waters (12-35 m) of Costa Rica at Isla del Coco. Black coral surveys in Ecuador have been focused mainly on the Galapagos Islands, where antipatharians were threatened by the commercial harvest for jewellery during the 1970s (Martinez and Robinson 1983, Romero 1997). Despite the known presence of black corals along the continental coasts (provinces of Guayas, Manabí and Esmeraldas) (Martinez and Robinson 1983, Romero 1997), no specific studies were directed in these areas of Ecuador.

Machalilla National Park is located in the central-western region of the Ecuadorian coast in the province of Manabí, and it consists of more than 500 km² of protected land and coastlines. The area is known to host a high biodiversity of terrestrial fauna and also provides habitat to important marine species (Guzman and Cortes 1993, Glynn 2001, Cruz et al. 2003).

The main aim of this study was to estimate the species richness and abundance of black coral populations in the Park. Until now, few data have been available regarding the density, structure and environmental associations of antipatharian assemblages along the continental Pacific coasts of Central and South America. These data are particularly important because they represent a clear baseline for monitoring of the population dynamics of benthic organisms in an area subjected to periodic El Niño and La Niña events, which greatly affect the composition and abundance of marine communities (Arriaga 1999, Glynn 2001, Podestà and Glynn 2001). The second goal of this paper was to study the black coral forest as a center of marine biodiversity, describing, also from the molecular point of view, the benthic species living as epibionts on the antipatharians. Finally, new morphological details were observed and added to the description of the black coral species present in the area.

Materials and methods

Study area

The marine protected area included in Machalilla National Park stretches along the coast in front of the town of Machalilla and also includes several islands of volcanic origin, such as Islotes de Salango and Isla de La Plata (Fig. 1).

<<Fig. 1 here>>

The shallow-water marine biodiversity of Ecuador includes about 2000 species (Cruz et al. 2003), and is strongly influenced by several geomorphological, climatic and oceanographic factors (Glynn 2001). As a result of both ocean hydrodynamics and the orographic conditions, the area is characterized by strong seasonal climatic regimes. The Ecuadorian coasts are lashed by the Equatorial Front, a convergence zone where the warm, low-salinity Panama and Niño water masses moving southward, meet the cold, high-salinity Humboldt water mass moving northward. The northward shift of the Equatorial Front by a few degrees of latitude during the summer dry season allows the cold Equatorial Deep Current to rise and hit the coast in the region surrounding Machalilla National Park. The strong seasonal sea surface temperature (SST) variations (15-25 °C) and upwelling phenomena, together with periodic El Niño and La Niña events, greatly affect the composition and abundance of marine communities (Arriaga 1999, Glynn 2001, Podestà and Glynn 2001). Corals in particular may undergo mass mortality or adapt to such changes by exhibiting slower growth rates compared to other tropical regions (Martinez and Robinson 1983).

In the studied area, small coral gardens composed mainly of antipatharians, scleractinians and gorgonians, generally grow on gently inclined rocky walls or sparse rocks laid on sandy bottoms. The bathymetric extension of these coral assemblages is generally limited to a depths shallower than 40 m, below which hard substrate becomes limited as bottoms are mostly sandy.

Sampling sites

Black corals were studied between July and October 2009, by SCUBA dives conducted at 10 sites: four sites located around Isla de la Plata, and six situated along the coast of Machalilla National Park (Fig. 1).

Isla de la Plata is a small island (about 14 km²) 32 km from the continental coast. Four sites were investigated on the western side of the island: **Bahia Drake** (Fig. 1) is a small bay characterized by very slow currents and a sandy bottom with scattered large rocks between 20-25 m depth; **Punta del Faro** (Fig. 1) is characterized by a small reef of hard corals between 4-8 m depth followed by a rocky slope with moderate currents that is composed of sparse rocks mixed with sandy patches until a depth of 35 m. Below 45 m the bottom turns into a sandy plain. The first 10 m of both **Roca Honda 1** and **2** (Fig. 1) are entirely rocky, and then sand stretches down to 20 m, subsequently followed by large scattered rocks to a depth of 30 m. Both of these sites are subjected to strong currents.

The remaining six sites were selected in the area representing the continental part of Machalilla Park, comprising both coastal and insular zones. The northernmost site is **Machalilla** (Fig. 1), located off the coast of the village of Machalilla. The rocky bottom, with moderate currents, is colonized in the first 10 m by a dense meadow of gorgonians (*Muricea* spp.), which mix with black corals up to a depth of 17 m, below which the bottom turns into a wide sandy plain. **Los Frailes** (Fig. 1) is situated in front of Los Frailes beach: scattered rocks and sandy patches characterize the bottom up to a depth of 17 m where a sandy plain starts. Currents at this site are moderate. The bottom of **Punta Mala** (Fig. 1), with strong currents, is characterized until 22 m by rocks and sandy patches ending with a sandy plain. The southernmost site is located between the two small islands of **Islotes de Salango** (Fig. 1). The bottom is characterized by weak currents and contains scattered rocks from 11-26 m depth, below which is a gently inclined sandy area.

Los Ahorcados 1 and 2 (Fig. 1) are located on the eastern and western sides of the islands of Los Ahorcados. The rocky cliffs, characterized by weak currents, stretch from 3 to 23 m depth, below which they give rise to sandy areas occupied by a dense meadow of gorgonians.

Taxonomic analysis of black corals

The taxonomic identity of black corals and their associated fauna was analyzed using morphological characters and was conducted on 24 black coral colonies. Colonies were photographed underwater, and the height, width and basal diameter of each colony were measured using a tape and a caliper. Portions of colonies were cut and each sample was then fixed partly in 4% formaldehyde for analyses of polyps, cnidomes and epibionts, while the remaining material was kept dry to study the patterns of ramification, spines and epibionts.

The cnidome of each specimen (considered in different portions - mouth, tentacles and interpolyar coenenchyme) was studied using an optical microscope by squeezing the tissues onto a slide. For scanning electron microscope (SEM) analysis, fragments of branches were washed with distilled water, dehydrated in a graded ethanol series, and dried in a Critical Point Dryer. Finally, they were coated with gold-palladium in a Balzer Union evaporator and examined with a Philips XL20 SEM.

Analysis of epibiontic zoanthids

To ascertain the taxonomic identification of the zoanthids observed on the black coral colonies, seven samples were collected and preserved in 70° ethanol for morphological and phylogenetic analyses. Three samples corresponded to a red zoanthid phenotype growing on *Antipathes galapagensis*, one sample corresponded to a cream phenotype growing on *Myriopathes panamensis*, and three samples were of both zoanthids phenotypes growing on the

same colonies of *M. panamensis*. *In vivo* aspect was documented with underwater photographs and measurements of the living polyps were gathered using a caliper. Height, diameter and number of tentacles were obtained using a stereomicroscope.

Phylogenetic analyses were carried out on four zoanthid samples, two red phenotypes (sample numbers: DNA1, DNA7) and two cream phenotypes (DNA3, DNA4). DNA extraction was made as in Reimer et al. (2008a) using a Qiagen (Santa Clarita, CA, USA) Dneasy Blood and Tissue Extraction kit following the manufacturer's protocol. Mitochondrial 16S ribosomal DNA (mt 16S rDNA), cytochrome oxidase subunit I (COI), and the internal transcribed spacer of nuclear ribosomal DNA (ITS-rDNA) were amplified using primers and protocols from previous zoanthid-focused research (Reimer et al. 2004; 2007; Sinniger et al. 2005). Amplified products were visualized by 1.5% agarose gel electrophoresis, and sequenced at MacroGen Japan (Tokyo, Japan). New zoanthid sequences obtained in the present study were deposited in GenBank (accession numbers XXX-XXXX), and compared for similarity with previous zoanthid sequences both by National Center for Biotechnology Information's Basic Local Alignment Search Tool (NCBI BLAST, Altschul et al. 1990) and by manual visual comparison using the software Se-Al v2.0a11 (University of Edinburgh). Sequences were only compared by similarity, and no further phylogenetic analyses were performed, as the utilization of mt 16S rDNA and COI sequences in tandem (along with ITS-rDNA) has been shown to be generally accurate for barcoding of zoanthids to the species level (Sinniger et al. 2008).

Coral distribution and epibiosis

The bathymetric and geographic distributions (colonies $m^{-2} \pm SE$ and percentage abundance) of the black corals were studied through visual census using a modified Belt Transect Method (Bianchi et al. 2003), which has previously been employed to investigate

antipatharian communities in Indonesia (Tazioli et al. 2007). Visual census was performed by two divers swimming along a linear 50 m horizontal transect (indicated by a line) for each considered depth range, for a maximum of eight ranges, depending on the site (5-10-15-20-25-30-35-40 m). The divers counted the number of colonies visible in a distinct 1 m wide visual field on each side of the line (for a total investigated surface of 100 m² at each depth range).

Similarly, to study the level of epibiosis of the zoanths and to obtain an indicative percentage data of colonized coral colonies, two transects (each 100 m long and 2.5 m wide) were performed by two separate divers at a depth of about 20 m at the Punta Mala site (where both coral species are well represented). The divers counted the total number of coral colonies and noted the presence/absence of zoanths on each colony. For each colonized colony, the following data were gathered: antipatharian species, zoanthid species and an estimate of the percentage surface of the antipatharian colony covered by zoanths (25%, 50%, 75%, 100%).

Results

Species description

Of the 24 collected specimens, six belonged to *Antipathes galapagensis* (family Antipathidae) and 18 to *Myriopathes panamensis* (family Myriopathidae).

The morphology of the *Antipathes galapagensis* colonies from Machalilla National Park, is in accordance with the descriptions previously reported in literature (Deichmann 1941, Kerstitch 1989, Opresko 2003, 2009, Hickman 2008). Colonies show an arborescent morphology characterized by numerous, dense ramifications ending, in the most peripheral zones of the corallum, with short rigid branchlets inclined upwards (Fig. 2A-F). Adult colonies may reach large dimensions, as indicated by the underwater measurements of 17 specimens: a height of 10-78 cm, width between 15-95 cm and basal stem diameter between 0.8-3.2 cm. Larger colonies

have been previously reported, up to 1 m high (Hickman 2008) and 2 m in diameter (Kerstitch 1989). The colour of the polyps varies between different tonalities of yellow (Fig. 2). Spines on the major branches are triangular-conical with a rounded and papillose apex, while on the pseudo-pinnules they are more triangular with a pointed, tuberculated tip (Fig. 2G-J). Polyps are radial with cylindrical tentacles, irregularly arranged in one line (side by side) on the pseudo-pinnules, but sometimes occur on two opposite lines (generally on the major branches) (Fig. 2K-M). The cnidome consists of basitrich isorhizae (19.0-21.7 x 3.0-4.0 μm) (Fig. 2N), microbasic mastigophores (18.6-24.5 x 4.0-5.0 μm) (Fig. 2O) and spirocysts (12.0-16.6 x 3.0 μm) (Fig. 2P). <<Fig. 2 here>>

Similarly, the morphology of the specimens assigned to *Myriopathes panamensis* is generally in accordance with what has previously been reported in literature (Verrill 1869, Brook 1889, Opresko 1976, 2001, Hickman 2008). The examined colonies show a flabellate, ramified and pinnulated morphology (Fig. 3A-F). They reach notable dimensions, with heights between 22-150 cm, widths between 28-340 cm, and basal diameters between 0.8-5.9 cm. Colonies tend to grow in width, getting thicker thanks to the successive overlapping of ramified fans, which arise from a short basal stem (Fig. 3A-B). Colonies show a great variability of colours: the most common type is brown (dark or light), but there are also orange, pink, green or grey phenotypes (Fig. 3A-C). Spines on branches are cylindrical, pointed, with a narrow base and a slightly papillose distal surface (Fig. 3G-H). On the major branches, spines are distributed irregularly. Spines on pinnules present a typical horn-shaped morphology (not laterally compressed), with a convex proximal side (Fig. 3I). Polyps are elongated in the transversal direction with cylindrical tentacles (Fig. 3J-N). They are arranged in one row on the same side of the colony, but on the stem and principal branches they may also contain smaller or incomplete zooids arranged irregularly (Fig. 3L-N). The cnidome of this species is made up of basitrich isorhizae (17.6-23.0

x 3.0-3.5 μm) (Fig. 3O), microbasic mastigophores (16.6-20.9 x 6.0-7.0 μm) (Fig. 3P) and spirocysts (12.0-18.0 x 3.0 μm) (Fig. 3Q).

<<Fig. 3 here>>

Associated fauna

Dead portions of all coral colonies were frequently encrusted by algae, ascidians, sponges, barnacles and bryozoans, while occasionally bivalves were found hanging on the branches of the colonies with their shells covered by the coral skeleton. The most representative organisms recorded as epibionts on the living portions of the corals were stalked barnacles (Fig. 4) and zoanthids (Fig. 5).

Myriopathes panamensis hosted numerous barnacles belonging to the genus *Oxynopsis*, commonly described in association with antipatharian colonies by previous authors (Totton 1923, Tazioli et al. 2007, Molodtsova and Poltarukha 2008) (Fig. 4A). Individuals were 0.7 cm long, with a 0.4 cm long capitulum and a 0.3 cm long peduncle. They grew mainly on stems and major branches of the colonies with an average density of 2.3 ± 0.3 specimens cm^{-1} (range between 1-5 individuals cm^{-1}) (Fig. 4B). Juvenile barnacles were occasionally observed, near the adult specimens (Fig. 4C). These epibionts anchored on the coral skeleton, which covered them with a thin sheet bearing spines and coenenchyme, from which polyps emerged (Fig. 4D-F). The coral spines covering the barnacles showed a different morphology with respect to the typical one. They were small (0.05 mm high), tuberculated and densely arranged near the aperture of the capitulum, where cirri emerged (Fig. 4G-H). On the remaining part of the valves, spines were more sparsely and irregularly distributed (Fig. 4I). The peduncle of the barnacle, finely corrugated, was covered by long (0.13 mm), tuberculated, densely packed spines (Fig. 4J-K).

The peduncle emerged from a flared opening of the tubular sheath formed on the axis of the coral branch (Fig. 4J). At times this cover caused adjacent ramifications to coalesce.

<<Fig. 4 here>>

The phylogenetic and morphological analyses identified two species of parasitic zoanthids: *Antipathozoanthus* cf. *hickmani* Reimer & Fuji, 2010 and *Terrazoanthus onoi* Reimer & Fuji, 2010 (Fig. 5). Specimens of *A.* cf. *hickmani* had small white-yellow polyps, uniformly arranged along the ramifications (4-6 polyps cm⁻¹), 1.5-3 mm high from the coenenchyme, with an oral disk 1.5-2.5 mm in diameter, and 27-28 white tentacles (1.8-2.5 mm long when expanded) emerging from a light yellow column entrapping sand inclusions (Fig. 5A-B). The large crowded polyps of *T. onoi* (3-4 polyps cm⁻¹), bright red to red-brown in coloration, were 1.5-7 mm high from the coenenchyme, with an oral disk 1.5-4 mm in diameter, and 32-33 red tentacles (2-3.5 mm long when expanded) emerging from a light brown column with densely packed sand inclusions (Fig. 5C-D). The polyps of both species were often covered by a thin red film of algae (Fig. 5B, D).

Acquired novel DNA sequences (n=2 each) of mitochondrial 16S ribosomal DNA (mt 16S rDNA), cytochrome oxidase subunit I (COI) and nuclear internal transcribed spacer of ribosomal DNA (ITS-rDNA) for the red phenotype zoanthids all matched 100% with previously reported sequences for *Terrazoanthus onoi* from the Galapagos Islands (Reimer et al. 2008, Reimer & Fujii, 2010). mt 16S rDNA and COI sequences (n=2 each) for the cream phenotypes matched 100% with previously reported specimens of *Antipathozoanthus* from the Galapagos (= *A. hickmani*; Reimer et al. 2008; Reimer and Fujii 2010) and Cape Verde (= *A. macaronesicus*). PCR reactions for ITS-rDNA sequences for these specimens were unsuccessful despite repeated attempts.

During the visual census, 117 black coral colonies were counted, 44 of which hosted parasites (corresponding to 38% of the total colonies). Of the two black coral species, *M. panamensis* was most affected (51% of the counted colonies) (Fig. 5E), while *A. galapagensis* was less colonized (30% of the counted colonies) (Fig. 5F-G). The majority of the colonized colonies were covered by up to 50% of their surface (34% and 39% of colonized colonies, respectively, for 25 and 50% coverage of the colony) (Fig. 5E). 20% of the colonized colonies were covered by up to 75% of their surface (Fig. 5F), while only a small proportion (7%) was completely covered. At all sites, most covered colonies lacked numerous thinner ramifications, with the zoanthids crowding the stem and major branches, while only apical portions of pseudo-pinnules were parasite-free (Fig. 5G).

Generally only one species of zoanthid was recorded on each black coral colony, but a few *M. panamensis* specimens hosted both zoanthid species (Fig. 5H-K). In these cases, zoanthids clearly occupied different regions of the colony, with *T. onoi* on the stem and major branches, and *A. hickmani* on the thinner branchlets and pinnules (Fig. 5J-K). Occasionally the two zoanthid species were observed mixed in the areas of contact (Fig. 5K). The observed colonies of *A. galapagensis* hosted only the red polyps of *T. onoi*. This species was also occasionally seen on various gorgonian species (Muriceidae) present in the area.

<<Fig. 5 here>>

Black corals distribution

A total of 4294 colonies were counted along the 20 transects (2 for each site). The average density of the black corals in the Machalilla area is 0.5 ± 0.05 colonies m^{-2} . The highest values were recorded for insular sites in the area of Isla de la Plata (Punta del Faro and Bahía Drake with average densities of 0.9 ± 0.1 colonies m^{-2} and 1.0 ± 0.5 colonies m^{-2} respectively)

and at Islotes de Salango (0.9 ± 0.3 colonies m^{-2}) (Fig. 6). The localities showing lower abundances are the coastal site of Machalilla (0.2 ± 0.1 colonies m^{-2}) and the insular sites close to the coast, namely Los Ahorcados 1 with an average density of 0.1 ± 0.04 colonies m^{-2} , and Los Ahorcados 2 (0.2 ± 0.07 colonies m^{-2}).

<<Fig. 6 here>>

Antipathes galapagensis was the species with the highest density (3.3 ± 0.4 colonies m^{-2} , with a maximum of 185 colonies in a single track), while *Myriopathes panamensis* had a density of 1.5 ± 0.4 colonies m^{-2} , for a maximum of 138 colonies in a single track. Consistent with this, 62% of the total investigated colonies belong to *A. galapagensis*, and 38% to *M. panamensis*.

The total average density distribution of antipatharians at different depths in Machalilla National Park shows a progressive increase from 5-30 m depth range (1.2 ± 0.2 colonies m^{-2}) when the substrate was generally rocky (Fig. 7).

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The bathymetric trend of the black coral density varied as a function of the availability of rocky substrate, but similar patterns between sites were observed. In the area of Isla de La Plata different distributions were observed (Fig. 8A-D): at Bahia Drake the community was dominated by *A. galapagensis* (maximum average density of 3.3 ± 0.4 colonies m^{-2} at 25 m depth) while *M. panamensis* was almost absent (Fig. 8A). At Punta del Faro (Fig. 8B) both species were present with *M. panamensis* more common in shallow transects (1.5 ± 0.4 colonies m^{-2} at 20 m depth) and *A. galapagensis* more common in deep waters (1.8 ± 0.07 colonies m^{-2} at 40 m depth). A very similar situation was recorded in both sites of Roca Honda (Fig. 8C-D).

<<Fig. 8 here>>

In the coastal area of the park, the distribution pattern described for Isla de La Plata, characterized by a higher abundance of *A. galapagensis* and by a preference of this species for the deeper regions, was confirmed for all sites (Fig. 8E-J).

The height and width ratio of the studied specimens gives some indications about the growth strategy of these species. *M. panamensis* has a typical flabellate colony with maximum expansion in width (average ratio height/width =0.6). There was no evident difference with respect to the collection site, however, considering width, the colonies of Los Frailes, Punta Mala, Machalilla and Punta Faro (characterized by stronger currents) were larger. As for *A. galapagensis*, the height to width ratio was 0.8, indicating that the colonies tend to have a more arborescent morphology, with no evident difference in width and height. *A. galapagensis* colonies were also larger at sites with stronger currents (Machalilla and Los Frailes).

Discussion

This work characterized the taxonomy and ecology of the black coral fauna found in Machalilla National Park (Ecuador), considered a high biodiversity area of the Ecuadorian coast (Cruz et al. 2003). The taxonomic study supplied new information about two black coral species, *Myriopathes panamensis* and *Antipathes galapagensis*. These species had previously been recorded from Ecuador (mainly Galapagos Islands) (Opresko 1976, Opresko and Grigg 1977, Martinez and Robinson 1983, Kerstitch 1989, Romero 1997, Opresko 2001, Opresko 2003, Hickman 2008), however, here we add a description of morphological details of living colonies, as well as new information on their distribution and associated fauna, which seems typical of the continental assemblages.

Antipatharians hold an important ecological role in benthic communities, in particular due to their branched morphology, which can host a rich, sometimes species-specific, associated

fauna (Totton 1923, Wirtz et al. 2001, Love et al. 2007, Tazioli et al. 2007). For example, for the Ecuadorian black corals, Kerstitch (1989) reported some mimic vagile fauna living on the branches of *A. galapagensis*, such as the shrimp *Periclimenes infraspinis* (Rathbun, 1902) and the fish *Oxycirrhites typus* (Bleeker, 1857). The recorded stalked barnacles of the genus *Oxynapsis*, epibionts of *Myriopathes panamensis*, exploit the net of branches as a settling space. This genus is described as exclusively associated to antipatharians, with 14 species found on eleven black coral genera (Molodstova and Poltarukha 2008). Our record confirms that these organisms prefer densely reticulated colonies (Molodstova and Poltarukha 2008) like those of *M. panamensis*, where the filtering efficiency is higher.

The morphological modifications of the black coral's spines growing on the stalked barnacles have already been documented in the literature (Molodstova and Poltarukha 2008). In our case, the barnacle's settling induces a skeletal reaction resulting in an outgrowth of skeletal tissue with modified spines. The different morphology of the spines on the carbonatic plates of the barnacle may depend on some kind of chemical reaction caused by the crustaceans (Molodstova and Poltarukha 2008), however explanations concerning the settling, growth and spine modification of these organisms are still lacking.

Parasitic zoanthids are known to occur on black corals, such as those of the recently established genus *Antipathozoanthus* (family Parazoanthidae) (Sinniger et al. 2009), which is currently limited to species associated with antipatharians, including *A. macaronesicus* (Ocaña and Brito, 2003) living on *Tanacetipathes cavernicola* Opresko, 2001. The parasitic zoanthids recorded on Ecuadorian black corals belong to two species, *Antipathozoanthus* cf. *hickmani* and *Terrazoanthus onoi*, and were first described from the Galapagos Islands (Reimer and Fujii 2010).

Some morphologic and ecologic differences were observed for the zoanthids with respect to their original descriptions. The morphometric analysis of our cream phenotype did not completely match with what has been previously been reported for *A. cf. hickmani*. Moreover, *A. galapagensis*, has been considered to be the specific host of *A. hickmani* (Hickman 2008, Reimer et al. 2008, Sinniger et al. 2009, Reimer and Fujii 2010), but in this study *A. cf. hickmani* was always found associated with *M. panamensis*. Thus, due to morphological differences in tentacle number, plus preference for a different substrate, the identity of the cream phenotype specimens as truly *A. hickmani* or instead a closely related but different species remains unknown. Furthermore, both *A. hickmani* from the Galapagos and *A. macaronesicus* from the Atlantic have identical mt 16S rDNA and COI sequences, suggesting that DNA barcoding using these DNA markers may not always be accurate for *Antipathozoanthus* spp.

Both black coral species were instead covered by *T. onoi*, a zoanthid known to occur from the Gulf of California to Galapagos (3-76 m depth), but described as usually settling on abiotic substrates (Kerstitch 1989, Hickman 2008, Reimer and Fujii 2010), and, occasionally, encrusting gorgonian stalks (Kerstitch 1989). In the study area, however, it was never recorded on abiotic substrate, although we did not specifically search in areas without antipatharians. However, all three DNA markers, including the faster-evolving ITS-rDNA marker, as well as morphological data for red phenotype zoanthids fit with *T. onoi*, and thus we are confident that red phenotype specimens in this study belong to this species.

The parasitic association between zoanthids and black corals was the most common symbiotic relation in the study area, and apparently greatly effects the survival of antipatharians. A large proportion of antipatharian colonies hosted zoanthids (38%), and approximately 7% were completely covered by their polyps. It seems that there was a slight preference for *M. panamensis* for both zoanthid species, but *A. galapagensis* was more often completely covered.

The thick pseudo-pinnules of *A. galapagensis* may enhance the expansion of the large polyps of *T. onoi* up to the most peripheral region of the colony.

The epibiosis of *T. onoi* on black corals generally starts from the stem of the colony and progressively covers the branches until the complete death of the coral. The colonies with skeleton greatly covered by *T. onoi* showed a more simple ramification pattern due to the breakdown of small branches where the large zoanthid polyps were not able to expand. *A. cf. hickmani*, perhaps because of its smaller polyps, is more adapted to expand on the smaller branches and pinnules of *M. panamensis*. A similar differentiation in the growth strategy by two zoanthids on a host has been recently described in Indonesia for two *Hydrozoanthus* species growing on the hydroid *Dentitheca habereri* (Stechow, 1909) (Di Camillo et al. 2010), suggesting that polyp size may be a constraining factor for zoanthid expansion.

Due to their rigid arborescent structure black corals are prone to epibiosis from encrusting filter feeders. In Hawaii more than 80% of the large bushy colonies of *Antipathes griggi* Opresko, 2009 and *A. grandis* Verrill, 1928 were described as affected by the epibiosis of the alien octocoral *Carijoa riisei* (Duchassaing & Michelotti, 1860) (Kahng and Grigg 2005). The parasitic overgrowth was reported to start on patches of bare skeleton, and then extended to the entire surface of the corals causing their death. Similarly to zoanthids, the epibiosis of *C. riisei* also started on the basal major branches and stems, expanding towards the distal ends of the colonies. In contrast to what is reported here, the octocoral didn't cause the breakdown of thinner ramifications, but continued to rapidly grow along all pinnules as a thin sheet, such that it completely covered the corals, which kept their original branching pattern unchanged (Kahng and Grigg 2005). Finally, because of the similar corallum morphology, no different overgrowth patterns were observed between the two Hawaiian black coral species (Kahng and Grigg 2005).

The visual census revealed that black corals, together with gorgonians, are among the most important 3 dimensional-structuring organisms of the Ecuadorian reefs. The dominant species was *A. galapagensis* with 62% of the total counted colonies distributed along the entire depth range with a peak of abundance towards the maximum surveyed depths (25-35 m). Also at Galapagos Islands this species is mainly distributed at major depths (Kerstitch 1989, Hickman 2008). On the contrary, *M. panamensis* is more distributed between 15-25 m depth. These data partially corroborate previous observations conducted on vertical rocky faces at Galapagos, where the maximal density of black coral species is at 15 m depth (Martinez and Robinson 1983). Indicatively, on the basis of the same studies, the observed coral colonies of Machalilla (with special reference to *M. panamensis*), showing a basal diameter of 0.8-6.0 mm, were between 3 and 14 years old.

The observed site-related differences in black coral abundances, strongly depend on the topography of the bottom. Particularly, differences were observed between insular sites and coastal sites, with the former being characterized by higher densities. With the exception of Bahia Drake, insular sites present a more extended rocky slope, while coastal sites are characterized by rocky masses up to 15-20 m depth with wide sandy patches. It therefore seems that the availability of rocky substrate is the most important abiotic factor limiting the settling of black corals in this area.

At some sites (Machalilla, Los Ahorcados, Punta Mala, Islotes de Salango) the first 10 m are dominated by fan-shaped gorgonians, which progressively give way to black corals in the deeper depth ranges. Fan-shaped gorgonians occur again on the deep sandy plains, where antipatharians cannot settle. A similar octocoral-antipatharian distribution has been recorded at some Colombian reefs where zooxanthellate gorgonians, better adapted to tidal action, inhabited

shallow areas, while azooxanthellate species shared the deeper ranges with antipatharians (sciophilous and adapted to greater inclinations) (Sanchez et al. 1998, Sanchez 1999).

The studied antipatharian community shows an average total density of 0.5 ± 0.53 colonies m^{-2} , higher than what has been reported in literature for different areas: 0.25 colonies m^{-2} for Indonesian reefs (Tazioli et al. 2007), up to 0.2 colonies m^{-2} for a Hawaiian community (Grigg 1965) and 0.25 colonies m^{-2} for an *Antipathella fiordensis* (Grange, 1990) meadow in the New Zealand fjords (Grange 1985). Density data reported from a Colombian community including both octocorals and antipatharian corals was 3.2 colonies m^{-2} , but black corals only contributed a minor part of this value (Sanchez et al. 1998). Up to now only a Mediterranean meadow of *Antipathella subpinnata* (Ellis & Solander, 1786) has been found to have a higher density (1.4 colonies m^{-2}) (Bo et al. 2009).

Despite the high abundance of black corals, the species richness of the Ecuadorian shallow-water community is low and consists of only two species (Martinez and Robinson 1983). This low coral diversity is probably due to the oceanographic features of the area characterized by a complex hydrographic system creating strong seasonal variations in water temperature and salinity (Glynn 2001). This, together with the periodic events of El Niño and La Niña, induces a stress in benthic organisms that reduces the marine biodiversity of the area.

Moreover, on an oceanic scale, it is possible to identify a decreasing biodiversity gradient of shallow-water black coral fauna from the Indonesian “hot spot” (several dozens of species; Schultze 1886, Brook 1889, Cooper 1909, Silberfeld 1909, van Pesch 1914, Tazioli et al. 2007), through to Hawaii (7 species; Grigg and Opresko 1977, Grigg 2001, Fenner 2005), to the Ecuadorian coast (2 species).

Black corals have been intensively collected in Ecuador, from the 1980s, and have completely disappeared at several sites (Martinez and Robinson 1983, Romero 1997). Currently

collection is strongly reduced, both because of the over-exploitation of the resource stock, and also likely due to management within the marine protected areas, such as the Galapagos Islands and Machalilla National Park. The data presented here may represent a benchmark for future studies focused on the coral reef management in Machalilla Park and to aid in developing recovery plans for black coral populations.

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Figure captions

Fig. 1 Sampling sites. Map of the boundaries of Machalilla National Park and location of sampling sites. **1.** Bahia Drake, **2.** Punta del Faro, **3.** Roca Honda 1, **4.** Roca Honda 2, **5.** Machalilla, **6.** Los Frailes, **7.** Punta Mala, **8.** Islotes de Salango, **9.** Los Ahorcados 1, **10.** Los Ahorcados 2.

Fig. 2 *Antipathes galapagensis*. **A-B-C.** Underwater photographs of *Antipathes galapagensis* colonies. **D.** Juvenile colony. **E-F.** Pattern of pinnulation of the colonies. **G-H-I-J.** Arrangement of the triangular, tuberculated spines on the ramifications and close-up views of the spines ornamentation. **K-L.** *In vivo* pictures of the expanded polyps showing the irregular arrangement of the zooids on both one and two opposite rows. **M.** Polyps with sagittal expanded tentacles. **N-O-P.** Cnidome composed of three typologies: isorhiza (**N**), mastigophore (**O**), spirocyst (**P**). Scale bar: A-B-C: 20 cm, D-E: 5 cm, F: 1 cm, K-L-M: 2 mm, G: 0.5 mm, H: 0.2 mm, I-J: 0.1 mm, N-O-P: 5 μ m.

Fig. 3 *Myriopathes panamensis*. **A-B.** Underwater photographs of *Myriopathes panamensis* colonies. **C.** Underwater photograph of the grey, single-fan variation of a *M. panamensis* colony. **D.** Pattern of ramification. **E-F.** Different patterns of pinnulation, with pinnules respectively more or less densely arranged. **G-H.** Arrangement of the cylindrical spines on major ramifications. **I.** Arrangement of the horn-shaped spines of a pinnule. **J.** *In vivo* picture of the expanded polyps. **K.** Polyps with elevated oral cones and sagittal tentacles inserted at a lower level. **L.** Radial polyps irregularly distributed on a branch. **M-N.** Transversally elongated polyps arranged in one row on the pinnules. Cnidome composed of three typologies: isorhiza (**O**), mastigophore (**P**), spirocyst (**Q**).

Scale bar: A-B-C: 20 cm, D-E-F: 1 cm, J: 5 mm, L-N: 1 mm, K-M: 0.5 mm, O-P-Q: 5 μ m.

Fig. 4 Associated stalked barnacles. **A.** Dry sample of *Myriopathes panamensis* with densely crowded associated barnacles. **B.** Arrangement of stalked barnacles on a ramification. **C.** Juveniles on the coral. **D.** Black coral polyps on the cirriped's valves. **E.** Barnacle anchored on the coral ramification. **F.** SEM image of the coral skeletal tissue covering the stalked barnacle. **G.** Spines along the aperture of the barnacle. **H. I.** Tuberculated spines on the capitulum surface. **J.** Skeletal gall near the anchoring area of the barnacle. **K.** Spines on the peduncle.

Scale bar: A: 2 cm, B-C: 0.5 cm, D-E-F-J: 1 mm, I-K: 0.5 mm, G: 100 μ m, H: 50 μ m.

Fig. 5. Associated zoanthids. **A.** Underwater photograph of *Antipathozoanthus* cf. *hickmani* on the pinnules of *Myriopathes panamensis*. **B.** Close-up view of the polyps of *A.* cf. *hickmani*. **C.** Underwater photograph of *Terrazoanthus onoi* on the pseudo-pinnules of *Antipathes galapagensis*. **D.** Close-up view of the polyps of *T. onoi* **E.** *T. onoi* completely covering a lateral branch of *M. panamensis*. **F.** *T. onoi* covering the stem and major branches of *A. galapagensis*. **G.** Free distal pseudo-pinnules of an *A. galapagensis* colony with parasitic *T. onoi* **H.** Colony of *M. panamensis* covered by both zoanthid species: *T. onoi* on the major branches and *A.* cf. *hickmani* on the lateral branches and pinnules. **J-K.** Co-mixture of the two zoanthid species along a branchlet of *M. panamensis*. Black arrow: *T. onoi*, White arrow: *A.* cf. *hickmani*.

Scale bar: F-G: 10 cm, E: 2 cm, H: 1 cm, A, B, C, D, J, K: 5 mm.

Fig. 6. Total average density (colonies $m^{-2} \pm SE$) in the 10 explored sites of Machalilla National Park.

Fig. 7. Bathymetric distribution of the total average density (colonies $\text{m}^{-2} \pm \text{SE}$) of black corals in Machalilla National Park.

Fig. 8. Bathymetric distribution of the species average densities (colonies $\text{m}^{-2} \pm \text{SE}$) in the explored sites: **A.** Bahia Drake, **B.** Punta del Faro, **C.** Roca Honda 1, **D.** Roca Honda 2, **E.** Machalilla, **F.** Los Frailes, **G.** Punta Mala, **H.** Islotes de Salango, **I.** Los Ahorcados 1, **J.** Los Ahorcados 2. *Myriopathes panamensis*, black; *Antipathes galapagensis*, white.

Figure 1

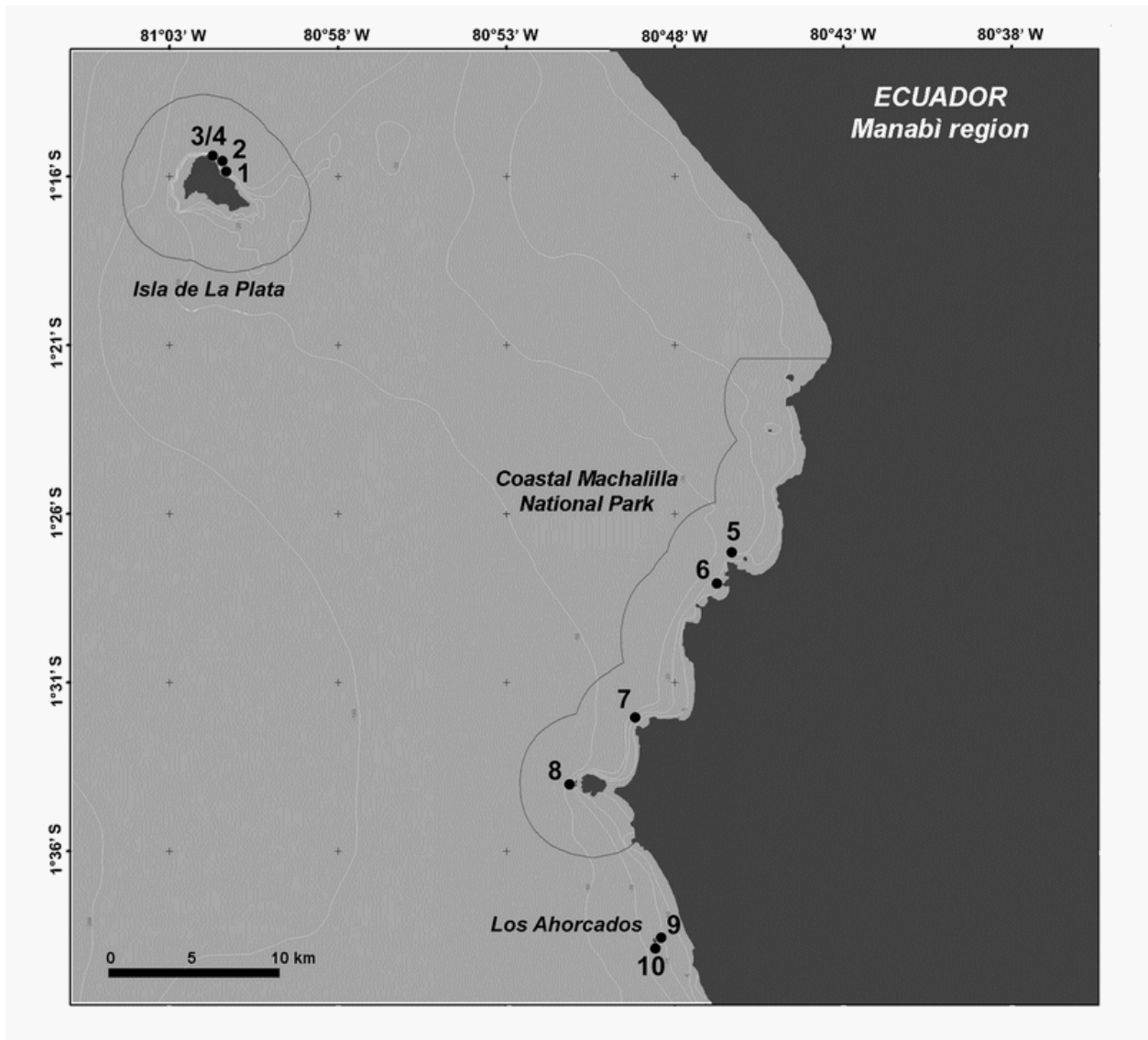


Figure 2

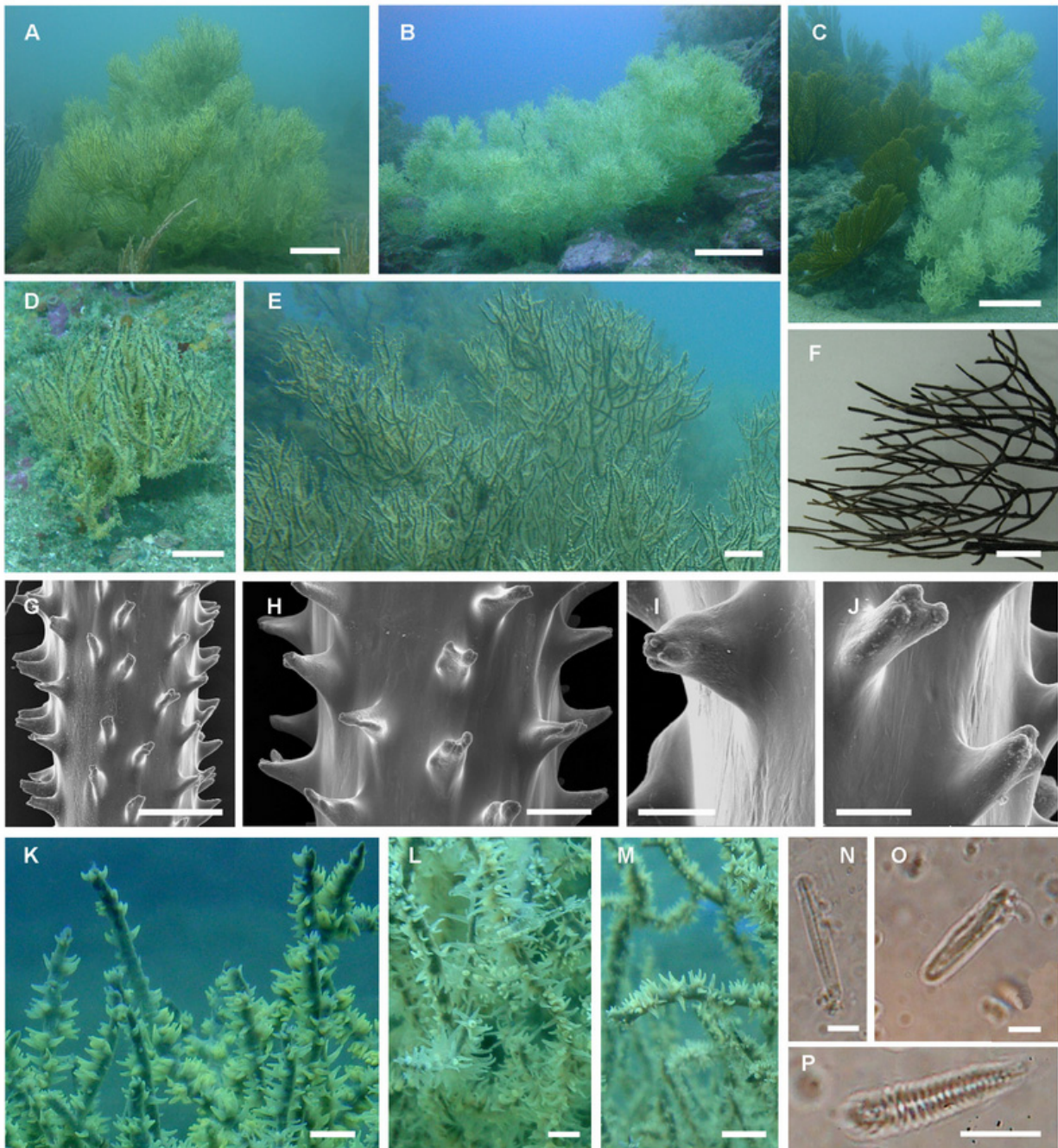


Figure 3

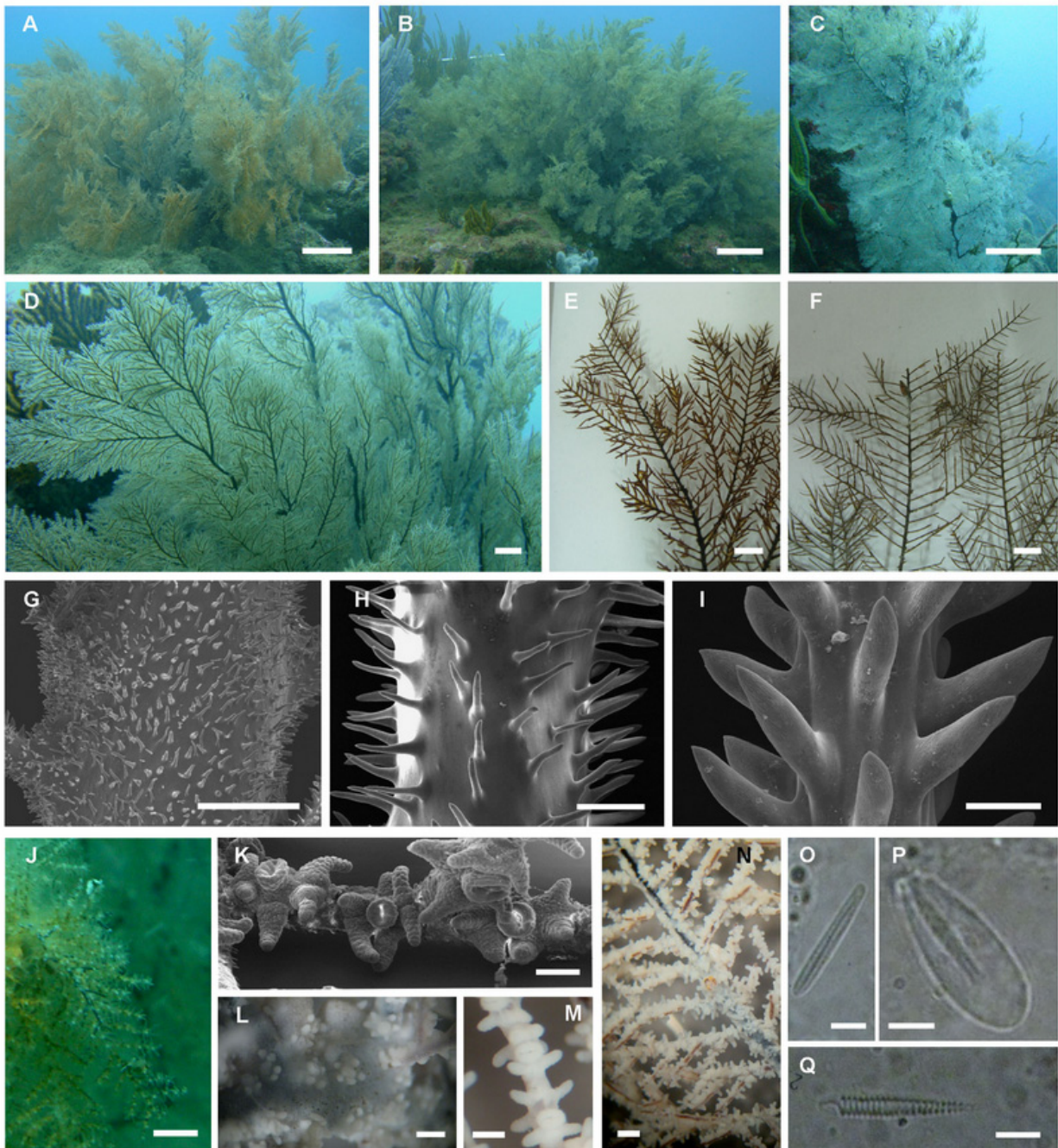


Figure 4

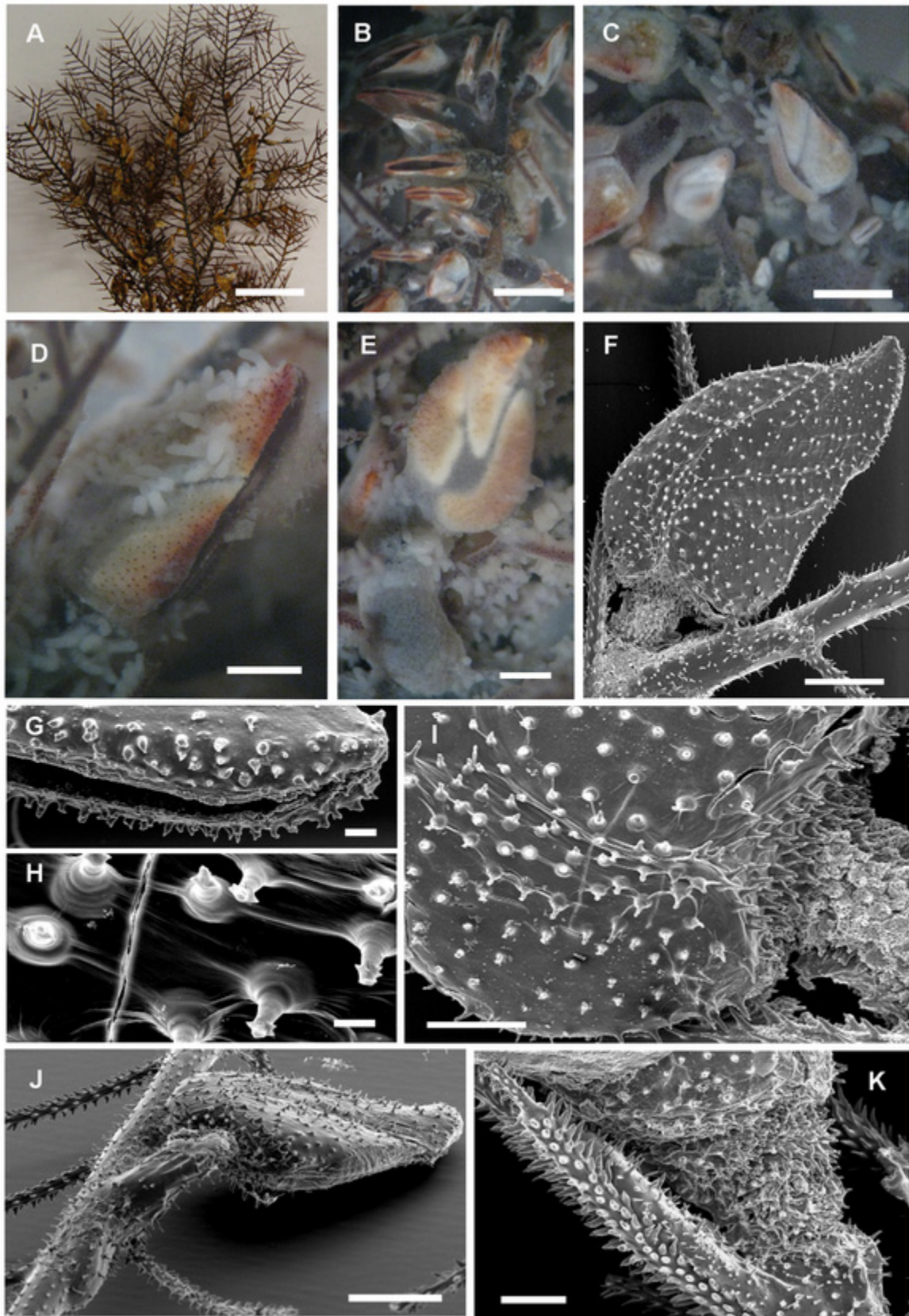


Figure 5

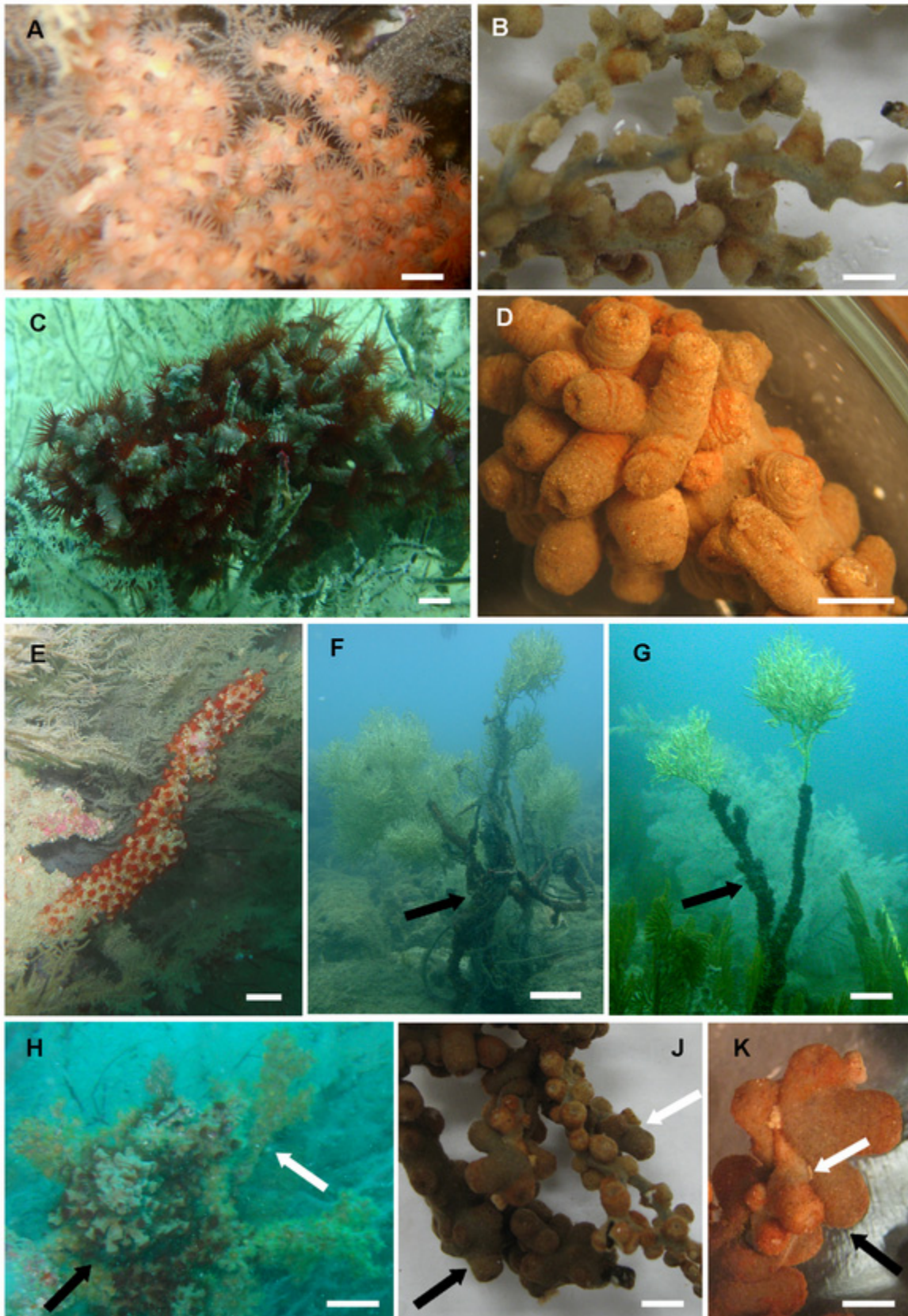


Figure 6

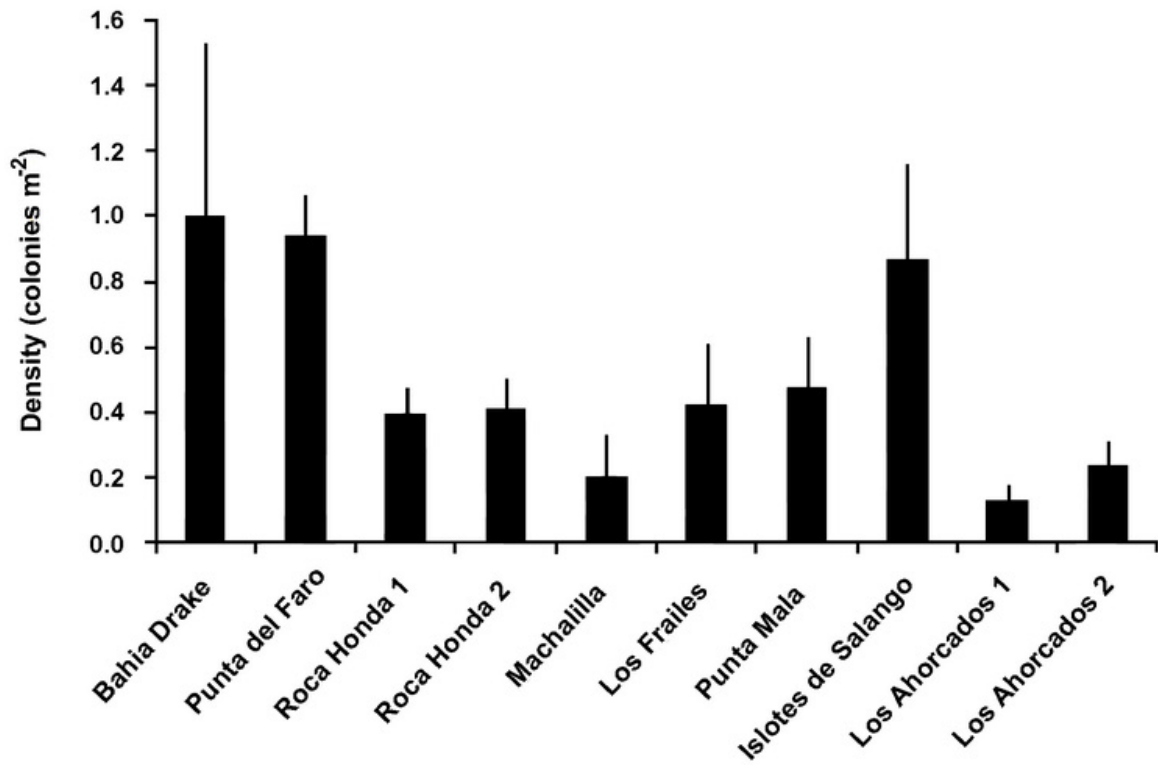


Figure 7

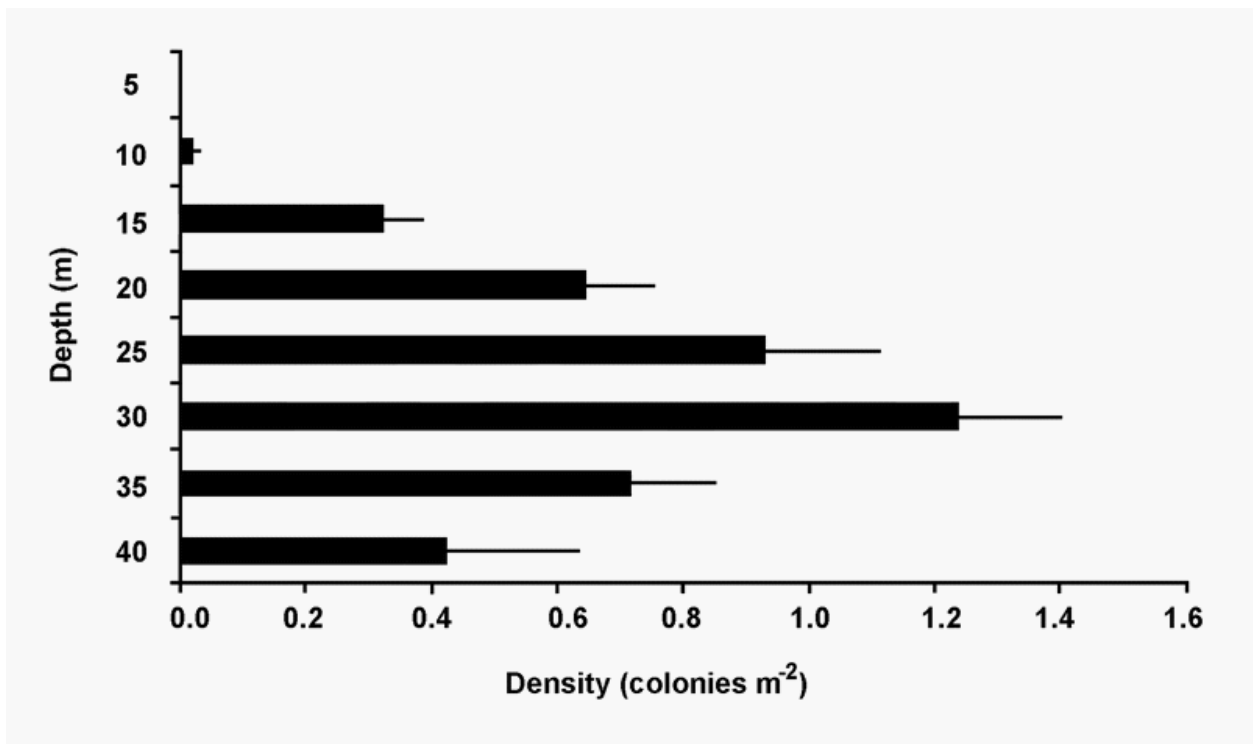


Figure 8

