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## RESEARCH ARTICLE

### **An enigmatic new octocoral species (Anthozoa, Octocorallia, Malacalcyonacea) from Isla del Coco National Park**

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**Running head:** An enigmatic new octocoral species from Isla del Coco

## Abstract

*Aliena parva* **gen. nov. et sp. nov.** is described from Cocos Island, Costa Rica. The species was found at various islets and rocky outcrops north and NW of the island, 20–30 m in depth. The genus is characterised by polyps retracting into low mounds forming thin encrusting mats extending on dead or live substrate. Sclerites are mostly asymmetrical spindles Anthocodial rods are arranged in points, not forming a collaret. Colonies and coenenchymal sclerites are red. Using an integrative taxonomic approach, we found the new genus to be different morphologically and genetically from all other described taxa. The molecular phylogenetic analyses provide strong support for the placement of this new genus in Order Malacalcyonacea, family Pterogorgiidae. Morphologically it is unlike any of the other members of this family, necessitating an amendment to the diagnosis of Pterogorgiidae. Like several other known taxa of octocorals with simple, encrusting growth forms, *Aliena* **gen. nov.** appears to have evolved from a gorgonian ancestor by loss of an internal skeletal axis. It is the first member of Pterogorgiidae to be reported from the eastern Pacific, contributing further to the knowledge of marine biodiversity in the eastern tropical Pacific and to the octocoral biodiversity of Cocos Island in particular.

## Keywords

Biodiversity, new species, new genus, oceanic island, soft corals, taxonomy

## Introduction

The occurrence of shallow water octocorals in Isla del Coco National Park is apparently rare in comparison to other oceanic islands like the Galápagos Islands (Ecuador) or the Revillagigedo Archipelago (México) where the number of reported species is higher (Williams & Breedy 2004, Bedolla 2007, Breedy & Cortés 2008, 2011, Breedy et al. 2009, Hickman 2008, Olvera et al. 2018). The reason for this difference is still unknown but could be due to high predation on these corals by other organisms or simply that more exploration is needed. The octocoral diversity in Isla del Coco increases towards mesophotic depths (Breedy et al. 2012, Cortés 2019, Breedy et al. 2021) and the fauna is different from the shallow communities. Presently five species have been reported from 10 to 35 m in depth: the gorgoniids *Leptogorgia alba* Verrill, 1868, *Leptogorgia tricolorata* Breedy & Cortés, 2008 and *Pacifigorgia curta* Breedy & Guzman, 2003, and two stoloniferans, *Carijoa riisei* (Duchassaing & Michelotti, 1860) and *Rhodolitica occulta* Breedy, McFadden, Murillo & Vargas, 2021. For the time being, three of them are considered endemic to Isla del Coco.

Recently a new octocoral was collected and photographed at several sites in the north part of the Island. Herein, we describe a new genus and species using an integrative taxonomic approach, combining morphological and molecular analyses in order to phylogenetically position this monospecific genus within Octocorallia. This study is a contribution to the knowledge of the octocoral biodiversity of Isla del Coco and marine biodiversity of the eastern tropical Pacific oceanic islands.

## Materials and methods

### Study site and collection methods

Isla del Coco National Park is an oceanic island located between 5°30'–5°34'N and 87°01'–87°06'W in the eastern Tropical Pacific (ETP) approximately 500 km southwest of Costa Rica and more than 600 km northeast of the Galápagos Islands, Ecuador (Cortés 2016, Breedy et al. 2021) (Fig. 1). The specimens were collected by scuba diving down to 30 m in depth from various points north and north-west of the Island. The colonies were observed and photographed *in situ* during four different trips, August 2021, October 2021, December 2021 and January 2022. Samples were collected and preserved in 95% ethanol for further analyses. The holotype and paratypes are deposited at the Zoology Museum, University of Costa Rica, Costa Rica (MZUCR).

### Morphological analysis

For taxonomic identification, external characters of the colony were analysed from the *in situ* photographs and dissection of collected samples under a stereoscope. For internal characters, sclerites from polyps and coenenchyme were obtained by dissolving the tissue in 5% sodium hypochlorite; dissociated sclerites were washed several times in distilled water until organic matter was completely removed, dehydrated with 100% ethanol, and subsequently dried in an oven. Sclerites were prepared for light microscopy, mounted in glycerine, and photographed with an Olympus LX 51 inverted microscope. For scanning electron microscopy (SEM), sclerites

were mounted on SEM stubs by double stick carbon tape and silver paint, then sputter-coated with gold, 30–60 nm layer, in EMS 550X Ion Coater; the images were obtained using a FESEM Zeiss Sigma 300 and a Hitachi NSEM 3700 (at 15kV). Measurements of the sclerites were obtained from optical and SEM images. Taxonomic terminology follows Bayer et al. 1983.

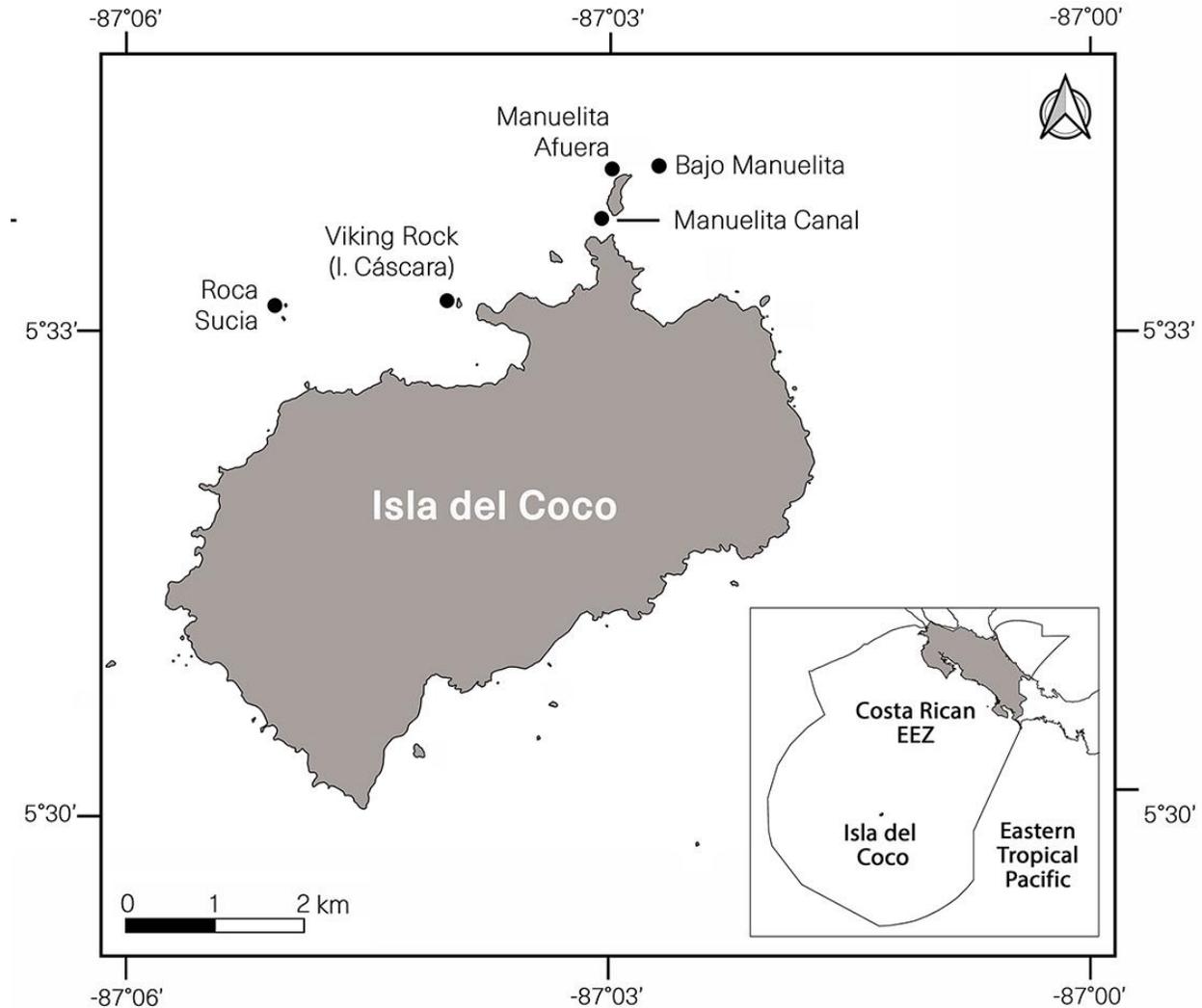


Figure 1. Map showing the locations where *Aliena parva* **gen. nov. et sp. nov.** was found in Isla del Coco, Costa Rica. Map by Beatriz Naranjo, University of Costa Rica.

#### Molecular phylogenetic analysis

DNA was isolated from ethanol-preserved tissue of two specimens using the modified salting-out protocol of Herrera (2022). The mitochondrial *mtMutS* and nuclear *28S rDNA* markers commonly used for DNA barcoding of octocorals were amplified using PCR with previously published primers (ND4-2599F and mut3458R for *mtMutS*; 28S-Far and 28S-Rar for *28S rDNA*) and protocols (McFadden et al. 2011; McFadden and van Ofwegen 2013). Amplicons were purified by PEG-precipitation (Sánchez et al. 2003) and Sanger-sequenced. Sequences for *mtMutS* were aligned by eye to the reference alignment of McFadden et al. (2022). This alignment includes representatives of all genera of octocorals for which *mtMutS* sequences are

available. Preliminary maximum likelihood phylogenetic analyses using PhyML (Guindon and Gascuel 2003) supported the placement of the new species in Order Malacalcyonacea. Further phylogenetic analyses of *mtMutS* were conducted using just the subset of the alignment that included taxa from that Order. Sequences for 28S *rDNA* were aligned to a reference dataset that included as many as possible of the same individuals that were included in the *mtMutS* alignment. 28S *rDNA* sequences were not available for 23% of the taxa in the *mtMutS* dataset, and for 9% of the taxa we included a 28S *rDNA* sequence that was from a different specimen of the same species (Supplemental Table 1). 28S *rDNA* sequences were aligned using MAFFT (Kato et al. 2005) and subsequently trimmed internally to remove regions of poor alignment using gblocks (Castresana 2000) as implemented at Phylogeny.fr (Dereeper et al. 2008). Phylogenetic analyses were run separately for each alignment. Maximum likelihood analyses were run using IQTree v.2.1.2 (Nguyen et al. 2015) with the model of evolution (TVM+F+R5 for *mtMutS*; GTR+F+R4 for 28S *rDNA*) selected by ModelFinder (Kalyaanamoorthy et al. 2017) and support from 1000 ultrafast bootstrap replicates (Hoang et al. 2018). MrBayes v.3.2 (Ronquist et al. 2012) was used for Bayesian phylogenetic reconstruction using a GTR+I+G model of evolution. MrBayes was run for  $8 \times 10^6$  (*mtMutS*) or  $5 \times 10^6$  (28S *rDNA*) generations (until standard deviation of split partitions  $<0.01$ ) with a 25% burnin and default Metropolis coupling parameters.

## Results

### Systematics

sub-Phylum Anthozoa Ehrenberg, 1831

Class Octocorallia Haeckel, 1866

Order Malacalcyonacea McFadden, van Ofwegen & Quattrini, 2022

Family Pterogorgiidae McFadden, van Ofwegen & Quattrini, 2022

Genus *Aliena* gen. nov.

**Diagnosis.** Colonies are thin encrusting mats of irregular shape. Polyps arise directly from the substrate, without axes, forming mats of 3 to 25 polyps in clusters. The mats may form individual patches or may be interconnected by thin coenenchymal extensions forming bridge-like bands between mats. Polyps are totally retractile into apertures that are slightly raised on calyx-like mounds covered by a dense layer of small sclerites producing a granular appearance. When the polyps expand, the large gastric cavities extend high over the polyp mounds. The polyp oral disk prolongs into eight rays marked by small red rods along the intertentacular margins. Tentacles are long, thin, and transparent with yellow sclerites. Coenenchyme is thin without differential sclerite layers. The coenenchymal sclerites are irregular spindles with short spines and irregular ends, up to 0.35 mm long. Anthocodial sclerites are mostly red flat rods, with serrated or spiny borders and sparse warts on the surface, and spine-like rods, up to 0.30 mm long. They are arranged ‘*en chevron*’, forming points but not a collaret. Flat rods form longitudinal rows along the polyp body. Tentacular sclerites are mostly yellow biscuit-like rods, up to 0.10 mm long.

Colonies are dark red in life and preserved in ethanol. Coenenchymal sclerites are mostly red.

**Type species.** *Aliena parva* sp. nov. by original designation.

**Etymology.** *alienus* (L) foreign, strange, not related. The generic name refers to the unexpected appearance or unnoticed presence of a new shallow water taxon. It also alludes to its surprising phylogenetic relationship to a group of gorgonian octocorals, a relationship not predicted by its morphology. Gender feminine.

***Aliena parva* sp. nov.**

Figures 2–6

Material examined. **Holotype.** MZUCR 3679, lot 1, ethanol preserved, Manuelita Afuera, Isla del Coco, 05°33.791' N 87°02.934' W, 22 m depth, J. Cortés and M. Cruz, 4 December 2021.

**Paratypes.** MZUCR 3680, lot 2, same data as holotype. MZUCR 3681, lot1, lot2, ethanol preserved, Manuelita Canal, Isla del Coco, 05°33.524' N 87°02.940' W, 20–30 m depth, B. Naranjo, 12 October 2021. MZUCR 3682, ethanol preserved, Bajo Manuelita, Isla del Coco, 05°33.849' N 87°02.676' W, 22.5 m, J. Cortés and A. Klapfer, 6 December 2021. MZUCR 3683, Manuelita Afuera, Isla del Coco, 05°33.791' N 87°02.934' W, 29 m depth, O. Breedy, 28 September 2022. MZUCR 3684, Manuelita Afuera, Isla del Coco, 05°33.791' N 87°02.934' W, 25 m depth, O. Breedy, 4 October 2022.

**Type locality.** Isla del Coco, Pacific Costa Rica, 20–30 m in depth.

**Description.** The holotype is formed of 15 scattered clusters of polyps encrusting the surface of a barnacle about 4 cm in diameter; the barnacle plates are covered by many epibionts and several small unbranched hydroids (Fig. 2A). The polyps arise directly from the substrate, without axes. They are in clusters composed of 3–20 polyps. The polyps are closely spaced; they reach up to 0.20 cm tall when partially expanded (from the base to the proximal border of tentacles). The polyps are totally retractile into apertures that are slightly raised on calyx-like mounds. The polyp mounds are up to 0.75 mm in diameter, and up to 0.5 mm tall when the anthocodia is retracted. The surface of the mound is covered by a dense layer of small sclerites giving it a slight granular appearance. When polyps are fully expanded, the large gastric cavities of the polyps extend high over the polyp mounds and the oral disk prolongs into eight rays marked by small red rods along the intertentacular margins (Figs. 3B–D). The tentacles are long, thin, and transparent with yellow sclerites. When retracted the yellow sclerites can be observed at the polyp-mound summit (Fig. 2C). Coenenchyme is thin without differential sclerite layers; it extends over the substrate. Coenenchymal sclerites are irregular spindles with variable ends: pointed, blunt, bifurcated or a combination. They are straight or slightly curved, 0.16–0.35 mm long and 0.02–0.08 mm wide (Figs. 4A, 5A), with short spines, not very crowded on the surface; they do not have complex tubercles or waists. Anthocodial sclerites are mostly red flat rods, 0.20–0.30 mm long and 0.02–0.06 mm wide, with serrated or spiny borders and sparse warts on the surface (Fig. 4C, 5B) and spine-like rods with a bent end, 0.14–0.29 mm long and 0.02–0.03 mm wide (Fig. 5C). Anthocodial rods are arranged ‘*en chevron*’, forming points but not a

collaret (Fig. 2B). The flat rods form longitudinal rows along the polyp body (Fig. 2B). Tentacular sclerites are mostly yellow, biscuit-like rods, 0.06–0.10 mm long and 0.02–0.03 mm wide (Figs. 4B, 5D).

Colonies are dark red in life or preserved (Fig. 2A-C). Coenenchymal sclerites are mostly red (Fig. 4A).

The paratypes' characteristics are very consistent with the holotype.

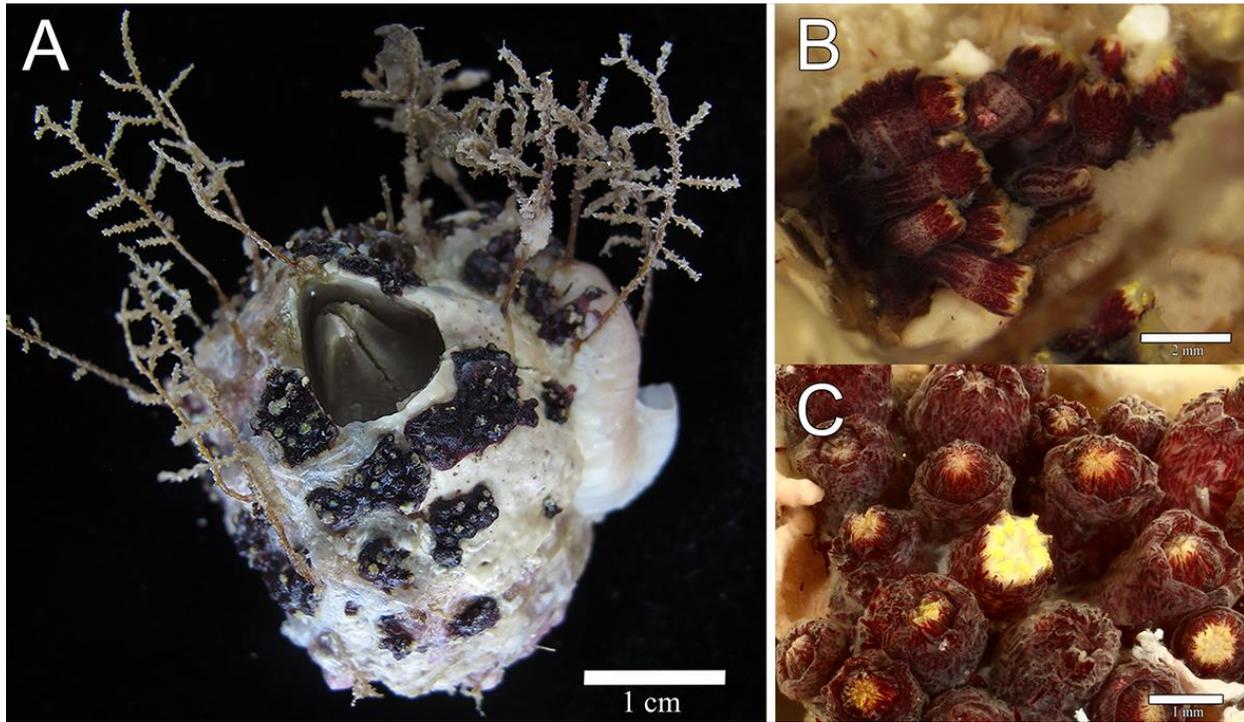


Figure 2. *Aliena parva* **gen. nov. et sp. nov.** A, Holotype MZUCR 3679. B, Polyps, partially retracted showing anthocodial sclerites. C, Polyp mounds (Photographs by Fiorella Vásquez, University of Costa Rica).

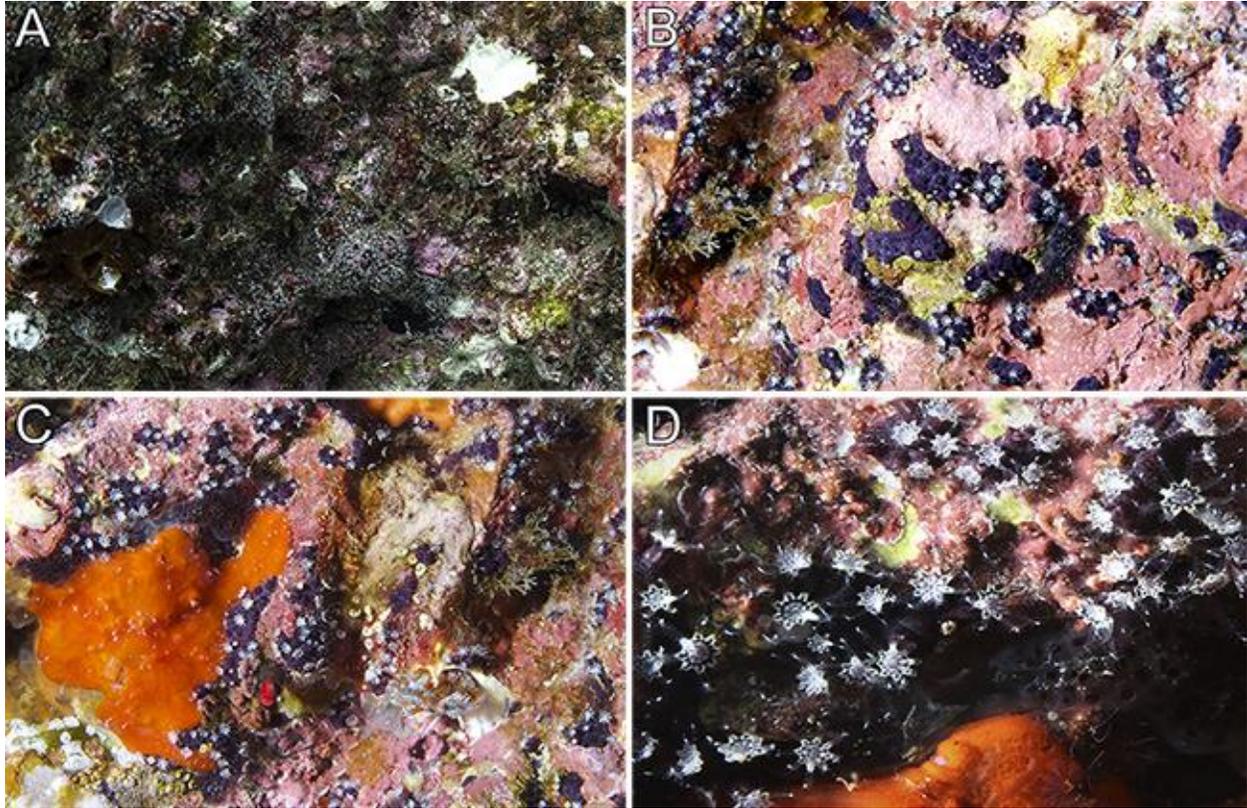


Figure 3. Colonies *in situ*. A, Manuelita Afuera, panoramic view of the wall, 25 m Photograph by Anuar Patjane. B-D, Manuelita Canal, 25 m deep. Photographs by Avi Klapfer.

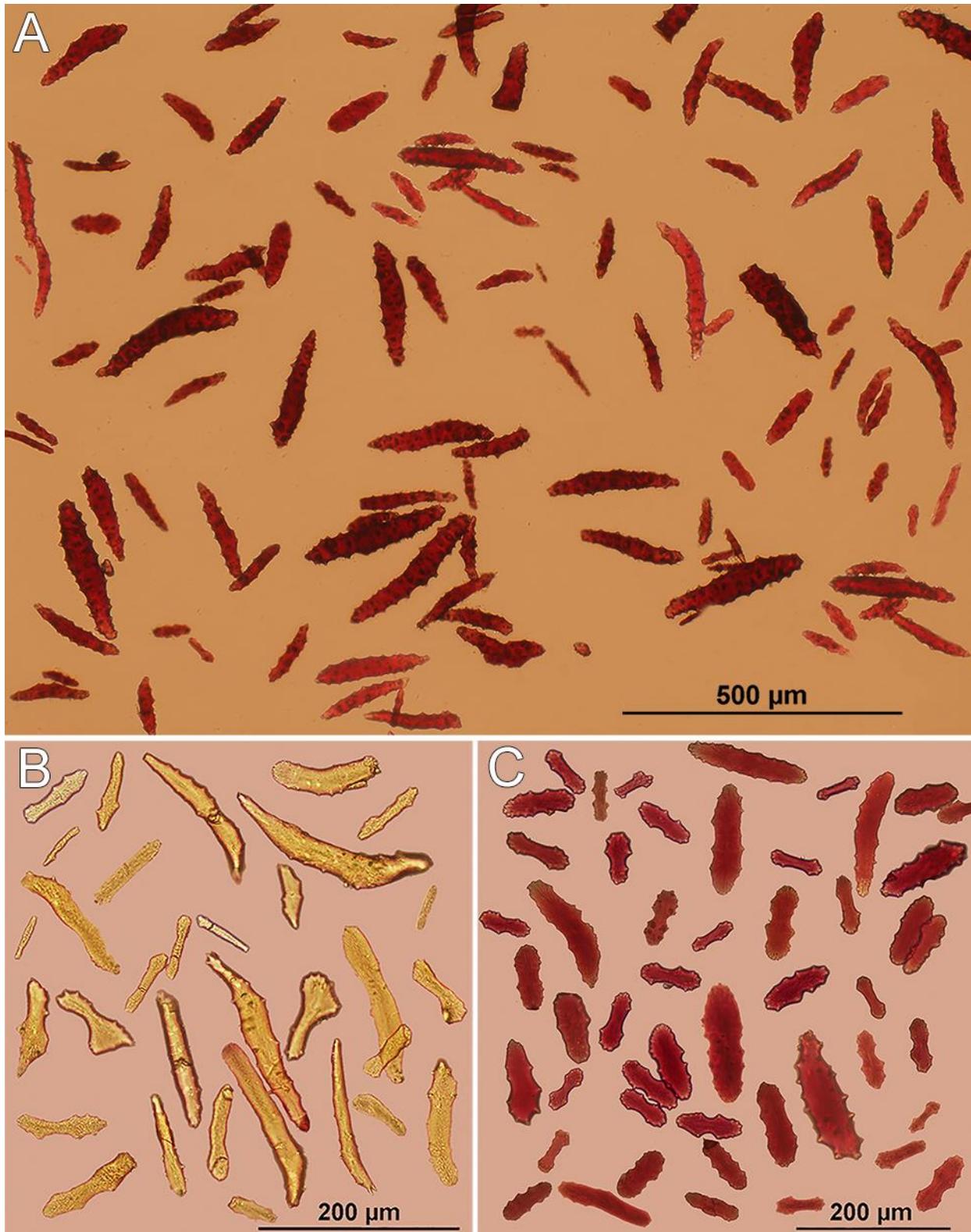


Figure 4. *Aliena parva* **gen. nov. et sp. nov.**, holotype MZUCR 3679 sclerites. A, Assorted coenenchymal sclerites. B, Tentacular sclerites. C, Anthocodial sclerites.

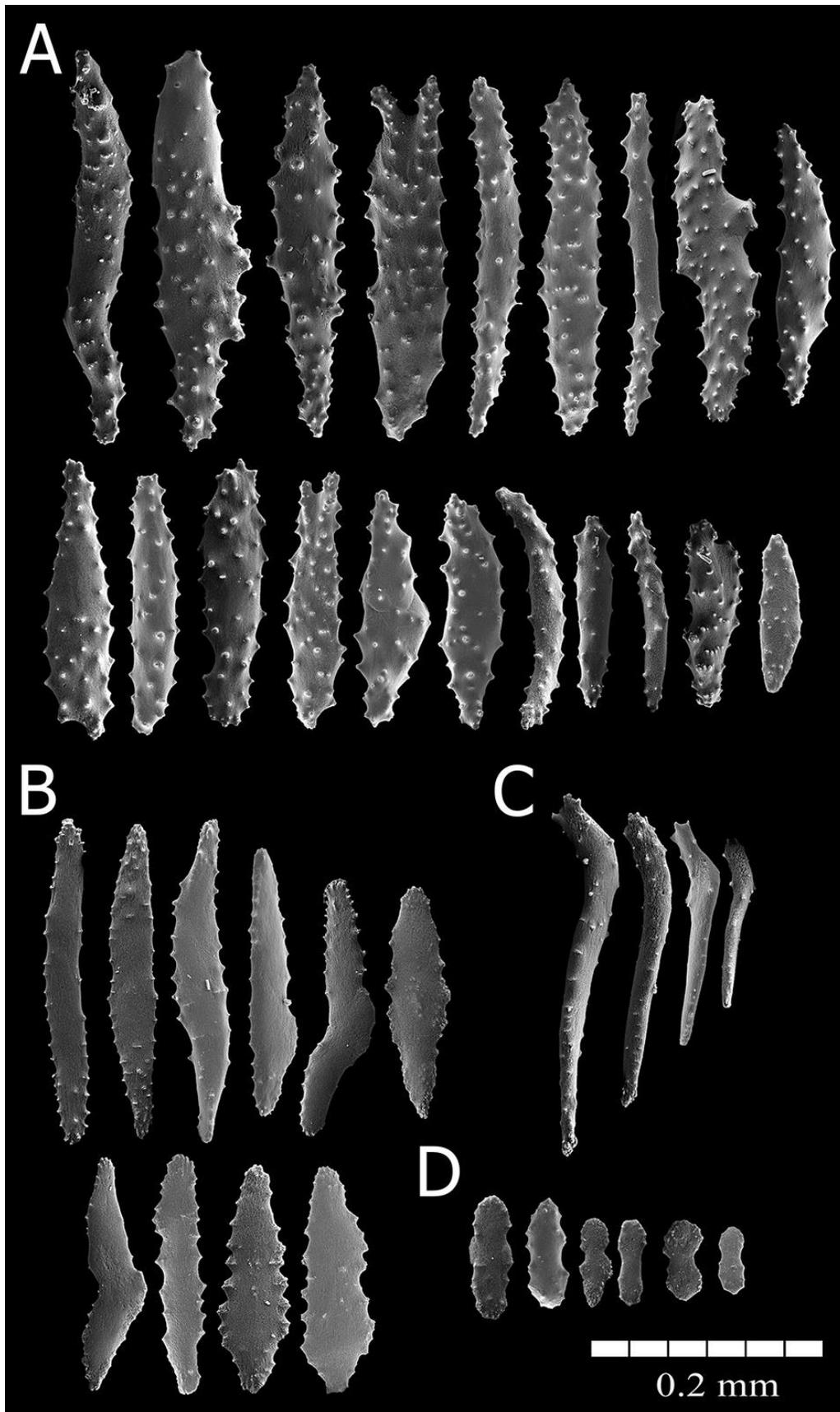


Figure 5. *Aliena parva* gen. nov. et sp. nov., holotype MZUCR 3679 SEM sclerites. A, Coenenchymal sclerites. B-C, Anthocodial sclerites. D, Tentacular sclerites.

**Remarks.** The colonies are overgrowing dead or live substrate, encrusting small rocks, barnacle plates, shells or among turf. They were frequently found among the worm tubes occupied by the endemic fish *Acanthemblemaria atrata* from Isla del Coco (Figs. 3C, 6B). We noticed during the January 2022 and September-October 2022 trips that the polyps have one differentiated long tentacle that was not present in the colonies observed during the other trips (in 2021) (Fig. 6A-B).

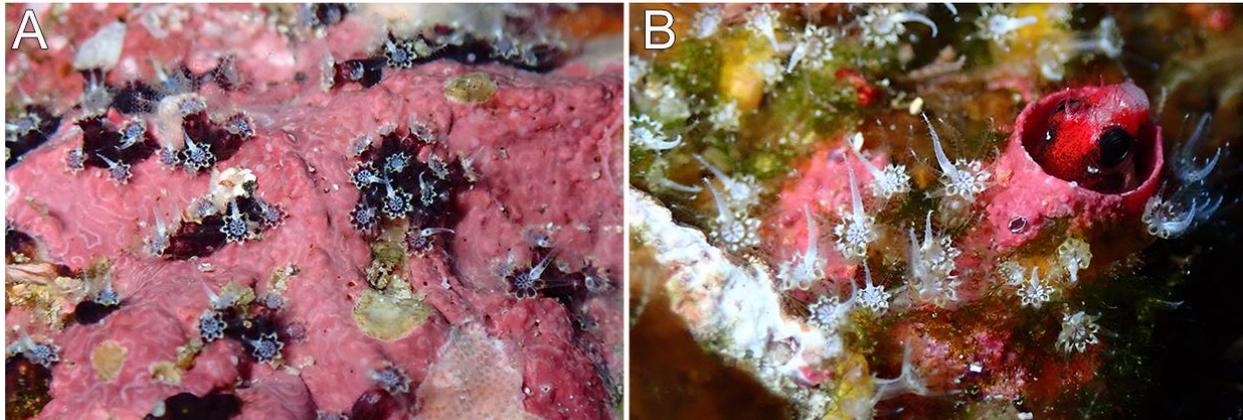


Figure 6. Colonies *in situ* with modified tentacle, January 2022. A. Manuelita Canal, 25-30 m deep. B. Roca Sucia, 25 m deep.

**Distribution.** Colonies were found at various localities north of Isla del Coco around Manuelita Afuera, Manuelita Canal and Bajo Manuelita; and northwest at Roca Sucia, 5°32.875' N 87°04.956' W and Viking Rock (Isla Cáscara), 05°33.006' N 87°03.865' W, NW of the Island (Fig. 1). Only known from the type locality. The bathymetric range was 20–30 m.

**Etymology.** *parvus* (L). In allusion to the small size of the polyps. Gender feminine.

#### Phylogenetic analysis

Phylogenetic analyses of both the mitochondrial *mtMutS* and nuclear *28S rDNA* genes strongly supported the placement of *Aliena parva* gen. nov. et sp. nov. in the octocoral family Pterogorgiidae (Fig. 7). All analyses supported *A. parva* as the sister to a clade of Pterogorgiidae that includes the gorgonian genera *Pterogorgia* Ehrenberg, 1834, *Pinnigorgia* Grasshoff & Alderslade, 1997 and *Muriceopsis* Aurivillius, 1931. Many of the relationships among and within other families of Malacalcyonacea were only very poorly supported by the *28S rDNA* maximum likelihood (ML) tree (Suppl. Fig. S1) and by both Bayesian trees, and differed from the *mtMutS* ML tree. Nonetheless, all phylogenetic analyses of both genes independently offered strong support (bootstrap values >99%; posterior probabilities >0.9) for the monophyly of Pterogorgiidae and the position of *A. parva* within that clade (Fig. 7). The moderately long branch length separating *A. parva* from other genera in Pterogorgiidae in both *mtMutS* and *28S rDNA* trees further supports its status as a new genus.

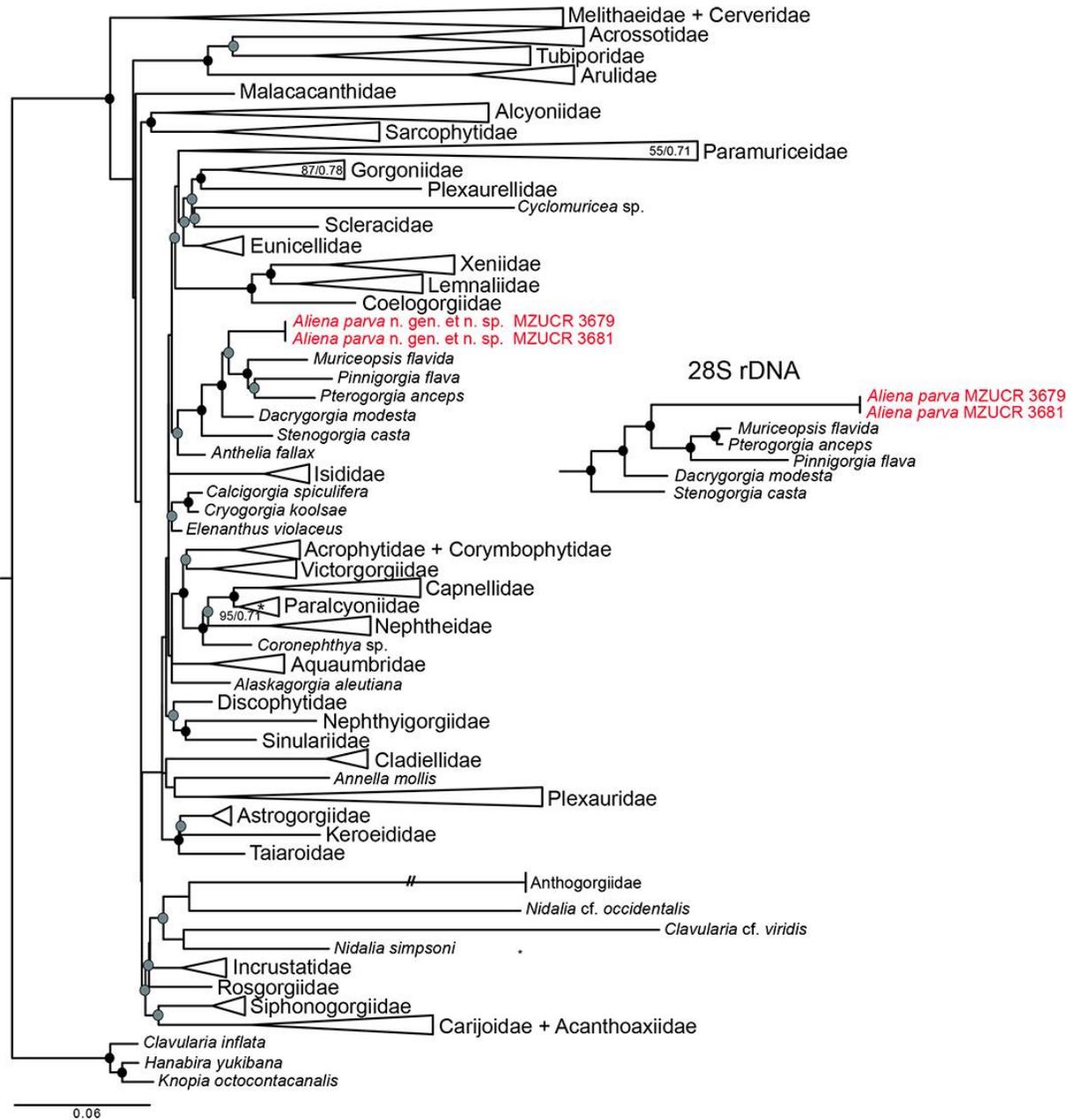


Figure 7. Maximum likelihood tree of Malacalcyonacea based on *mtMutS*. Support values indicated by symbols at nodes. Black circles: maximum likelihood bootstrap value (bs) > 70% and Bayesian posterior probability (pp) > 0.9; gray circle: bs > 70, no support (pp < 0.9) from Bayesian analysis. Families have been collapsed to facilitate readability. All collapsed clades have bs > 70% and pp > 0.9 unless otherwise noted. The branch leading to Anthogorgiidae has been shortened to fit the page. Inset shows Pterogorgiidae clade from analysis of 28S rDNA.

## Discussion

The external morphology of *Aliena* gen. nov. suggests it could belong to one of the families of stoloniferous octocorals, several of which (e.g., Carijoidae, Incrustatidae, Sarcodictyonidae,

Tubiporidae) include genera of encrusting colonies that produce mats extending on hard substrata (McFadden et al. 2022). It bears a particular superficial resemblance to some of the genera in family Tubiporidae such as *Stragulum* Ofwegen & Haddad, 2011, but all members of that family have sclerites in either the coenenchyme or polyp body wall that are fused. The molecular evidence from both a mitochondrial (*mtMutS*) and nuclear (28S *rDNA*) gene, however, suggest that *Aliena* gen. nov. does not belong to any of the families that comprise mostly stoloniferous taxa, but instead strongly support its placement in the gorgonian family Pterogorgiidae.

The gross colony morphology of *Aliena* gen. nov. is unlike any of the other genera in Pterogorgiidae, all of which have internal skeletal axes of gorgonin that support an erect growth form. Its sclerome is, however, reasonably consistent with that of the other Pterogorgiidae, all of which have asymmetrically spiny or curved spindles in the coenenchyme and flattened rods in the polyps (McFadden et al. 2022). The polyp sclerites of *Aliena* gen. nov. are similar in particular to those of *Pterogorgia* and *Stenogorgia*, which are usually small spindles or flattened rods that are occasionally arranged as collaret and points or just points as in *Aliena* gen. nov. *Aliena* gen. nov. lacks the additional sclerite forms that typify some of the other genera of Pterogorgiidae, such as the capstans of *Pterogorgia* and *Stenogorgia* Verrill, 1883 or the balloon clubs of *Dacrygorgia* (McFadden, van Ofwegen and Quattrini, 2022). The simple thorn-like ornaments on the coenenchymal spindles of *Aliena* n. gen. also differ markedly from the asymmetrical spines and high, complex tubercles that characterize the spindles of *Muriceopsis* and *Tobagorgia* (Sánchez, 2007).

*Aliena* gen. nov. is one of several genera of octocorals with a simple, encrusting growth form that belong to clades whose other members are all gorgonians, i.e. species with an internal skeletal axis of proteinaceous or calcareous material. Other examples include the encrusting genera *Thrombophyton* (McFadden & Hochberg, 2003, which falls within the gorgonian family Paramuriceidae, and *Discophyton* (McFadden & Hochberg, 2003 (recently assigned to the monotypic family Discophytidae) which belongs to a larger clade that consists almost exclusively of gorgonians (McFadden et al. 2022). The most parsimonious interpretation of these surprising phylogenetic relationships is that these three genera have evolved independently from gorgonian ancestors by the secondary loss of an axis, a scenario that has been supported by ancestral state reconstruction of skeletal evolution in Octocorallia (Quattrini et al. 2020).

Placement of *Aliena* gen. nov. in Pterogorgiidae requires amending the diagnosis of that family to accommodate a species that lacks an axis (changes underlined):

**Diagnosis.** Octocorals with (or rarely without) a proteinaceous skeletal axis. Axis hollow with wide, cross-chambered central core. Colonies encrusting or erect, sparsely to profusely branched (dichotomous, pinnate), planar or bushy; branches may be flattened, oval or triangular in cross-section. Polyps monomorphic, retractile into coenenchyme or into low calyces, distributed evenly over branch surface or arranged biserially in recessed grooves along branch margins. Polyp sclerites small, flattened rods or slender spindles only rarely arranged as collaret and points. Sclerites of coenenchyme typically include asymmetrically spiny or curved spindles with or without complex tubercular ornamentation; capstans, asymmetrical clubs or balloon-clubs,

double-heads or plates may also be present. Zooxanthellate or azooxanthellate (modified from McFadden et al. 2022).

In addition, *Aliena* gen. nov. is the second genus of Pterogorgiidae known to have a distribution in the Pacific, and the only one recorded so far from the eastern Pacific. The majority of the taxa in this family are distributed in the tropical Atlantic.

Although several studies have reported agonistic interaction structures such as sweeper tentacles in scleractinians (e.g. Richardson et al. 1979), corallimorphians (Chadwick 1987, Miles 1991) and black corals (Goldberg et al. 1990), the only previous report in octocorals is in *Erythropodium caribaeorum* (Duchassaing & Michelotti, 1860) (Sebens and Miles 1988). In that species, all eight tentacles of a polyp become modified (elongated with reduced pinnules and the tips swollen with nematocysts) in response to contact with spatial competitors. Similar elongation of the tentacles has been observed in the gorgonians *Corallium rubrum* (Linnaeus, 1758) and *Paramuricea clavata* (Risso, 1827), but hypothesized to function in feeding (Abel 1970; López-González et al. 2018). The single enlarged tentacle observed in *Aliena* gen. nov. differs from the threadlike structures seen in these other species, but could nonetheless represent a case of a modified tentacle whose main function is for competitive interactions or nutrition (Y. Benayahu pers. comm. 2022). But, the possibility that they serve other functions related to reproductive strategies could also be explored. Some Mediterranean *Clavularia*-like soft corals brood their embryos below their tentacles in a kind of a surface brooding. Alternatively, the modified tentacle could function in transferring sperm to neighbouring polyps or colonies, thus resembling the hectocotylus of some cephalopods (Y. Benayahu pers. comm. 2022). Studies of the competitive interactions and reproductive strategy of *Aliena* gen. nov. will be necessary to further elucidate the function of the elongated tentacle.

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## Figure legend

Figure 1. Map showing the locations where *Aliena parva* **gen. nov. et sp. nov.** was found in Isla del Coco, Costa Rica. Map by Beatriz Naranjo, University of Costa Rica.

Figure 2. *Aliena parva* **gen. nov. et sp. nov.** A, Holotype MZUCR 3679. B, Polyps, partially retracted showing anthocodial sclerites. C, Polyp mounds (Photographs by Fiorella Vásquez, University of Costa Rica).

Figure 3. Colonies *in situ*. A, Manuelita Afuera, panoramic view of the wall, 25 m Photograph by Anuar Patjane. B-D, Manuelita Canal, 25 m deep. Photographs by Avi Klapfer.

Figure 4. *Aliena parva* **gen. nov. et sp. nov.**, holotype MZUCR 3679 sclerites. A, Assorted coenenchymal sclerites. B, Tentacular sclerites. C, Anthocodial sclerites.

Figure 5. *Aliena parva* **gen. nov. et sp. nov.**, holotype MZUCR 3679 SEM sclerites. A, Coenenchymal sclerites. B-C, Anthocodial sclerites. D, Tentacular sclerites.

Figure 6. Colonies *in situ* with modified tentacle, January 2022. A. Manuelita Canal, 25-30 m deep. B. Roca Sucia, 25 m deep.

Figure 7. Maximum likelihood tree of Malacalcyonacea based on *mtMutS*. Support values indicated by symbols at nodes. Black circles: maximum likelihood bootstrap value (bs) > 70% and Bayesian posterior probability (pp) > 0.9; gray circle: bs > 70, no support (pp < 0.9) from Bayesian analysis. Families have been collapsed to facilitate readability. All collapsed clades have bs > 70% and pp > 0.9 unless otherwise noted. The branch leading to Anthogorgiidae has been shortened to fit the page. Inset shows Pterogorgiidae clade from analysis of 28S *rDNA*.