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Callogorgia spp. and Their Brittle Stars: Recording Unknown Relationships in the Pacific Ocean and the Caribbean Sea

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The habitat formed by the *Callogorgia* species, with their abundance and colony sizes, provides an important refuge for a variety of brittle stars which are recognized as the epibionts of octocorals in both shallow and deep environments. In such a relationship, ophiurans benefit directly from being elevated because they facilitate their feeding by suspension, while octocorals do not seem to benefit or be harmed. During three different expeditions developed in the Colombian Pacific from 2012 to 2013 and in the Caribbean Sea during 1998 and 2012 by the INVEMAR - Marine and Coastal Research institute, different samplings were carried out on soft bottoms through trawls with an epibenthic net. For the Pacific Ocean, 33 fragments of the octocoral *Callogorgia* cf. *galapagensis* Cairns, 2018 with 178 specimens of the ophiuroid *Astrodia* cf. *excavata* (Lütken and Mortensen, 1899) were found in two stations at depths 530 and 668 m. Considering the abundance of *A*. cf *excavata*, other biological characters such as size, presence of mature gonads, and evidence of arm regeneration were also detailed. In contrast, in the Caribbean Sea, *Callogorgia gracilis* (Milne Edwards and Haime, 1857) was found with ophiuroids belonging to the genera *Asteroschema* and *Ophiomitra*. The octocoral *Callogorgia americana* (Cairns and Bayer, 2002) was also found, but without associated brittle stars. These findings constituted the first specific association reported in the Eastern Tropical Pacific, and new relationships for the Caribbean Sea. This further reflected a possible specific association between the *Callogorgia* and *Astrodia* species that needed to be further explored. Thus, the *Callogorgia* species and the brittle star *A.* cf. *excavata* represented new records for the Colombian Pacific Ocean and the southern Caribbean Sea.

Keywords: commensalism, *Callogorgia* cf. *galapagensis*, *Astrodia* cf. *excavata*, *Callogorgia gracilis*, *Callogorgia americana*, Colombia, Eastern Tropical Pacific, Caribbean Sea

INTRODUCTION

The structural complexity that octocorals provide in deep habitats facilitates and increases biodiversity by providing biogenic habitats for settlement and colonization by crustaceans, echinoderms, annelids, and bryozoans, among other organisms (Buhl-Mortensen et al., 2010; Watling et al., 2011; Bourque and Demopoulos, 2018). The symbiotic association with octocorals seems to be related to the advantages of the morphologically enhanced feeding of gorgonians that, when rising from the bottom, are oriented with the flow of water to maximize the capture of food from the water that flows through their polyps. This is believed to benefit the diverse assembly of facultative and potentially obligated symbionts of octocorals (Buhl-Mortensen et al., 2010; Allcock and Johnson, 2019). Additionally, it is believed that some symbionts could receive passive protection against predation from the defense system of octocorals through secondary metabolites (Watling et al., 2011; Allcock and Johnson, 2019).

The biological interaction between octocorals and brittle stars has been commonly documented, with its type of association seeming to vary among species, such as between commensal, mutualist, and parasitic relationships (Cairns, 2010; Carreiro-Silva et al., 2011; Watling et al., 2011). Historically, commensalism has been recognized as the usual type of association between brittle stars and octocorals (Watling et al., 2011). In this association, the ophiurans benefit directly by being elevated through facilitating their feeding by suspension, while the octocorals do not seem to benefit or be harmed by this relationship (Fujita and Ohta, 1988). Nevertheless, recent investigations in the Gulf of Mexico suggested some benefits to the octocorals with this association, such as receiving a cleaning action by the ophiuroids guest (Girard et al., 2016). For the brittle stars, the symbiotic interaction could be facultative or obligate. In some cases, a close relationship occurs between cohabiting species, with the interaction beginning from their juvenile stages. This is thought to occur with the ophiuran *Ophiocreas oedipus* Lyman, 1879 that colonizes the octocoral primnoid *Metallogorgia melanotrichos* (Wright and Studer, 1889) when both are young and grow up together until death, with the brittle star being the only symbiont of the colony (Mosher and Watling, 2009). However, at least for shallow-water species such as *Ophiothela mirabilis* (Verrill, 1867), it has been suggested that a negative effect could exist for the coral host due to the high densities of the brittle stars (Mantelatto et al., 2016).

In a review and compilation of the information related to deep-sea octocorals, including their symbiotic interactions, a study by Watling et al. (2011) recorded symbiotic relationships, which were commensal and parasitic, in 17 families of Alcyonacea out of the 31 existing as of 2011. For the octocoral family Primnoidae, 17 symbionts were recognized among amphipods, copepods, polychaetes, zoanthids, and brittle stars, which were mostly commensals and a few parasites (Watling et al., 2011; Cairns, 2016). However, only the ophiuroids from the order Euryalida (Asteronychidae Ljungman, 1867 and Asteroschematidae Verrill, 1899, currently Euryalidae Gray, 1840, according to O'Hara et al., 2017) form associations with octocorals (Watling et al., 2011). Despite the relevance of these associations, there are few records where brittle stars are identified to the species level (Emson and Woodley, 1987; Fujita and Ohta, 1988; Fujita, 2001; Mosher and Watling, 2009). All the Euryalida specimens recorded by the study of Watling et al. (2011) were identified to the class or genus level of *Asteroschema*.

The genus *Callogorgia* Gray, 1858 (Alcyonacea: Primnoidae) is considered a habitat-forming coral due to its abundance and morphology, which create habitats for a diverse array of fauna in the Gulf of Mexico and the northeast Pacific Ocean (Etnoyer and Morgan, 2003; Etnoyer and Warrenchuk, 2007; Quattrini et al., 2013). Several records of the associations of *Callogorgia* spp. from the Atlantic and the Pacific Ocean involved zoanthids, copepods, and scale worms that were identified to a genus or species level (Grygier, 1980; Pettibone, 1991; Carreiro-Silva et al., 2011). However, the brittle stars were still not identified even if they were usually mentioned (Quattrini et al., 2013; Bayer et al., 2014; Cairns, 2018b; Cordeiro et al., 2018). Records of the ophiuroid genus *Astrodia* in the Pacific Ocean have also been presented in studies by Sellanes et al. (2008) and Okanishi and Fujita (2014). Nevertheless, the study by Sellanes et al. (2008) recorded *Astrodia tenuispina* (Verrill, 1884), which is an Atlantic species according to a study by Okanishi and Fujita (2014).

In the present study, we recorded for the first time the previously unknown relationship between the two species of the octocoral *Callogorgia* and their associated brittle stars based on specimens from the Colombian Pacific Ocean and the Colombian Caribbean Sea. Furthermore, we presented the detailed morphological characteristics of the associated Pacific species and described their characteristics in regard to the size, arm regeneration, and mature gonads of the Pacific ophiuroids.

MATERIALS AND METHODS

The reviewed specimens were collected during expeditions developed by INVEMAR - Marine and Coastal Research Institute in the Colombian Pacific and Caribbean Sea (**Figure 1**). The Pacific specimens were collected during the Tumaco Offshore expedition from 2012 to 2013. These samplings were carried out on the soft bottoms of 15 localities in an unexplored area offshore of Tumaco (Nariño department, **Figure 1**) using an epibenthic trawl net (with an opening of 9 × 1 m, 2.5 knots by 10 min). On the other hand, the Caribbean specimens were collected during both the Macrofauna I expedition (see Reyes et al., 2005; Santodomingo et al., 2013) using a semi-balloon trawl net (with an opening of 9 × 1 m, 3 knots by 20 min) in the off shore continental shelf of Tayrona National Natural Park (Magdalena department; **Figure 1**) and the ICP expedition developed in the offshore continental shelf of La Guajira department (**Figure 1**) using a semi-balloon trawl net (9.5 m opening, 3 knots by 10 min). In the **Supplementary Material**, the coordinates of the trawling in the collection locations were expressed as well-known text representations (WKT footprint) according to the Darwin Core Standard (TDWG, 2018), thus the line string will be $latitude_1$ $longitude_1$, $latitude_2$ $longitude_2$. The line string method means that the sampling was a transect, where the first pair of coordinates indicates the beginning point and the second pair corresponds to the ending point.

The collected octocorals and their associated ophiurans were submerged in magnesium chloride ($MgCl_2$) to relax the structures of taxonomic importance and facilitate their observation. The samples were also preserved in alcohol 96 % so that there would be available tissues for molecular studies in the future. Specimen vouchers were then cataloged and stored in collections at the Marine Natural History Museum

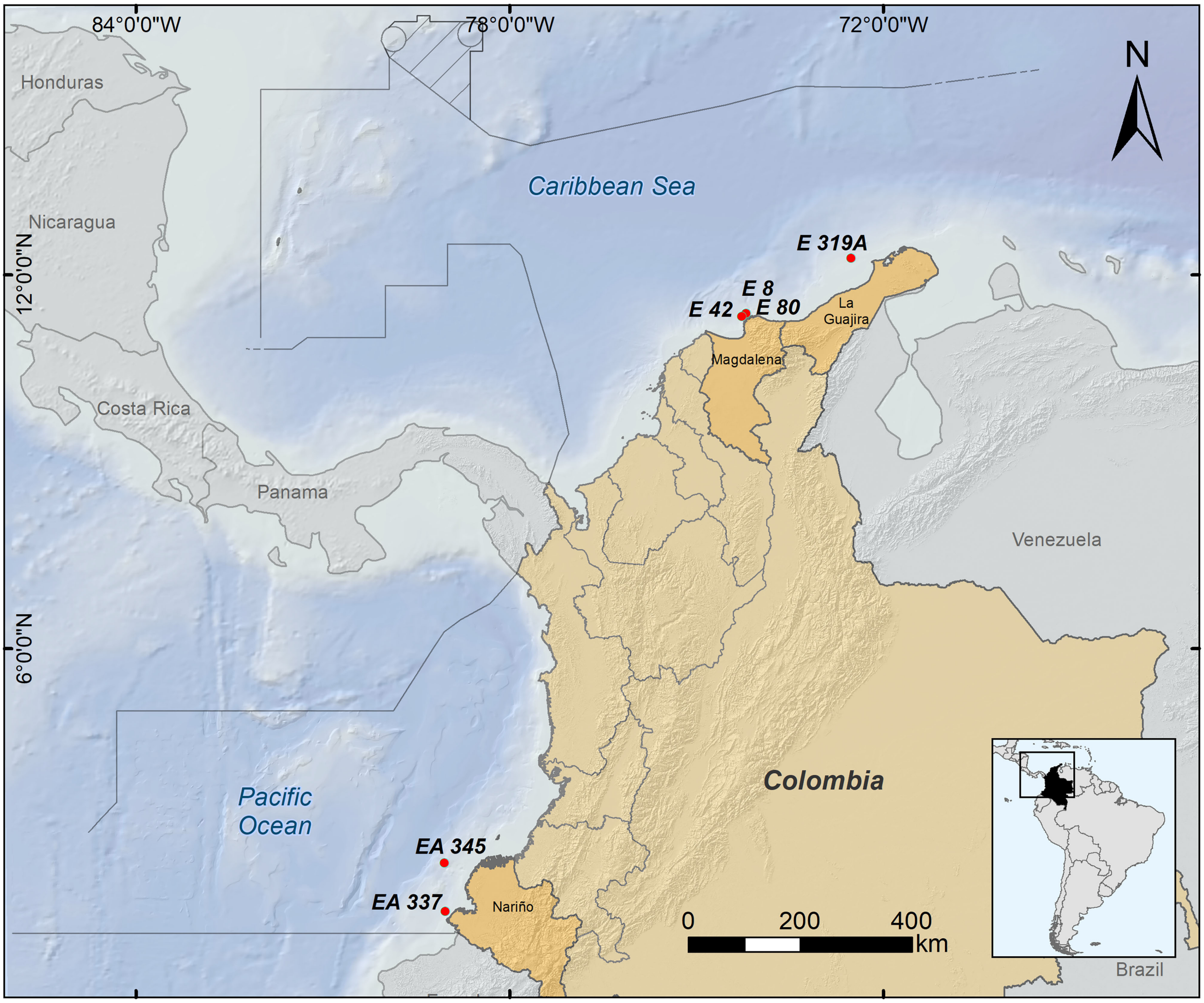

FIGURE 1 | Sampling sites in the Colombian Pacific and Caribbean Sea where samples of *Callogorgia* spp. in association with brittle stars were collected. Collections were made in different stations (red points) during different INVEMAR expeditions: Macrofauna 1 (Magdalena sites), ICP (La Guajira sites), and Tumaco Offshore (Pacific sites, Nariño). Stations names are the same used in each expedition.

of Colombia (MHNMC) of INVEMAR (**Table 1**). Furthermore, the octocoral species were identified according to the colony morphology and characteristics of the polyp scales, following the works made by Bayer (1982), Cairns and Bayer (2002), Bayer et al. (2014), and Cairns (2018a). The brittle stars were then identified based on their external morphological characteristics following the taxonomic information presented by the studies of Fell (1960), Okanishi and Fujita (2014), and O'Hara et al. (2017). The external characteristics of *Astrodia* include dorsal disc with thickened skin with few or no scales, bar-like radial shields that almost meet in the disc center, long and tapering arms that are covered by thickened skin, lacking dorsal plates, and never hooked arm spines (After Okanishi and Fujita, 2014; O'Hara et al., 2017; Okanishi et al., 2017). Biological information based on the specimens collected in the Pacific Ocean, such as the size of the ophiuroids by disk diameter (dd), presence of mature gonads by direct observation, and macroscopic evidence of arm regeneration, were reviewed and reported. Octocoral fragments were also reviewed for predation signs, while the stomach contents in the ophiuroids that were randomly selected were observed by microscopy.

RESULTS

Pacific Ocean Associations

During the deep-sea explorations carried out on the Colombian Pacific continental shelf, one species of *Callogorgia* and one of the brittle stars were captured in symbiotic association. The octocoral species was identified as *Callogorgia* cf. *galapagensis* Cairns, 2018 (**Figures 2A,B**) and the associated was the ophiuroid *Astrodia*

TABLE 1 | Commensal relationship between *Callogorgia* spp. and ophiuroids found in the Colombian Pacific and Caribbean Sea based on material deposited in the Marine Natural History Museum of Colombia (MHNMC) of INVEMAR.

***Callogorgia* species**	**Ophiuroid species found in the *Callogorgia* species**	**Number of fragments / No. of ophiuroids**	**Depth (m)**	**Location (Figure 1)**	**Catalog numbers of *Callogorgia***	**Catalog numbers of ophiuroids**
C. cf. *galapagensis*	*Astrodia* cf. *excavata*	28/176	550 –670	Pacific Ocean (Tumaco off - EA345)	INV CNI3425 INV CNI3426 INV CNI3427 INV CNI3428 INV CNI3429 INV CNI3430 INV CNI3431	INV EQU4097 INV EQU4098 INV EQU4099 INV EQU4100 INV EQU4101 INV EQU4102 INV EQU4103 INV EQU5014
C. cf. *galapagensis*	*Astrodia* cf. *excavata*	5/2	530	Pacific Ocean (Tumaco off - EA337)	INV CNI4406	INV EQU5015
C. gracilis	*Asteroschema oligactes*	9/6	200–206	Caribbean Sea (Magdalena off - E80)	INV CNI1850	INV EQU5111
	Ophiomitra valida	9/1				INV EQU5114
C. gracilis	*Asteroschema oligactes*	4/11	200–206	Caribbean Sea (Magdalena off - E8)	INV CNI1409	INV EQU1086 INV EQU1087 INV EQU1088 INV EQU5112
	Ophiomitra valida	4/2				INV EQU1089 INV EQU5115
C. gracilis	*Asteroschema oligactes*	1/1	230–270	Caribbean (La Guajira)	INV CNI3648	INV EQU5113
C. americana	No association	1/0	500	Caribbean (Magdalena off - E42)	INV CNI1410	

cf. *excavata* (Lütken and Mortensen, 1899) (**Figures 2C–E**). This symbiotic association was found among the 2 out of 15 stations sampled in Tumaco (Nariño), with a total of 33 octocoral fragments of *C.* cf. *galapagensis* and 178 individuals of the ophiroid *A.* cf. *excavata* found (**Table 1**). The species abundance varied between the two stations. In EA 345 (northern and deepest station, **Figure 1**), 28 coral fragments and 176 brittle stars were captured between 550 and 668 m depths. In contrast, in station EA 337, only five coral fragments and two ophiurans were captured at a depth of 530 m. Considering the abundance difference, the association and the biological information of the ophiuroids were described mostly based on the samples collected at station EA 345 (**Figure 1**).

The octocoral and brittle star associations found in this study were considered as commensalism since no injury symptoms were found in the coral fragments and no sclerites, i.e. octocoral microstructures, were found in a random sampling of the stomachs of the brittle stars. The reviewed 176 ophiurans allowed the recognition of some biological traits, such as the disc diameters of *A.* cf. *excavata* residing in the octocoral fragments ranged between 6 and 23.46 mm, with a mean of 16.23 mm (**Figure 3A**). The sexes of the specimens were also not determined, but 60 % of the individuals that were reviewed showed mature gonads. Most of these gonads were between 14–17 mm sizes (**Figures 3A–C**), with 13 mm as the smallest size. Macroscopic signs of regeneration were also observed in 11.5 % of the 176 specimens reviewed, where most of them were larger than 18 mm in the disc diameter. Only one specimen showed a disc in regeneration (**Figure 3D**), while the other individuals only had regenerations in the arms, which were usually from the distal portions (**Figures 3E,F**).

Caribbean Sea Associations

In the Colombian Caribbean, the coral species *C. gracilis* (Milne Edwards and Haime, 1857) was found associated with two brittle stars, *Asteroschema oligactes* (Pallas, 1788) and *Ophiomitra valida* Lyman, 1869 (**Figure 2F**). These symbiotic associations were registered in three of the four stations sampled in both La Guajira and Magdalena locations. In total, 14 fragments and one small colony were reviewed. In all cases, the octocoral was identified as *C. gracilis* (Milne Edwards and Haime, 1857) except for one fragment that corresponded to *C. americana* (Cairns and Bayer, 2002). This species was collected in station E 42, but it was not found to be associated with any ophiuroid. Thus, *C. gracilis* (**Figures 2F,G**) was commonly associated with the brittle star *A. oligactes* (Pallas, 1788) (**Figures 2I,J**), with 18 ophiuroids found attached to the coral, while only three ophiuroids of *O. valida* Lyman, 1869 (**Figures 2H,K**) were found among all the specimens reviewed.

Both brittle star species explored in this study, *A. oligactes* and *O. valida,* were found co-occurring at the same colony or fragments (**Figure 2F**), reflecting no exclusion between guest species. Additionally, one juvenile of *O. valida* (disc diameter = 2.73 mm) was found together with specimens of *A. oligactes.* Collection data and other detailed information are presented in **Table 1**. For detailed information about species taxonomy see Borrero-Pérez et al. (2008), Benavides-Serrato et al. (2011).

DISCUSSION

The habitat formed by the *Callogorgia* species with their large colony sizes provides an important refuge for different species (Etnoyer and Morgan, 2003). In this case, they provide for a

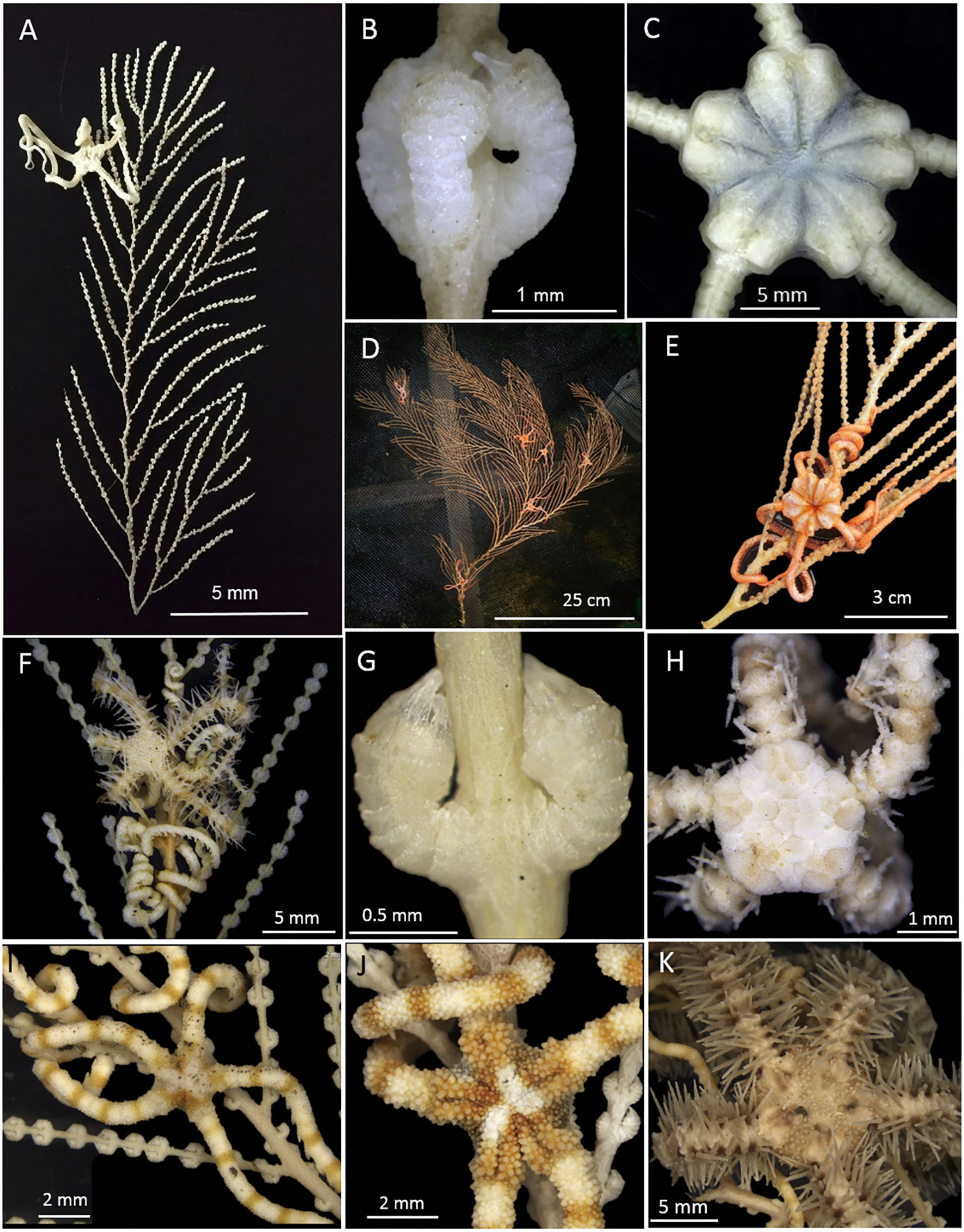

FIGURE 2 | Octocorals and brittle stars found associated in the Colombian Pacific and Colombian Caribbean Sea. Pacific specimens: **(A)** Fragment of the octocoral *Callogorgia* cf. *galapagensis* associated with the brittle star *Astrodia* cf. *excavata*. **(B)** Whorl of polyps of *C.* cf. *galapagensis*. **(C)** Disc of *A.* cf. *excavata* **(D,E)** Color of *C.* cf. *galapagensis* and *A.* cf. *excavata* in live specimens. Caribbean specimens: **(F)** Association among the octocoral *Callogorgia gracilis* and the brittle stars *Asteroschema oligactes* and *Ophiomitra valida*. **(G)** Whorl of polyps of *C. gracilis*. **(H)** Juvenile of *O. valida*. **(I,J)** *A. oligactes*. **(K)** Adult specimen of *O. valida*.

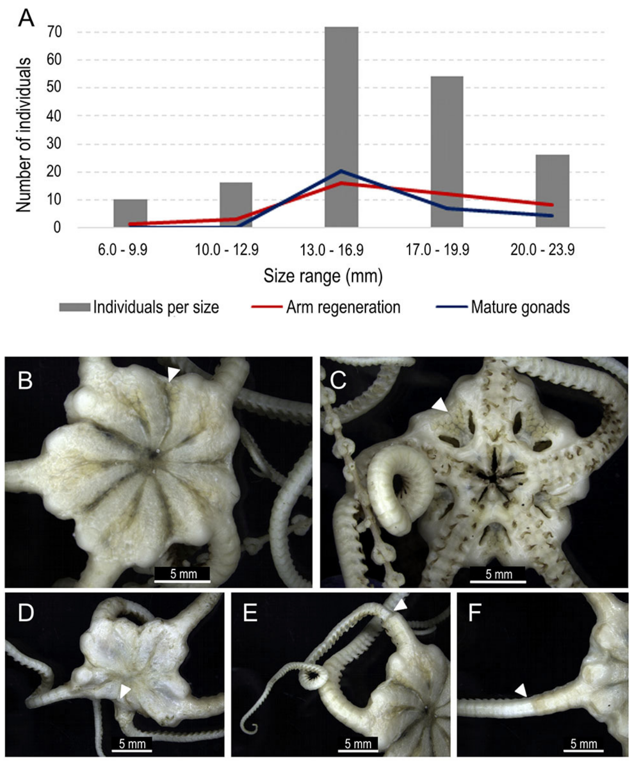

FIGURE 3 | *Astrodia* cf. *excavata* biological information. **(A)** Plot of number of individuals per size range, number of individuals showing evidence of arm regeneration, and mature gonads **(B,C)** Dorsal and ventral view showing mature gonads by direct observation. **(D)** Evidence of disc regeneration. **(E,F)** Evidence of arm regeneration.

variety of brittle stars living in the Eastern Tropical Pacific (ETP) and the Caribbean Sea. The exploration of deep waters to the southwest of the Colombian Pacific allowed for the recognition of a symbiotic relationship between the octocoral *C.* cf. *galapagensis* and the ophiuroid *A.* cf. *excavata*, thus being the first record of this symbiotic relationship in the ETP.

Although the species involved in the symbiosis maintain the taxonomic status "to be confirmed – cf." they have a close affinity with the mentioned species and only a few morphological variations (described in the remarks of each species as presented in the **Supplementary Material**) based on their original descriptions, make us maintain this caution in both species. *Callogorgia galapagensis* is a recently described octocoral species, only reported for Galapagos and Cocos Island (Cairns, 2018a), which now would be present in the deep continental platform of Colombia. In particular, *A.* cf. *excavata* has been recorded mainly in the northeastern Pacific, from California, USA to Tres Marias Islands, Mexico, with only two records from Perú and Chile (Okanishi and Fujita, 2014; Natural History Museum, 2020). This study confirmed the presence of this species in the southeastern Pacific, specifically in Colombia.

The symbiotic relation found between *C.* cf. *galapagensis* and *A.* cf. *excavata*, was considered here as commensalism since the ophiurans benefited from the host but did not seem to affect them. This conclusion is supported since the coral fragments that were reviewed did not show signs of dead tissue. Additionally, no sclerites were found in the stomach contents of the ophiuroids during a random sampling. This case would prove predation by the brittle star. However, more information is needed about the possible benefits for the coral host since their consequences in a symbiotic relationship are poorly known (Girard et al., 2016). A recent study demonstrated the positive influence of the brittle star *Asteroschema clavigerum* in the recovery of the octocoral *Paramuricea biscaya* after an oil-spill event. This study revealed that the coral benefits from the removal of the deposited oil material by the ophiuroid actions (Girard et al., 2016). Regarding the benefits to the brittle stars from being elevated to facilitate their feeding by suspension, it was expected that the octocoral host acted as a refuge from predators due to the chemical protection provided by its secondary metabolites (Watling et al., 2011). However, predation in associated brittle stars was expected, considering the need to unravel their arms from the coral and extend them into the water to catch their food from the water column. For this reason, ophiuroids were recognized in a study by Watling et al. (2011) as the one group for whom the chemical protection by octocorals is of only a limited benefit. This phenomenon could explain the 11.5 % regeneration observed in the arms and discs of the *Astrodia* specimens that were collected in this study. Nevertheless, there is no information available to compare if this is a high or low percentage.

For the *Callogorgia* genus, many different associations with brittle stars have been documented, but specific relations are still unknown since the majority of the brittle stars were unidentified (Quattrini et al., 2013; Bayer et al., 2014; Cairns, 2018b; Cordeiro et al., 2018). However, we think that some brittle stars entwined to *Callogorgia* spp. could belong to the genus *Astrodia,* with four species currently accepted in the genus depending on their geographic distribution (Okanishi and Fujita, 2014), although they may also be new species. *Astrodia* identification was performed by comparing external morphology, as detailed in the Material and methods section, and geographic distribution with the other three genera in the family Asteronychidae, namely, *Asteronyx* Müller and Troschel, 1842 (six species, around the world), *Astronebris* Downey, 1967 (one species, Alaska), and *Ophioschiza* H.L. Clark, 1911 (one species, Alaska). Further identification was done by comparing *Astrodia* with the other families in the order Euryalida (Euryalidae Gray, 1840 and Gorgonocephalidae Ljungman, 1867), according to the new Ophiuroidea classification proposed by O'Hara et al. (2017) and other authors (Okanishi et al., 2017; Horvath and Stone, 2018; GBIF, 2019). However, since these specimens were identified by photos and videos, the material needs to be confirmed with collected or museum specimens.

Astrodia spp. were identified to be associated with *Callogorgia lucaya* and *C. arawak* colonies in the Atlantic Ocean (Bayer et al., 2014; Cordeiro et al., 2018), with *C. gracilis*, *C. americana,* and *C. americana delta* colonies in the Gulf of Mexico being reported by the Lophellia II Expedition 2009 (Brooks et al., 2012; Quattrini et al., 2013). They were also identified to be associated with the holotype of *C. cracentis* in the central Pacific, with at least a dozen of ophiuroids registered (Cairns, 2018b). These Caribbean and Pacific reports, along with our record, reflected a possible specific association between *Callogorgia* and *Astrodia* species that needs to be further explored. The information gathered about this relationship in the Atlantic and Pacific, especially the records presented in the studies by Brooks et al. (2012), Quattrini et al. (2013), and Cairns (2018b), and our findings in *C.* cf. *galapagensis* corroborated with the observation that many ophiuroids of *Astrodia* can live on the same colony. The differences in the sizes of ophiurans (between 6 and 23.9 mm diameter discs) and their reproductive stages (**Figure 3**) on a unique big octocoral fragment suggested that both juveniles and adults could live on one colony, completing a life cycle in the coral host. The presence of several small ophiuroids specimens (10 individuals between 6 and 10 mm diameter disc) in the reviewed material may indicate that *A.* cf. *excavata* recruit frequently on the coral host, contrary to the report presented in a study by Emson and Woodley (1987) for *A. tenue*. This could be expected in *A.* cf. *excavata* since these species live aggregated together on the host in high densities, consequently ensuring fertilization after spawning, as is the reproductive mode of most echinoderms (Hendler et al., 1995). Other information such as the sexes and proportions of the specimens and the size of the oocytes would help to better understand this association. It is possible that the species is hermaphroditic, or that males and females are present on the same octocoral colony. However, a more detailed analysis of the gonads would be needed. In this study, we were only able to say that reproduction is taking place, considering the presence of mature gonads.

The Caribbean associations found between the Atlantic octocoral *C. gracilis* and the ophiuroids *A. oligactes* and *O. valida* could also be considered as commensal relationships, which have not been previously reported. In these relationships, only two specimens of *O. valida* were found in the octocoral fragments, while the common ophiuroid seemed to be *A. oligactes* with 11 brittle stars entwined to the coral host. In particular, *Ophiomitra valida* is not an obligate octocoral guest and could also be found across the deep-sea environment, which has been reported for ophiocanthid ophiuroids in a study by Watling et al. (2011). Contrastingly, the brittle stars of the *Asteroschema* genus (Family Asteroschematidae, Order Euryalae) could be considered obligate octocoral symbionts since their association appears to be essential for brittle star survivorship (Emson and Woodley, 1987); it has also been commonly associated with them (Emson and Woodley, 1987; Watling et al., 2011). Other Atlantic species such as *A. arenosum* and *A. tenue* had been seen on colonies of the octocoral families Primnoidae (without a taxonomic species description), Plexauridae, and Ellisellidae (Emson and Woodley, 1987; Frensel et al., 2010). For *C. gracilis*, a study by Quattrini et al. (2013) showed that, in addition to cf. *Astrodia* that were entwined in a colony in the Gulf of Mexico, other brittle stars were present, which were possibly asteroschematids and ophiacanthids (See Figure 2C in Quattrini et al., 2013).

In this article, we reported three *Callogorgia* species, two of them hosting three different species of brittle stars in the Colombian Pacific Ocean and the Caribbean Sea. The three corals and one brittle star species presented in this study were recorded for the first time from these regions. Since little is known about the symbiotic relationships between the associated species found, and even among other species that are not usually identified, this study contributed to the knowledge of the common symbiotic association between octocorals and brittle stars and increased the knowledge of the marine biodiversity of Colombia, especially in the Pacific Ocean.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

EM-C was the principal investigator of the Tumaco Offshore Expedition and collected and processed the data during the expeditions in 2012. GB-P and KM-Q identified the specimens from the expeditions. KM-Q, GB-P, and EM-C wrote the manuscript. All authors read and accepted the manuscript.

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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.2021.735039/full#supplementary-material

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