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Growth in two deep-sea associates: the octocoral *Pseudogorgia bellona* and the euryalid snake star *Asteroschema ajax*

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Abstract

The deep-sea octocoral *Pseudochrysogorgia bellona* was recently described from specimens sampled on the Chesterfield Plateau, off New Caledonia. It is morphologically and genetically similar to the con-familial *Metallogorgia melanotrichos*, which is known to closely associate with a species of brittle star, *Ophiocreas oedipus*. These latter two species have never been observed separately and are thought to grow synchronously. The morphological similarity between *M. melanotrichos* and *P. bellona* makes the latter another possible host for ophiuroids. However, no brittle star was associated with *P. bellona* specimens from the type collection. In 2017, 130 *P. bellona* colonies were sampled near the type locality, and 98% were associated with *Asteroschema ajax*, a species closely related to *O. oedipus*. Mitochondrial DNA analysis confirmed the morphological identifications of both *P. bellona* and *A. ajax*. Uni- and multivariate statistical analyses were used to characterize the morphological space of both species to test if larger ophiuroids are associated with larger corals. Two variables were measured to estimate the size of the coral (total height and diameter of the skeletal axis at its base) and 9 variables were used to characterize the brittle star (disc and arm morphology). Morphological variables representing the size for both species were significantly correlated (Spearman rank correlation coefficient: 50%, $p < 0.001$), suggesting that larger ophiuroids indeed associate with larger corals. This is one of the rare studies that allowed comparison of growth in associated deep-sea invertebrates.

Introduction

Shallow-water corals are known to associate with a large array of species, creating rich ecosystems (Knowlton *et al*., 2010). In comparison, interactions among deep-sea corals and other invertebrates are still poorly known, as they are difficult to observe (Buhl-Mortensen and Mortensen, 2004; Roberts *et al*., 2009). Chrysogorgiidae is among the most speciose and ubiquitous deep-sea octocoral families (Watling *et al*., 2011, Pante *et al*., 2012). Its members associate with many species including ophiuroids, crustaceans (ascothoracid barnacles, copepods, shrimp, chirostylids), pycnogonids, annelids, and ring anemones; their branches can also be used by cephalopods and fish to attach their eggs (Watling *et al*., 2011). Chrysogorgiids include the genera *Metallogorgia* and *Pseudochrysogorgia*, which live below 500 m (Pante *et al*., 2012). *M. melanotrichos* is associated with the brittle star *Ophiocreas oedipus*, and the two partners are thought to grow and senesce together (Mosher and Watling, 2009). Their relationship seems to be obligate commensal, benefitting the ophiuroid and apparently having no effect on the coral. Perched amid the branches of the coral, the brittle star is able to feed off of planktonic organisms and could use the coral for protection (Mosher and Watling, 2009).

The chrysogorgiid *Pseudochrysogorgia bellona* was recently described based on specimens collected off the shores of New Caledonia (Pante and France, 2010). Its close phylogenetic relationship and morphological resemblance to *M. melanotrichos* makes it another possible host for brittle stars. As *M. mellanotrichos*, *P. bellona* has a tall (up to 45 cm) skeletal axis with well-spaced secondary branches offering a perch for ophiuroids. However, the few specimens available to describe the species at the time were not associated with ophiuroids (Pante and France, 2010). As *P. bellona* was described using museum specimens, it is possible that associates living on the corals were lost during trawling or subsequently removed.

In 2017, the type region of *P. bellona* was revisited as part of the deep-sea cruise KANADEEP. During this cruise, an exceptional haul of 130 colonies of *P. bellona* was made. Most of the specimens collected were occupied by one single brittle star. The goal of this study was to determine whether larger corals harbor larger ophiuroids, as previously observed for *M. melanotrichos* and *O. oedipus* in the Atlantic Ocean (Mosher and Watling, 2009).

Material and Methods

Sampling

One hundred and thirty colonies of *Pseudochrysogorgia bellona* were trawled south of Landsdowne Bank (station CP5052, 20° 57' S, 161° 3' E, 684-715 m depth), off the NW coast of New Caledonia (Figure 1), on 09/24/2017 during the deep-sea cruise KANADEEP1 (under the authority of the Museum National d'Histoire Naturelle and the Institut de recherche pour le développement; BasExp reference: https://expeditions.mnhn.fr/campaign/kanadeep; Sismer field study DOI and associated data: 10.17600/17003800). The Government of New Caledonia approved the work permits for this research (No. 08/2017 and No. 2017-1851/GNC). Sampling was performed 265 km from the type locality for *P. bellona* (Pante and France, 2010). Coral colonies and their associated ophiuroids were photographed on deck with a Panasonic Lumix DMC-FT2 camera for future measurements (Figures S1-2). Specimens from both species had died prior to photography, probably because of the temperature shock when arriving on deck. Multiple pictures were taken of the pair; the ophiuroid and the coral where then separated and photographed multiple times individually. Care was taken as to photograph the specimen perpendicularly to the morphological characters of interest. A metric scale was used on all pictures. Each measure, for all characters except coral base width, ophiuroid central plate and disc area, was replicated 5 times and averaged. Due to limited resources (space and ethanol), tissue from 33 specimen pairs were preserved in ethanol (MHNH voucher numbers IK.2012.13655-87). One additional lot of ophiuroids was placed in 4% formalin and later transferred in 70% ethanol for future investigation.

Morphological and molecular species identification

Corals were morphologically identified by examining colony branching, polyp morphology, and sclerite form and zonation (Pante and France, 2010). Ophiuroids were identified based on the original description of Clark (1949) and the recent review of taxonomic characters for the genus Asteroschema by Nethupul et al. (2022). The morphology of an Australian specimen whose sequence of 16S was compared in this study has been observed by MO (second author), who registered the sequence (AB605078.1). The size and density of the granules of the disc, the degree of concave of inter-radial lateral disc, and the swollen parts of arm segments were almost identical to our examined specimens. Three ophiuroids and their associated corals were chosen at random for genetic analysis. Whole genomic DNA was extracted from 4mm portions of ophiuroid arms, and 3-4 coral polyps, using the NucleoSpin® Tissue kit (Macherey-Nagel), following their standard protocol for animal tissue. Samples were digested overnight in 25 ml of 23 mg.L-1 proteinase K at 70°C. Samples were then put in 20 ml of 23 mg.L-1 proteinase K at 56°C for 3 additional hours. Extracted DNA was quantified using a NanoDrop 2000 (Thermo Scientific) and checked for quality on a 1% agarose gel.

For the coral, the primers ND4L2475F and MUT3458R amplified the mitochondrial gene *mtMutS*, a small portion of *nad4l* (Table 1), as well as the intergenic space separating these genes

(Pante and France 2010). For ophiuroids, amplification of a portion of 16S using the primers reported in Okanishi and Fujita (2013) (Table 1). As DNA extractions from corals and ophiuroids can contain PCR inhibitors, template gDNA was diluted 10x prior to amplification. We followed Pante and France (2010) and Okanishi and Fujita (2013) for PCR amplification parameters for corals and ophiuroids, respectively (reported in Table S1). PCR product size and concentration were checked on a 1% agarose gel. The amplified fragments with the sharpest PCR bands were sent for sequencing in both directions at Eurofins genomics. Sequences were quality-controlled with Geneious 10.2.5. The primer sequences were trimmed. The resulting contigs were blasted using blastn with default parameters (Altschul *et al*., 1990). For ophiuroids, as the sequences obtained here were not identical to sequences on GenBank (see results), we constructed a partial phylogenetic tree for the Euryalidae. First, we downloaded the sequences for *Asteroschema*, *Ophiocreas*, and *Astroceras* based on the GenBank accession numbers listed in Okanishi and Fujita (2013). We aligned them using MAFFT v. 7.467 (Q-INS-I method, optimized for ribosomal RNA; Katoh and Toh, 2008), and inferred the tree using the Maximum-Likelihood (ML) method implemented in IQ-TREE v. 2.0.5 (Minh *et al*., 2020). We used automatic model selection (ModelFinder; Kalyaanamoorthy et al., 2017), and estimated node support with 1,000 bootstrap replicates (UFBoot method; Hoang et al., 2018).

Morphometric analysis

Previous research suggests that the relationship between age and colony total height is linear in *Chrysogorgia agasizii* (Vinogradov, 2000). Since sampling was performed by trawling, most of the octocoral skeletal axes (i.e. the “stem”) were broken at ground-level, and most colonies were missing their holdfast (the calcified disc or root system used by octocorals to anchor themselves to the substrate); the exact height of the colonies was therefore not measurable. Consequently, we measured the skeletal axis diameter at the lowest point possible to estimate the size of the corals. Finally, for coral colonies having an intact holdfast and distal tip (n = 10), we measured the height of the ophiuroid on the coral (distance from the coral holdfast to the middle of the ophiuroid disk), as to test whether its position amid the coral branches is predictable.

For ophiuroids, the following characters were used for morphometric analyses. (1) disc diameter (Gage and Tyler, 1981; Packer *et al*., 1994; Piepenburg and Schmid, 1996; Mosher and Watling, 2009; Alitto, 2019); (2) mouth length (Alitto, 2019). In Alitto (2019), the third dorsal "free" arm segment from the disc is measured with respect to arm length and width. However, arms of *A. ajax* are greatly swollen from the third (or around) arm segments, and the degree of swelling varies with each arm (e.g. Fig. S1 Aboral view). Therefore, we decided to measure (3) the length and (4) width of the first arm segment that just emerged from the disc, despite the risk of being overgrown by the disc. The length of the radial shields (5) was estimated from the prominent area on the disc surface. In the order Euryalida, this character is often covered by thick skin. In contrast, in other brittle stars, this character is more easily measured because it is bare on the dorsal disc. Therefore, as in Stewart (1996), it is most desirable to measure the radial shield by cleaning skin tissue with bleach to expose the radial shields. However, since *A. ajax* has a relatively thin skin and the prominences of the radial shields can be observed close to the center of the disc, we thought we could measure this character (length of radial shield) in this study by measuring the prominent parts without cleaning the skin.

Additionally, we set the following characters for morphometry in this study. As mentioned above, as a character of the aboral disc, there is a possibility that the radial shield is not entirely measured. Therefore, to compensate for this, (6) the entire area of the aboral disc was selected as a character that can be measured reliably (Disc area). In brittle stars, the length of the oral shield, a plate at the peripheral end of the mouth, has been used for morphometric analysis (e.g. Sanvicente-Añorve et al., 2021). However, as mentioned above, the thick skin of euryalid brittle stars makes it difficult to externally observe this structure, and its size is relatively smaller than that of other brittle stars. Therefore, (7) the length from the tip of teeth to the edge of the ventral interradius was measured (oral length). In addition, (8) area of oral plates, aboral shields and oral shield (oral interradii area) were used as a character that may be related to oral length. Furthermore, we also measured (9) dorsal interradii area as corresponding to the aboral side of the "oral interradii area" (summarized on Figure S1).

For variables having several values for each specimen (*e.g.* five independent measures of arm width were taken), the mean value was calculated and used in downstream statistical analyses. The photographs taken of each of the pair of associates were analyzed using ImageJ 1.52s (Schindelin *et al.*, 2015), using the metric scale on the photograph to calibrate our measurements. We did not sex specimens; while deep-sea ophiuroids usually do not present sexual dimorphism (Gage and Tyler, 1982; Hendler and Tran, 2001; Mosher and Watling, 2009), in a future study size could be measured according to sex, based on the formalin-preserved specimens held at the MNHN in Paris.

Statistical Analysis

Using the ten corals that were unbroken after sampling, we performed a non-parametric Spearman rank test to determine the correlation between the length of the skeletal axis (total height, from holdfast to tip) and its base diameter (the base width data deviates from normality; the residual vs. fitted values plot suggests departure from homoscedasticity). This correlation indicated that base diameter is a good predictor for coral size, and therefore age (n = 10, Spearman's *S* = 32, *p* = 0.008, ρ = 0.81).

All the measured variables (coral and ophiuroid) were placed in a correlogram to explore the correlation strength among them (using the Spearman test, owing to the lack of normality in over half of our variables). The aboral side of all ophiuroids were taken (in cases where one coral colony was associated with one ophiuroid individual). However, only 52 photographs were taken from both the oral and aboral sides. We performed Spearman correlation tests on both sets of brittle star data, collected on each side of the samples (aboral: arm width, distance between spines, disc area, disc diameter, radial shield area, radial shield length; oral: mouth width, oral length, oral area). We were then able to choose the most useful set describing ophiuroid size. For the remaining statistics, only the most strongly correlated with the coral size was used.

We performed a Principal Component Analysis (PCA) on the nine ophiuroid variables listed above. The PCA is used here to collapse many correlated variables into two Principal Components used to represent ophiuroid size. The first two axes were used to perform a Spearman's rank correlation with coral base width to test whether the two species of invertebrates grow together.

All statistical tests were performed using R 4.3.1 (R Core team, 2023) at α = 0.05. All raw photographs, raw measurements, R codes and supplemental files are available on Zenodo (Open-Access archive doi :10.5281/zenodo.8186489).

Results

Based on morphology, the brittle stars examined in this study were identified as *Asteroschema ajax*. Their morphology conformed to the description of Clark (1949) and the review of Nethupul et al. (2022): discs are entirely covered by fine granules (Fig. S3B—F), inter-radial lateral discs are concave (Fig. S3A), radial shields are narrow, parallel, raised above the disc, and not meeting at center (Fig. S3B), proximal portion of arm, approximately between the 4th (just outside of the disc) and the 14th arm segments, are swollen (Fig. S3A). Corals were identified as *Pseudochrysogorgia bellona* based on their bottlebrush shape with monopodial, slightly zigzagging skeletal axis; dichotomously-subdivided branches originate in a mostly regular spiral; short (< 2cm) interbranch distance along the skeletal axis; L-shaped polyps with ornamented sclerites of irregular shape, in the form of plates, scales and rods (Pante and France, 2010).

In one out of three coral colonies amplified for *mtMutS*, the sequence was an identical match for the holotype of *P. bellona* (100% query cover to GQ868331.1, voucher MNHN-IC.2008-006). Three out of three ophiuroid specimens amplified at 16S DNA had the three sequences identical to one another (GenBank accession number: MT792000), and had a similarity of 99.8% with *Asteroschema ajax* (469/470 bp identical to AB605078.1, voucher MV:F99759). Inter-individual, intra-specific variation is expected between these specimens (ours and AB605078.1), as the sampling sites are over 1,000 km apart (AB605078.1 was collected off Lord Howe Island, Australia). Our 16S sequences match other Euryalidae species, including *Ophiocreas oedipus*, but in all cases, identity was lower than 95.5%. The ML tree was built based on 35 sequences and 559 bp of 16S, using the TIM3+F+G4 model of evolution (choice based on BIC), and was rooted to *Astroceras* sp. (Figure 2). The sequences produced here group with *A. ajax* and form a clade sister to three *O. oedipus* sequences; *Asteroschema* and *Ophiocreas* are polyphyletic (a fourth *O. oedipus* sequence grouping with *A. salix* in a sister clade also composed of *A. migrator*), as observed by Okanishi and Fujita (2013).

Among the 130 sampled coral colonies, 124 (95.4%) were occupied by a single ophiuroid, 4 (3.1%) had no echinoderm associate, and 2 (2.3%) had two ophiuroids amid their branches.

Sampling was done by trawling, which may have caused some ophiuroid to move across corals (resulting in a few colonies bearing zero or two ophiuroids). The correlation between coral height and ophiuroid height ("altitude" on the coral colony) was high ($n = 10$, Spearman's $S = 12$, $p = 0.0001$, $\rho = 0.93$). Ophiuroids indeed occupied a predictable position on the corals, being preferably placed towards the top of the colony, in a place of dense branching.

Coral height was positively correlated with all measured ophiuroid variables, except with spine distance ($n=10$, $S = 184$, $p = 0.7$, $\rho = -0.12$; Figure 3). A marginally significant positive correlation with coral height was found for radial shield length ($n=10$, $S = 26$, p-value $= 0.07$, $\rho = 0.69$). As stated above, only ten colonies were sampled with intact holdfast and distal tip; we therefore carried the rest of the statistical analyses based on coral colony base diameter as a predictor of coral age. Base width was significantly positively correlated with all ophiuroid metrics at $\alpha = 0.05$. Applying a strict Bonferroni correction for multiple testing (9 tests, bringing α to 0.006), the following ophiuroid variables remained significant: width of 1st arm segment ($n = 122$, $S = 196278$, $p < 0.0001$, $\rho = 0.35$), disc diameter ($n = 122$, $S = 193025$, $p < 0.0001$ $\rho = 0.36$) and disc area ($n = 122$, $S = 187941$, $p < 0.0001$, $\rho = 0.38$), radial shield length ($n = 120$, $S = 164128$, $p = p < 0.0001$ $\rho = 0.43$; Figure 4) and dorsal interradii area ($n = 120$, $S = 192426$, $p = 0.0002$, $\rho = 0.33$). These results suggest that most measured ophiuroid variables (particularly on the aboral side) had positive relationships with coral size, and that larger brittle stars were found in association with larger corals.

The first two axes of the PCA performed on nine ophiuroid variables explained 79% of total variance in the data ($n = 45$). The first axis (PC1, 59% of variance explained) captured brittle star size, with all variables contributing rather equally to it. After evaluation of photographs with similar PC1 scores and different PC2 scores, we established that the second axis (PC2: 20% of variance explained) probably captures brittle star shape. PC2 is affected by the unequal spacing of the arms, orientation of the arms, and reproductive status (all variables contributing rather equally to it, except width of 1st arm segment and mouth length, which had a weaker contribution; width of 1st arm segment was measured proximal to gonadal swelling). Spearman's rank correlation between ophiuroid PC1 ("size") and the coral's base diameter was significantly positive (n = 45, S = 22808, p = 0.0004, ρ = 0.50; Figure 4).

Discussion

First description of the *P. bellona* - *A. ajax* association

Morphological and molecular data confirmed the identification of the two associates as *Pseudochrysogorgia bellona* and *Asteroschema ajax*. This association is, to our knowledge, new to science. No mention of a coral host was made in the original description of *A. ajax* (Clark, 1949). In addition, the brittle star was not observed on the type specimens of *P. bellona*, collected in 2004. This could either be explained by the fact that the two invertebrates were associated in the field but separated during sampling or prior to archival in museum collections, or that these taxa were not sampled together in the field. The latter would imply that *P. bellona* is not obligatorily associated with *A. ajax*. This scenario is, in our opinion, unlikely, given that 98% of *P. bellona* colonies sampled in 2017 sheltered at least one *A. ajax*. In addition, ten specimens identified as *A. ajax* were collected at the type locality of *P. bellona* at the same station of its initial discovery (station CP2557, EBISCO cruise, 2005, specimen voucher MNHN-IE-2013-2585 of the echinoderm collection of the MNHN). *A. ajax* is not common in the MNHN collection: the only New Caledonian samples come from the Chesterfields (Lansdowne Bank and Bellona Plateau, stations CP2557 and CP2627 from the EBISCO cruise of 2005, CP5055 and DW4968 from the Kanadeep cruise of 2017). The only other occurrences of *A. ajax* in the

MNHN collection come from Tahiti and Madagascar (one station and one specimen each). It is therefore possible that *A. ajax* individuals were indeed originally attached to the coral specimens sampled at the same station. Likewise, the *P. bellona* specimens from NIWA museum collections (vouchers NIWA15611 and 16272 from Otara Seamount, stn TAN0413/35 and 41, respectively; Pante and France 2010) were not associated with ophiuroids, but *O. oedipus* is reported from the same stations in the echinoderm collection (NIWA13606 and 13608 from stn TAN0413/35 and 41, respectively). *Metallogorgia* occurs on Otara seamount (NIWA15642 from TAN0413/45), and some *P. bellona* specimens were initially mislabelled as *Chrysogorgia* and *Metallogorgia*. It remains to be checked whether the specimens from Otara seamount and labeled as *O. oedipus* are indeed that taxon or *A. ajax* (this could not be done during the preparation of this manuscript as Echinoderms from NIWA were not available for examination due to renovations of the collection rooms). In conclusion, the *P. bellona* specimens discussed in Pante and France (2010) may have been associated with *A. ajax* in the field and placed in the museum collections corresponding to their respective phyla.

The fact that the original species description of *A. ajax* does not include information on associate(s) is not surprising. Indeed, the original description and following studies on *O. oedipus* did not provide any information of host species, until 2009 (Mosher and Watling, 2009, and references therein). We do observe, however, that euryalids can associate quite specifically to octocorals. *Ophiocreas oedipus* seems obligate to *Metallogorgia melanotrichos* (Mosher and Watling, 2009). *Asteroschema clavigera* associates with gorgonian species of *Paramuricea* and *Paragorgia* (Cho and Shank, 2010). *Asteroschema tenue* seems obligate to *Ellisella barbadensis* (Emson and Woodley, 1987). *Squamophis amamiensis* associates with *Muricella* sp. (Okanishi and Fujita, 2009). Unidentified *Asteroschema* sp. were also reported on multiple *Riisea*, *Nicella* and *Callogorgia* species (Cairns, 2007; Cairns *et al*., 2018). This suggests that some euryalids have adapted (partly or entirely) to a life perched on coral branches.

Insights on the ecological nature of the association

The significant proportion of *P. bellona* colonies occupied by a single *A. ajax* individual (95%), as well as the observation that taller octocorals shelter bigger ophiuroids, perched higher in the branches, suggest that the relationship between these two species is obligate, at least at our study site. Indeed, while our present study site is the type locality for the octocoral *P. bellona*, and that both morphology and genetics perfectly match the data presented in Pante and France (2010), comparative data for the ophiuroid *Asteroschema ajax* are scant, and geographically more widespread. This species was originally described from Hawaii (Clark 1949), and to the best of our knowledge, no DNA sequence is available from the type series. The specimen genetically matching our material was collected off Lorde Howe Island. Given the geographical distance between the Norfolk Ridge and Hawaii, we therefore do not rule out, based on the information at hand, that our specimens from New Caledonia may belong to a species closely related to the Hawaiian type material. The obligate nature of the association between coral and ophiuroid therefore needs to be validated with data from more geographical sites, including the type locality for the echinoderm.

Few corals had either no associates (3%) or two associates (2%). This may reflect an ecological pattern and/or a sampling artifact. Ecological hypotheses for corals lacking associates include a non-obligate relationship and mortality (predation, disease, ageing). It is also plausible that few ophiuroids lost their host during trawling. Of equal interest are the two (out of 124) corals that harbored two ophiuroids. It can be hypothesized that two ophiuroids may be able to successfully live on a single coral colony, that ophiuroids may swim among nearby corals, or that ophiuroids crawled upon another host after sampling. This last hypothesis is plausible and probable: the percentage of coral colonies without associate is similar to the number of colonies with two associates (2 and 3%, respectively).

The nature of this strong association (in terms of the dependency of one species on the other) remains, then, speculative. *A. ajax* could use the octocoral branches as shelter, sitting amid stinging polyps that provide protection against predators (Mosher and Watling, 2009). Euryalid brittle stars are suspensivores (Warner 1982; Okanishi, 2016), and therefore probably benefit from sitting above the seabed, getting better access to particles on which they feed (Mosher and Watling, 2009). ROV observations of *O. oedipus* on *M. melanotrichos* show that the normal behavior of the brittle star is to wave its arms in the water column to catch food particles (Mosher and Watling, 2009; E. Pante, personal observation). The benefit of higher food availability while perched on corals is so important for another ophiuroid, *Astrobrachion constrictum* (Euryalidae), that when detached from its black coral host, *Antipathes fiordensis*, it immediately searches for a new coral on which it can live (Stewart, 1998).

The coral might also benefit from the ophiuroid. For example, the resilience of the octocoral *Paramuricea biscaya* after the Deepwater Horizon oil spill was improved in the presence of its symbiont *Asteroschema clavigerum*, which removed particles depositing on the polyps (Girard *et al*., 2016). Perhaps similarly, Grange (1991) suggested that *As. constrictum* contributes to the good health of *An. fiordensis* by consuming its excess mucus and removing other epizoics settling on it by eating their larvae. Mosher and Watling (2009) did not observe *O. oedipus* cleaning *M. melanotrichos* of particulate matter. In situ observation would be useful to understand whether *P. bellona* is cleaned by *A. ajax*. In addition, we do observe, both on *M. melanotrichos* and *P. bellona*, traces of broken branches at the base of the colonies (Figure 2 and page 607 in Pante and France, 2010); the prevalence of these stumps, higher on taller corals, may simply be related to the coral's growth, due to movements of the ophiuroids on coral colonies, damaging branches, or some other, unknown cause. There is no evidence that *A. ajax* feeds on the coral itself. However, it is possible that *A. ajax* occasionally steals food from *P. bellona*, as does *As. constrictum* from *An. fiordensis* (Grange, 1991), and *Eunice* worm living on *Lophelia* (Mortensen 2001). We could speculate that reducing the number of feeding polyps by breaking branches and occasional kleptoparasitism could reduce the coral's food intake, potentially affecting growth and reproduction and that there might therefore be benefits and costs for *P. bellona* to associate with *A. ajax*. Data from other geographic sites, such as ROV observations of the behavior of both species, in situ manipulative experiments, and gut content characterization could help determine whether this relationship is indeed obligate, and whether is it commensal, symbiotic, or parasitic in nature.

Are the growth of *P. bellona* and *A. ajax* synchronized?

Larger corals harbored larger ophiuroids. The size / age relationship and its linearity are here central to determining whether growth is synchronized in this species pair. *Pseudochrysogorgia*, as its close allies *Metallogorgia* and *Chrysogorgia*, is a calcaxonian octocoral, and therefore deposits calcite and aragonite along its axial skeleton. On the latter, Cairns (2002) noted about *C. antarctica* that "the concentric axial lamellae are assumed to be laid down with temporal regularity, but the time interval between each lamella can only be speculated." Vinogradov (2000), using photographs of young *C. agassizii* growing on the stern of the sunken R.M.S. Titanic, observed linear growth based on 3 time points taken 8 years apart. Looking at older (>500yr) colonies of *Chrysogorgia* sp., Prouty et al. (2016), found that this linear growth pattern levels off as colonies get older. Likewise, ophiuroid size (*e.g.* estimated by measuring disc diameter) was found to follow a sigmoid curve in the antarctic *Ophionotus victoriae*, and in the arctic *Ophiocten sericeum* and *Ophiura sarsii* with fast growth in early years, then linear growth, then slower growth (Dahm and Brey, 1998; Ravelo et al., 2017). As size was shown to be a predictor of age (and despite the non-linearity of the size / age relationship in early and late years) in some ophiuroids and octocorals, we can propose the hypothesis that the brittle star attaches itself to the coral early in its life, at least at the juvenile stage (the smallest *As. ajax* sampled had a disc diameter of 3 mm; this is probably a sexually immature, juvenile individual, Gordon Hendler, pers. com.). The pair may then grow together, or ophiuroids may switch partner if growth rates differ between the two taxa (*e.g.* small corals incapable of sheltering large brittle stars). Indeed, the lifespan of coral and ophiuroid may very well differ in scale; as stated above, *Chrysogorgia* colonies can live hundreds of years (Prouty et al. 2016), while ophiuroid maximum lifespan may be an order of magnitude lower (*e.g.* ~ 20 yr for *Ophionotus victoriae*, Dahm 1996, Dahm and Brey, 1998; ~ 20 yr for *Ophiura ljungmani* Gage and Tyler, 1981; 19 yr for *Ophioceres iizcipiens*, 25 yr for *Ophiurolepis brevirima*, 33 yr for *Ophiurolepis gelida*, Dahm 1996) with the exception of *Astrotoma agassizii* (91 yr, Dahm 1996). It is worth noting that the red sea urchin *Mesocentrotus franciscanus* counts among the longest-lived animals (up to 200 yr), while it was originally thought to live for seven to ten years (Ebert and Southon 2003). We can therefore not rule out that coral and ophiuroid have similar lifespan. Determining if *P. bellona* and *A. ajax* indeed grow and senesce together is therefore premature at this stage and would necessitate age and growth rate estimates for these two species (*e.g.* Prouty et al. 2016; Quiroga and Sellanes 2009 and references therein).

Unfortunately, the size range of octocorals and ophiuroids was narrow in our study (28.9 ≤ coral height ≤ 45.9 cm; 3 ≤ ophiuroid disc diameter ≤ 11 mm), preventing us to investigate the full size range for these species. Juveniles, however, were sampled for *M. melanotrichos* and *O. oedipus* in the study of Mosher and Watling (2009). The authors distinguished three growth stages for the octocorals (juvenile, intermediate, adult) that correlated with ophiuroid size. Most interestingly, they described a pair of extremely small specimens sampled in the Bahamas, the coral being 11 cm tall and the ophiuroid 1.5 mm in disc diameter (see their Addendum on page 88). This single pair speaks of the very tight relationship between the species. Mosher and Watling (2009) suggested that the *M. melanotrichos* / *O. oedipus* association have lasted for thousands of years. How, then, have two partners manage to stay together generation after generation? The authors propose that ophiuroid larvae could be chemically attracted to young corals on which they can settle and be able to sustain the coral's chemical and physical defenses (Mosher and Watling, 2009). Future investigations on the chemical and reproductive ecology of these species could help bring light to these questions.

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Figure Legends

Figure 1. Map of study area with sampling site of 130 octocoral / ophiuroid pairs (station CP5052), and type locality for *P. bellona* (station CP2557). Sampling depth range is provided below station numbers. Isobaths plotted from 0 to 1500 m depth, in 500 m increments (Pante and Simon-Bouhet, 2013).

Figure 2. Maximum likelihood tree based on partial 16S sequences for the genera *Asteroschema, Ophiocreas*, and *Astroceras* within the Euryalidae. The alignment contains 35 sequences and is 559 bp long. Support values for each node are ultrafast bootstrap values. Names of *A. ajax* specimens are bolded.

Figure 3. Correlogram showing the Spearman correlations among the coral (top 2 lines, left 2 columns) and brittle star (bottom 9 lines, right 9 columns) variables. Lower panel: blue gradient indicating a positive correlation (dark blue meaning high correlation) and red gradient indicating a negative correlation (dark red meaning a strong negative correlation). Diagonal: probability density functions for individual variables. Upper panel: biplots corresponding to each variable pair. Sample sizes: n=10 for lines 1-2, n=52 for lines 3-5 (with data on oral and aboral sides of the ophiuroids, see methods), n=124 for lines 6-10 (with data on aboral side only, see methods).

Figure 4. Ophiuroid radial shield length (top) and PC1 summarizing overall ophiuroid size(bottom) as a function of octocoral base width. Trendline: LOESS curve with alpha degree of smoothing set to two.