The Importance of Home Cleaning: Sediment Transport by Alpheid Shrimps Provides a Competitive Advantage to Their Host Anemones

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Bartholomea annulata is a facultative host of the *A. armatus* species complex. In the Mexican Caribbean it is commonly found in cracks and crevices located where the vertical walls meet the sandy bottom or on large coral patches away from the sand. To protect themselves from predators, anemones often contract their hydraulic body into a cavernous den and extend the stinging tentacles toward the entrance. The high sediment dynamics of the region, however, result in a permanent risk of animal shelters to be obstructed by sand. By both analysing field data and conducting laboratory experiments with artificial shelters, the present study explored the den cleaning behaviour widely extended amongst alpheid shrimp, and its role in the alpheid-anemone symbiotic interaction. Videorecordings showed that den cleaning was composed of three main behaviours: digging, tossing and tamping. It commenced as soon as 7.2 ± 10.5 min after anemones were recognised by alpheids, and behaviours were displayed systematically amongst all 12 replicates. Despite being completely burrowed in sand, *Alpheus* spp. were capable of finding the anemone and liberating the entrance of the artificial shelters in less than 2.5 h. In addition, manipulative experiments showed that anemones confronted with shelters that were obstructed with sediment had a 25% probability of fully retracting when *Alpheus* spp. were absent, compared to a 75% probability when shrimps had cleaned the shelter's entry and internal passage. The analysis of field data indicated that the presence of alpheid shrimps as symbionts of B. annulata was 30% higher amongst anemones in close contact with sandy bottoms than when inhabiting crevices on the top or lateral walls of hard substrates, away from the sediment. Overall, our study concludes that den cleaning constitutes a quick and effective mechanism to assure the anemone's full retraction into their den, and by keeping the sediment away, alpheids provide the necessary conditions that serve both

the anemone's and the shrimp's higher chances of acquiring maximum protection from predators. This advantageous exchange in protection partially explains why alpheids are more frequently present in *B. annulata* in interface microhabitats where the benefit of the interaction is maximised.

Keywords: symbiosis, ecological interactions, coral reefs, animal behaviour, *Alpheus armatus*, *Alpheus immaculatus*, snapping shrimps, *Bartholomea annulata*

INTRODUCTION

Coral reefs are heterogeneous environments, featuring a great variety of optimal and sub-optimal habitats (Richardson, 1999). The main variables that affect the community structure in coral reefs are luminosity, water currents flow, water temperature, intra and inter-specific competition, predation, and sediment transport (Glynn and Enochs, 2011; Alvarez-Filip et al., 2013). Anemones are an essential component of coral reefs' benthic fauna in the Caribbean Sea and are typically attached to hard substrates, such as coral rubbles, rocks and live or dead coral on which the pedal disc can be firmly fixed (Ellis, 1767; Le Sueur, 1817; Duchassaing de Fonbressin and Michelotti, 1860; Duerden, 1897; Manjarrés, 1977; González-Muñoz et al., 2013). Other species, such as *Actinostella flosculifera* or *Phymanthus crucifer,* are found in sandy patches and also attach to hard structures but maintain the column covered with sediment (Le Sueur, 1817; González-Muñoz et al., 2012).

Partnership and common objectives amongst individuals of different species are one way to survive against natural selective pressures (Bauer, 2004). Symbiosis could be the difference between life and death for dissimilar organisms living together (Baeza, 2015). The cost and benefit of the symbiotic interaction (i.e., parasitism, mutualism or commensalism) and the degree of dependence (i.e., facultative versus obligate) vary widely (Thiel and Baeza, 2001), and have been recognised as an important speciation mechanism that explains and maintains the biodiversity patterns found in underwater ecosystems (Watson and Pollack, 1999; Crandall et al., 2008; Dáttilo and Rico-Gray, 2018; Pérez-Botello and Simões, 2021).

Sea anemones are host to a wide variety of invertebrates, amongst which crustaceans, and more specifically shrimps, dominate (Herrnkind et al., 1976; Silbiger and Childress, 2008). Symbiotic relationships can act in two directions altering both symbiont and host fitness (Crawford, 1992; Roopin and Chadwick, 2009). Without exception, this symbiotic interaction is facultative for the anemones, hence the occurrence of anemones both with and without symbiotic crustacea. For the crustacea, however, the level of dependence varies amongst interactions, with some species being obligate anemone-symbionts. Such is the case of shrimps *Ancylomenes pedersoni, Periclimenes yucatanicus, Alpheus armatus,* and *Thor amboinensis,* whereas such as the crabs *Stenorynchus seticornis* and *Mithraculus cinctimanus* are facultative guests of anemones (Manjarrés, 1977; Silbiger and Childress, 2008; González-Muñoz et al., 2012; Hurt et al., 2013).

Interactions between anemones and alpheid shrimp have demonstrated benefits for both symbiotic species. Anemones obtain protection against benthic predators that are immune to cnida-toxins through the snapping water jet made by their alpheid guests (Smith, 1977; McCammon and Brooks, 2014). It has also been argued that the particles that alpheids discard after feeding constitute a source of nitrogen supply for the anemone's zooxanthellae (Spotte, 1996), increasing the number and efficiency of associated photosynthetic cells (Kropp, 1987; Day, 1994; LaJeunesse, 2002; Khan et al., 2003). In exchange, alpheids obtain a safe refuge from their pelagic predators derived from the defensive mechanism of the anemones' toxic sting (Romey et al., 1976; Vincent et al., 1980; Cestele, 2000; Sanchez-Rodriguez, 2001; McCammon, 2010; Mascaró et al., 2012).

The advantage of the anemone's chemical protection is restricted to shrimps that can acclimate to the host's toxins (Mascaró et al., 2012). These guests, however, may also benefit from other anemone defensive strategies that are not directly dependent of the chemical power of nematocysts. Anemones have three distinct defence strategies that do not directly involve cnida: (i) releasing the pedal disc from the substrate and swimming away from the hazard; (ii) sticking the tentacle to protect the oral disc and column; and (iii) contracting its hydraulic body and dodging any possible danger (Rosin, 1969; Edmunds et al., 1974; Harris and Howe, 1979; Shick, 1979; González-Muñoz et al., 2012). If body contraction occurs within a burrow, the defence efficiency of toxic cnida could be increased: anemones cluster the tentacles with the deadly nematocysts at the burrow's opening, while keeping vulnerable parts of the body sheltered and secure. By remaining close to the column and pedal disk when the anemone retracts into the burrow, alpheids may take advantage of the cover provided by the protective tentacles.

The shrimp genus *Alpheus* is a diverse and highly radiated group of fully benthic shrimp, distributed throughout all shallow tropical and subtropical marine regions of the world (0–50 m deep) (NMNH, 2001; Anker et al., 2006; CRED, 2008; Massuti et al., 2020). These shrimps are common in coral reefs, seagrass beds, mangroves, estuaries, and mixed sand-rock bottoms (Anker, 2001; Duarte et al., 2014). Some species are hard-substrates drillers, and others specialise on excavating soft substrates; but in every case digging behaviour influences the alpheid community structure and dynamics (Palomar et al., 2005; Anker et al., 2006). A remarkable behaviour amongst alpheid shrimp that are obligate symbionts of goby fish consists on building dens for their fish host and keeping them clear of sediment, an interaction that has been extensively documented in recent literature on crustacean symbiosis (Karplus, 1979, 1987, 2014; Karplus et al., 2011; Bauer, 2004). In this symbiotic relationship, the digging behaviour constitutes the primary shrimp behaviour on which the interaction is sustained (Karplus, 1987; Karplus et al., 2011). Whilst the goby fish watch over,

the snapping shrimps dig, construct and maintain clean the burrows that goby fish will use for breeding and shelter (Karplus, 1987, 2014; Karplus et al., 2011; Henmi et al., 2017). This association is reported to exist in all five oceans and across sediment types, ranging from silty mud to coral rubble (Karplus, 1987), suggesting that digging behaviour amongst alpheid shrimp is not only common within the group but also geographically widespread.

The sea anemone *Bartholomea annulata* has a symbiotic multi-specific interaction with several crustacea, such as *A. pedersoni, P. yucatanicus, T. amboinensis* and specially three alpheid species of the *Alpheus armatus* species complex (i.e., *A. armatus, A. immaculatus,* and *A. polystictus*) (Knowlton and Keller, 1983, 1985; Silbiger and Childress, 2008; McCammon, 2010; Hurt et al., 2013). *Bartholomea annulata* is distributed in the lagoon and back-reef zones of the Gulf of Mexico and the Caribbean inside caves and crevices of rocks and coral rubble with only its tentacles exposed into the water column (González-Muñoz et al., 2012; Rodríguez et al., 2014). It is common between 1 and 15 m deep, but has been observed as deep as 20 m (González-Muñoz et al., 2012). In contrast with other anemones common in tropical waters that are found attached to surfaces relatively distant of the sea bed, such as *Condylactis gigantea*, *B. annulata* is frequently found in the interface between the hard and soft substrate, a microhabitat exposed to abundant sediment and high wave intensity (Barrios-Suárez et al., 2002; González-Muñoz et al., 2012; Santos et al., 2020).

The *A. armatus* species complex are obligate symbionts of anemones that are occasionally found as one shrimp, yet most commonly are found as a pair of male-female reproductive shrimps occupying a large anemone or anemone cluster (Hurt et al., 2013). Because alpheids are markedly territorial (Schein, 1975; Schmitz and Herberholz, 1998; Rahman et al., 2004), groups of more than two reproductive adults are never found (Smith, 1977). The species common to the North Western Atlantic Ocean are *A. armatus* and *A. immaculatus,* distributed in the Gulf of Mexico and the Caribbean Sea from 1 to 25 m deep (Hurt et al., 2013). Whilst *Alpheus armatus* typically occurs at depths lower than 10 m, whereas *A. immaculatus* is generally found at depths between 13 and 25 m (Knowlton and Keller, 1983, 1985; Hurt et al., 2013). Both species are extremely similar morphologically and occur microsympatrically (Knowlton and Keller, 1983). They also occupy the anemone's column and protect their body by sharing their host's burrow (Huebner et al., 2019). The occurrence of *A. armatus* species complex is therefore restricted to the distribution of available of host anemones (Hurt et al., 2013; Huebner et al., 2019).

While the alpheid behaviour of building and maintaining the dens of goby fishes is well known, the contribution of similar digging behaviours to the symbiotic interaction with anemones has not been studied. If den cleaning behaviour is a common feature of alpheid shrimp that are obligate symbionts of *B. annulata* and the advantage for the anemone is confirmed, then symbiotic associations between alpheids and *B. annulata* should be most frequent in those microhabitats where the benefit of the interaction is maximised. To support this hypothesis the present study aimed at describing the distribution of *B. annulata* with and without *A. armatus* complex (*sensu* Knowlton and Keller, 1983, 1985) in different types of microhabitats within the reefs of the Mexican Caribbean from Isla Contoy to Punta Xcalac. Laboratory experiments were then conducted to characterise the den cleaning behaviour of *A. armatus* complex in the presence of *B. annulata* and provide evidence that this behaviour improves the retraction capability of anemones within artificial shelters built for this purpose.

MATERIALS AND METHODS

Microhabitat Distribution of *Bartholomea annulata* in the Mexican Caribbean
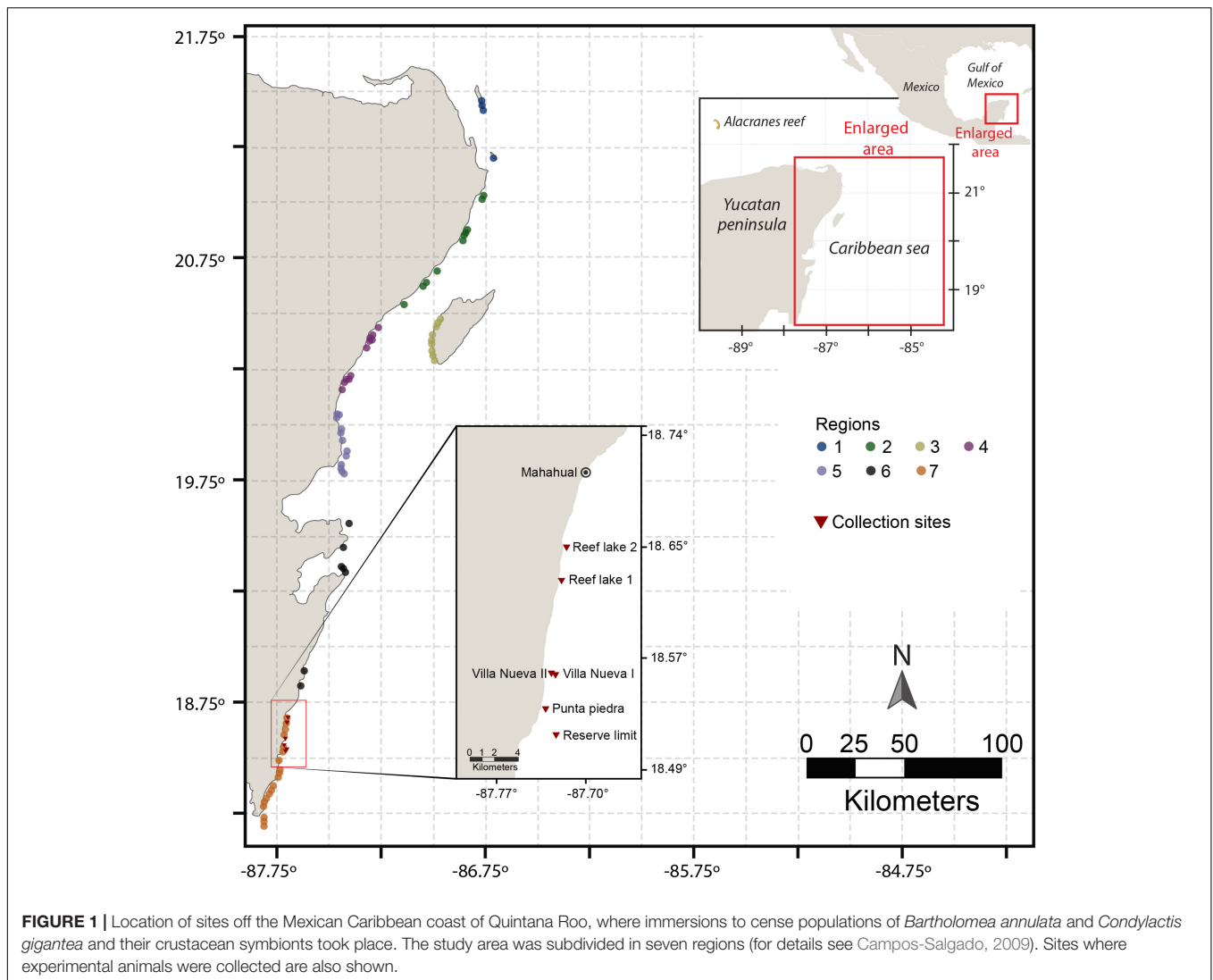
Information related to the type of substrate, structure and the position in which individuals of *B. annulata* were observed was extracted from Campos-Salgado (2009) field sampling reports. The study reported a total 179 immersions in 87 different sites along 7 regions in the Mexican Caribbean from *Isla Contoy* (21.6633°, −87.1000°) to *Punta Xcalak* (18.1500°, −87.8430°; **Figure 1** and **Table 1**). At each site two independent divers performed visual census of *B. annulata* and *Condylactis gigantea* and their crustacean symbionts following each a 45 min transect. Anemones, including *B. annulata*, were identified using the Reef Creatures identification book (Humann and DeLoach, 2002) and the micro-habitat in which they were found was noted. Micro-habitats were originally described as crevices, caves or small holes on coral fragments, sand, limestone massif, platforms or rocks; and in positions that varied from on the top or lateral surface of hard structures or at the interface between these hard structures and the sandy bottom. Each anemone was carefully inspected for crustacean symbionts and the number of individuals and species found was noted.

From this data base, we retrieved the number of *B. annulata* observed at each site and the position in which each individual anemone was found: i) in crevices, caves or small holes located on the top of lateral walls of coral fragments, limestone massif and rocks (**Figure 2A**); or ii) at the interface between hard substrate and the sandy bottom (**Figure 2B**). In addition, each anemone was classified as the host of none or at least one individual of the *A. armatus* species complex (Knowlton and Keller, 1983, 1985).

To test the hypothesis that the proportion of anemones with alpheid shrimps of the total number of anemones was higher in microhabitats with a sandy interface than amongst those found elsewhere, a logistic regression with the logit link function and the binomial distribution was applied to the field data (Powers and Xie, 1999). The frequency of anemones was transformed to the odds of finding an anemone with at least one alpheid shrimp present (*against* those without a shrimp) in each habitat and region. The odds (O) are defined as the ratio of the probability of one outcome (p) to another ($1 - p$)

$$O = \frac{p}{1 - p}$$

where p is the probability of finding an anemone with at least one alpheid shrimp. The underlying general lineal model

FIGURE 1 | Location of sites off the Mexican Caribbean coast of Quintana Roo, where immersions to cense populations of *Bartholomea annulata* and *Condylactis gigantea* and their crustacean symbionts took place. The study area was subdivided in seven regions (for details see Campos-Salgado, 2009). Sites where experimental animals were collected are also shown.

(GLM) had "habitat" (with 2 levels: Interface and Elsewhere) and "region" (with 7 levels) as additive terms. To assess the effect of habitat and region on the response variable, a hypothesis

TABLE 1 | The number of scuba-diving immersions performed in sites distributed throughout seven regions off the Mexican Caribbean coast of Quintana Roo and the total number of anemones *B. annulata* and associated *A. armatus* found in each region.

Region	Number of immersions	Number of anemones	Number of *A. armatus*
Region 1	14	19	29
Region 2	22	111	121
Region 3	20	127	77
Region 4	22	113	96
Region 5	30	177	157
Region 6	10	53	66
Region 7	61	569	361

Regions were delimited following Campos-Salgado (2009).

testing procedure that compared the residual deviance of a full versus a reduced model by means of a χ^2-test was applied for each term (Zuur et al., 2007). If the term "habitat" was significant, it would constitute evidence that the occurrence of shrimp-anemone associations differed between the two habitats more than expected by chance alone; and that these differences were irrespective of region. If the term "region" was significant, it would constitute evidence that the occurrence of shrimp-anemone associations, in either interface habitats or elsewhere, varied amongst regions.

Laboratory Observations and Experiments
Origin and Maintenance of Experimental Animals
A total of 27 sea anemones and 33 snapping shrimps of two cryptic species, *Alpheus armatus* and *A. immaculatus*, in the same study area where previous field work took place (Region 7; **Figure 1**). Because both species occupy a similar ecological niche (Knowlton and Keller, 1983), they were

treated as a single taxonomic unit also known as *A. armatus* species complex throughout experiments despite the ability to identify each one. Anemones were carefully removed from the substrata with a hammer and chisel, avoiding any damage to the column or the pedal disc, while snapping shrimps were collected using a plastic slurp pump. Both anemones and shrimp were then transported to laboratory facilities at the *Unidad Multidisciplinaria de Docencia e Investigación* of the *Universidad Nacional Autónoma de México* at Sisal, Yucatan, where experimental trials took place.

Experimental animals were placed in a system consisting of Perspex aquaria (length 26.5 × width 20 × depth 20 cm; 10.6 L) with a continuous supply of recirculating sea water treated with mechanical (25, 10, and 5 μm), biological (bio-balls) and UV filtration. Aquaria were equipped with an artificial shelter to provide anemones with a hard substrate for attachment and shrimps a safe place to hide. These rigid shelters were made of a mixture of sand and cement and had an arched entrance leading to a halfpipe gallery, thereby mimicking natural dens (**Figure 3**). Anemones were left to attach their foot to the end furthest from the shelter's entrance, resulting in the full extension of the column, oral disc, and the tentacles (**Figure 3**). Apart from the artificial shelter, no substrate was added to aquaria during maintenance.

Female and male shrimps of the *A. armatus* species complex were paired into 15 couples thereafter treated as experimental units. An attempt was made to keep naturally bonded pairs as they were originally found in the field, but new stable pairs were formed when this was not possible. Because male and female shrimp were not in equal numbers, three organisms were discarded from subsequent experimental trials. Stability in the newly formed couples was achieved by presenting pairs of individuals through controlled choice procedures, in which escalating agonistic interactions were interrupted and animals presented with an alternative partner until positive coexistence was achieved. Each couple was then placed with one sea anemone in an aquarium forming 15 distinct triads. The remaining anemones were kept in shelters without snapping shrimps. All experiments were conducted with couples of shrimps in order to keep conditions as similar as possible to those occurring naturally in reefs.

During maintenance (2–3 weeks), anemones were fed three times a day with 25 g of defrosted brine shrimp uniformly distributed amongst holding aquaria. Alpheids were fed twice a day a mixture of 5 g of mussel gonad and 20 g of squid cubes (9:00 a.m.) and 25 g of squid cubes. Uneaten food was extracted daily by syphon and water levels re-established to keep sea water conditions constant. Water in the aquaria was kept at 26 ± 0.5°C (mean ± standard deviation), salinity 37 ± 1 ups and gentle aeration. A 12:12 light:darkness period was kept throughout both maintenance and experimental trials.

Experimental Setup

Experiments took place in an isolated room where noise and other sources of interference were kept to a minimum. Four glass aquaria (length 23 × width 23 × depth 23 cm; 12 L) were filled with sea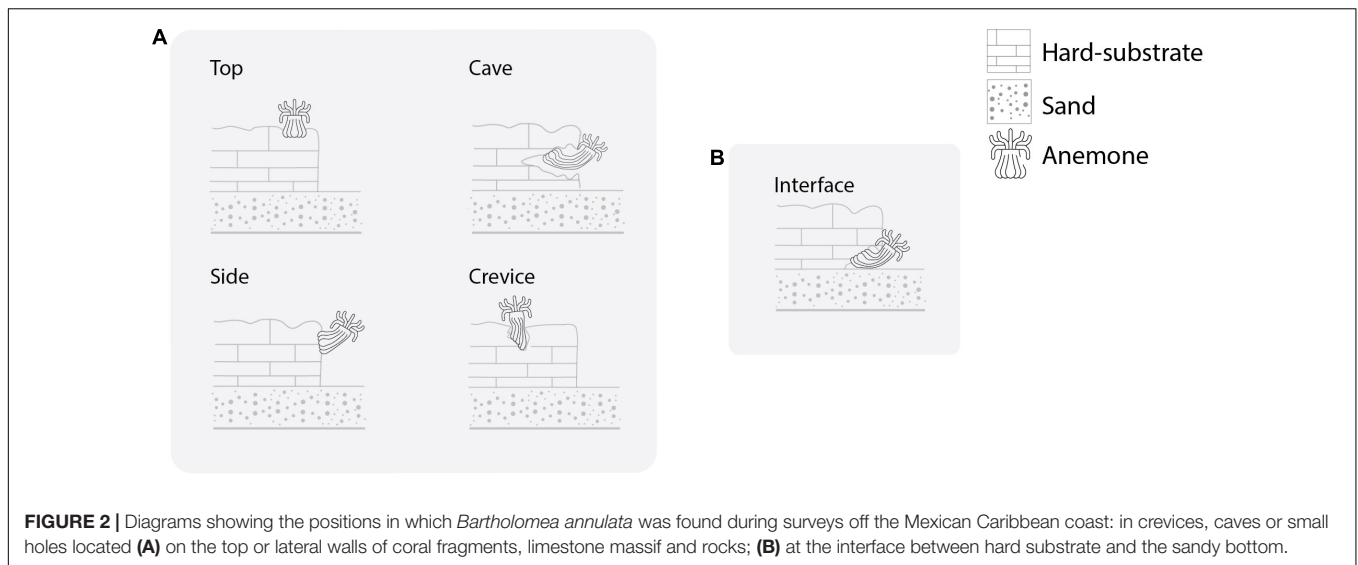 water from the maintenance system and fully exchanged at the end of each trial. Two of the aquaria were covered with a 5 cm layer of sand collected from Sisal, Yucatan, Mexico, and sieved through a 0.95 mm mesh to standardise particle size (<1 mm). The other two aquaria were kept free of sand throughout experiments.

A video recording system was implemented inside the room to register the behaviours of both anemones and snapping shrimp displayed throughout experiments. This system consisted of a closed-circuit television (CCTV) and a hand-held video camera (Canon Vixia Hf R11 Full HD). The CCTV was used to record activity continuously, and the handheld video camera was used to capture high-quality videos of specific behaviours of alpheid shrimp.

Effect of Den Cleaning Behaviour on the Retraction Response in *Bartholomea annulata*

To investigate the effect of the den cleaning behaviour of snapping shrimp on the retraction response of *B. annulata*, a manipulation experiment was designed using three different treatments: (i) anemone in a shelter with sand and a pair of *A. armatus* shrimps (BSA), (ii) anemone in a shelter with sand but without *A. armatus* shrimp (BS0), and (iii) anemone alone in its shelter, i.e., without shrimp or sand (B00). The 27 anemones, together with their artificial shelters, were transported four at a time to the experimental room and placed individually in an aquarium with or without sand depending on the treatment they would be receiving (BSA: $n = 9$; BS0: $n = 8$; B00: $n = 10$). Shelters in the BSA and BS0 treatments were manually buried with the sand available in the aquarium, partially covering the anemone. A 24 h period was allowed for anemones to habituate to the aquaria and for shrimp to remove the sand from the shelters before initiating trials. To simulate an aggression and trigger the anemone's defence behaviour of retracting the body into the shelter, a quick jet of seawater was then fired at the anemones' oral disk using a 3–5 ml hand pipette. The defence response was subsequently video recorded during 30 s by means of the hand-held video camera. In each case, the success or failure of the anemones' full contraction was noted. Only results for the first contraction response were considered.

A defence response was considered successful (p) when the anemone was able to fully retract both the column and the oral disc into the shelter, whereas a defence failure (q) was considered when the anemone could not retract the column or the oral disc either partially or totally within the shelter. This defence behaviour has been reported as the primary and immediate strategy used by *B. annulata* to avoid predation (Harris and Howe, 1979; González-Muñoz et al., 2013). Focus was directed to the protection of the oral disc and column as they constitute vital structures to the anemone and its exposure entails a high risk to survival (Ottaway, 1977; Harris and Howe, 1979). Finally, to compare the probability of successful defence responses between treatments, a χ^2-test was performed on a 3 × 2 contingency table (Zar, 2010). A higher probability of successful retractions by the anemone in treatments with the presence of alpheids would be considered evidence in support of our hypothesis.

FIGURE 2 | Diagrams showing the positions in which *Bartholomea annulata* was found during surveys off the Mexican Caribbean coast: in crevices, caves or small holes located **(A)** on the top or lateral walls of coral fragments, limestone massif and rocks; **(B)** at the interface between hard substrate and the sandy bottom.

Den Cleaning Behaviour in *Alpheus armatus* Complex

Once the manipulation experiment was terminated, the video recording of 12 triads of animals was performed to describe the cleaning behaviour. Shrimps were removed from triads in BSA treatment 24 h prior to the beginning of observations. Shelters were then manually buried with the sand available in the aquaria to assure all anemones were similarly covered when shrimp were introduced. Two additional *A. armatus*. couples were placed in aquaria that contained an artificial shelter and sand, but no anemone. Activity was recorded during the first 12 h of light immediately after shrimp pairs had been introduced into the aquaria.

Preliminary observations allowed to clearly identify three stages of *A. armatus* complex behaviour that took place chronologically and were operationally defined with the following criteria:

(1) Habituation: From the moment *A. armatus* complex shrimps were introduced in the aquaria to the moment both individuals ceased movement and remained still on the bottom.
(2) Searching: From the moment habituation ended to the moment both shrimp had recognised the anemone. Anemone/shelter recognition normally occurred by chemical stimuli and was characterised by shrimp moving directly toward the anemone, making physical contact with the pedal disc, column or tentacles and remaining in close contact with the host.
(3) Cleaning and maintenance: From the moment both *A. armatus* complex recognised the anemone/shelter to the end of the recordings. Four distinct behaviours states were displayed by shrimp during this stage and are categorised according to Palomar et al. (2005):

(a) Sweeping: vigorous fanning of pleopods resulting in loose and suspended sand being pushed from under the abdomen toward the rear (**Figure 4A**).
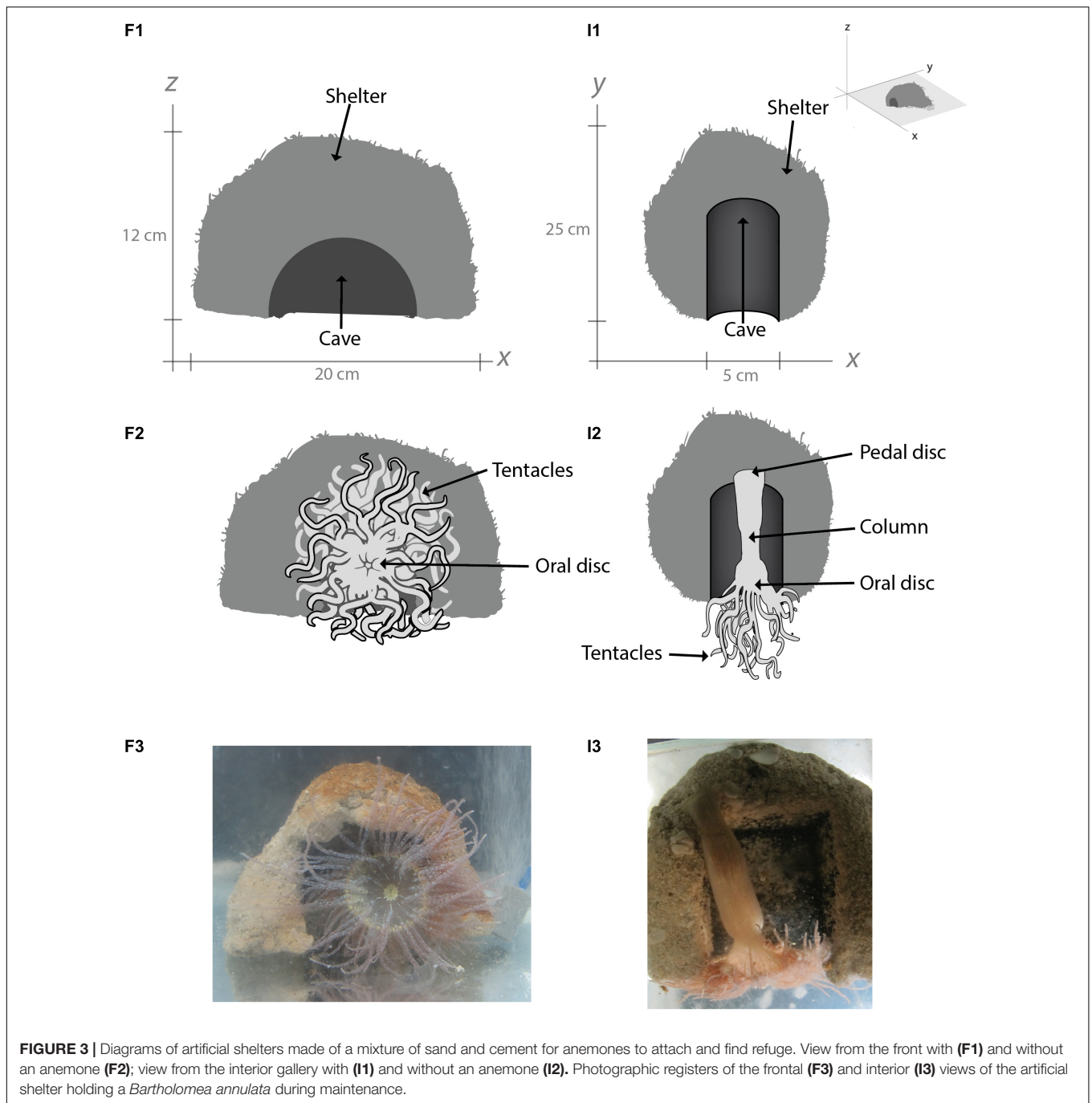
(b) Tamping: shrimps tamped the surrounding sediment down firmly using the second pair of chelated pereiopods (**Figure 4B**).
(c) Dumping: shrimps used the first minor chela to lift larger sediment particles and move them away from the shelter (**Figure 4C**).
(d) Resting: shrimps displayed no activity, entered the shelter or wandered shortly away in strolls that never lasted more than 15 s.

Video recordings were analysed to measure the duration of each stage and the latency of anemone/shelter recognition by *A. armatus* (i.e., the time elapsed from the moment shrimp were introduced into the aquaria to the moment both shrimp had recognised the anemone/shelter). Once anemone recognition had occurred, the number of times that at least one shrimp was observed sweeping, dumping, tamping or resting were registered during 10 min every 0.5 h. This produced the frequency distribution of all four behaviours in 24 consecutive moments throughout the observation period. Central tendency and dispersion parameters (mean ± standard deviation) were calculated for measures of duration and latency to add quantitative descriptors to the qualitative characterisation of the shrimps' cleaning behaviour.

RESULTS

Microhabitat Distribution of *Bartholomea annulata* in the Mexican Caribbean

The distribution of both anemones *Bartholomea annulata* and individuals of the *Alpheus armatus* species complex in the seven regions of the Mexican Caribbean is shown in **Table 1**. Overall, slightly more than half of the total number of individuals of this species (627 of 1169; 53.6%) hosted at least one shrimp, whereas 542 (46.4%) had no alpheid shrimps associated. There were 634 (54.2%) anemones observed inhabiting the interface

FIGURE 3 | Diagrams of artificial shelters made of a mixture of sand and cement for anemones to attach and find refuge. View from the front with **(F1)** and without an anemone **(F2)**; view from the interior gallery with **(I1)** and without an anemone **(I2)**. Photographic registers of the frontal **(F3)** and interior **(I3)** views of the artificial shelter holding a *Bartholomea annulata* during maintenance.

between the sandy bottom and hard structures, such as coral fragments, limestone massif and rocks, whilst 535 (45.8%) were found in crevices and fractures on the horizontal or lateral surface of hard substrates, but distant from the sand.

Results of the hypothesis testing procedure showed that both the "habitat" and "region" terms significantly contributed to explain the total deviance estimated by the GLM. The odds of finding a *B. annulata* with an alpheid shrimp as a symbiont differed significantly depending on the microhabitat in which anemones occurred ($\chi^2 = 5.25$; $p < 0.05$), and were 30% higher

in interface microhabitats than elsewhere irrespective of the region. The odds of finding a *B. annulata* with an alpheid shrimp in any type of microhabitat also varied significantly amongst regions ($\chi^2 = 115.95$; $p < 0.001$), the highest being in region 6, followed by regions 1 and 2, 4 and 5; regions 3 and 7 has the lowest odds. Despite the variability throughout sampling regions (**Figure 5A**), the data revealed a pattern that was remarkably consistent: anemones with alpheid shrimps were more frequent when anemones were in close contact with sandy bottoms than when inhabiting crevices on the top or lateral
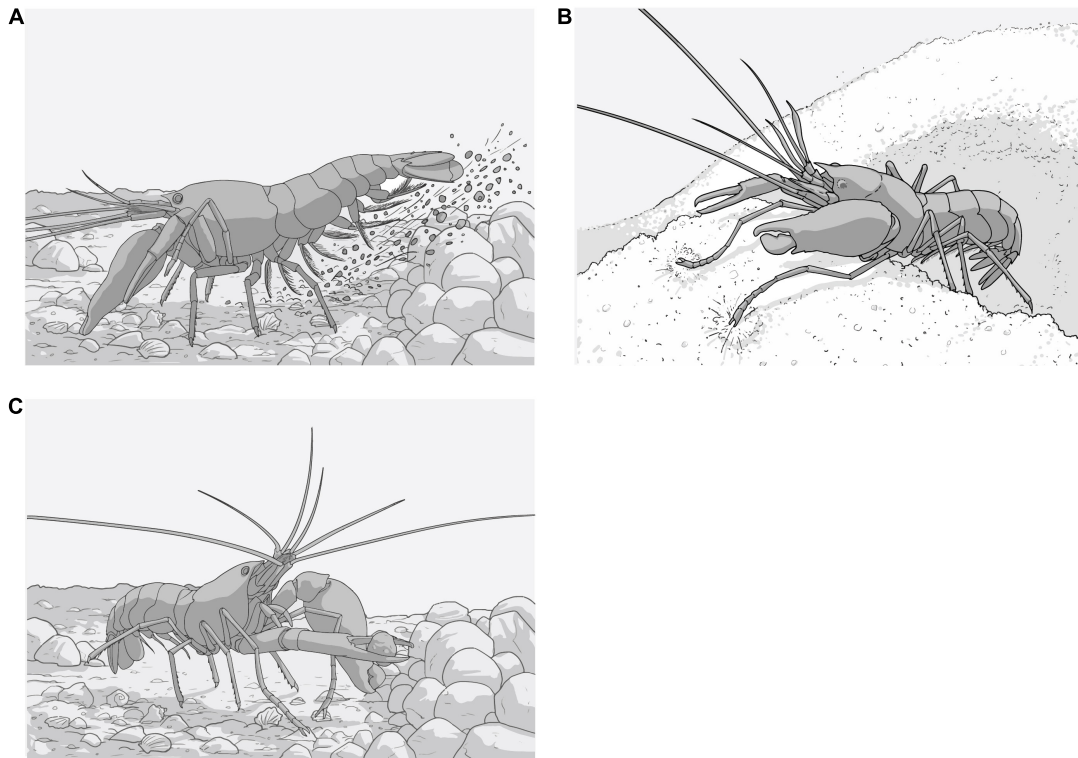
FIGURE 4 | Diagrams representing the body posture, movement and appendices used by *Alpheus armatus* complex when displaying three den cleaning behaviours during observations in experimental conditions. **(A)** Sweeping: shrimp used their pleopods remove the sand from under the abdomen toward the rear. **(B)** Tamping: shrimps tamped the surrounding sediment down firmly using the second pair of chelated pereiopods. **(C)** Dumping: shrimp used the first minor chela to lift larger sediment particles and move them away from the shelter.

walls of hard substrates (**Figure 5B**). The statistical analysis showed that these differences were larger than those expected by chance alone.

Effect of Den Cleaning Behaviour on the Retraction Response in *B. annulata*

Results of the experiment showed that the absence of snapping shrimps reduced the ability of *Bartholomea annulata* to retract successfully when sediment was obstructing the entry and/or free passage inside the shelter. When both alpheid shrimps and sand where absent from the aquaria (B00), all 10 anemones retracted fully within the shelter after receiving the jet of sea water (**Supplementary Material 1**; video recording). By contrast, in treatments where sand obstructed the free movement within the shelter, the success of retraction from stimuli was strongly associated to the presence of alpheid shrimps: 8 (88.8%) successful retractions were observed in treatment with alpheid shrimp (BSA), but only 2 (25%) were observed in those where shrimps were absent (BS0; **Supplementary Materials 2, 3**, respectively).
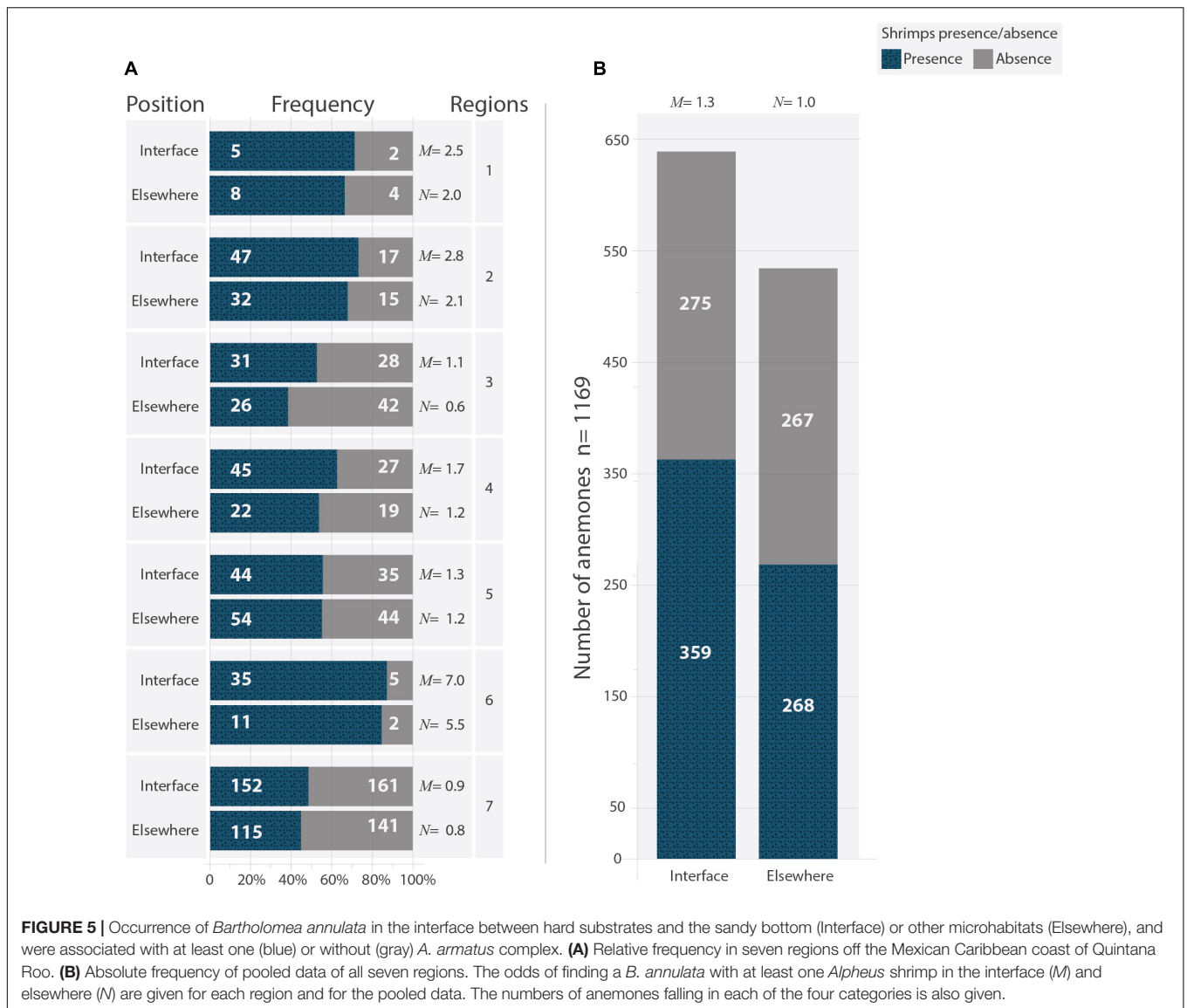
The frequency of successful retractions relative to those that failed varied significantly between treatments ($\chi^2 = 15.34$; $p < 0.01$; **Figure 6**). Anemones confronted with shelters that were obstructed with sediment had only a 25% probability of fully retracting when *Alpheus armatus* complex were absent, compared to a 75% probability when snapping shrimps kept the shelter's entry and passage free of sediment.

Den Cleaning Behaviour in *Alpheus armatus* Complex

As soon as they were placed in the observation aquaria, the *Aalpheus armatus* complex displayed frantic swimming in all directions in the water column and/or walked swiftly on the bottom sediment. This hectic behaviour often resulted in *A. armatus* colliding with the aquarium walls, with one another, or with the anemone or its shelter. Collisions with the anemone, however, did not appear to trigger host recognition immediately, and the shrimps kept moving until they eventually came to a complete standstill. The habituation stage had a mean duration of 3.9 ± 8.7 min (mean $\pm$ standard deviation; **Figure 7A**), but both shrimp in triads had completely ceased motion in the first 2.1 min since observation trials began. The sole exception was the case in which shrimps ceased motion after 30.9 min had passed.

Searching shrimps explored the surroundings by probing with the antennae, antennule and chelate pleopods as they moved in progressively widening stretches throughout the bottom of the aquaria. Movement was characterised as short, forward and backwards jabs as shrimp slowly advanced in their trajectories. *Alpheus armatus* often encountered each other during searching and would touch using the antennae and antennule. When this

FIGURE 5 | Occurrence of *Bartholomea annulata* in the interface between hard substrates and the sandy bottom (Interface) or other microhabitats (Elsewhere), and were associated with at least one (blue) or without (gray) *A. armatus* complex. **(A)** Relative frequency in seven regions off the Mexican Caribbean coast of Quintana Roo. **(B)** Absolute frequency of pooled data of all seven regions. The odds of finding a *B. annulata* with at least one *Alpheus* shrimp in the interface (*M*) and elsewhere (*N*) are given for each region and for the pooled data. The numbers of anemones falling in each of the four categories is also given.

occurred, physical interactions lasted no more than 1.2 min and searching would immediately be resumed. The searching stage had a mean duration of 5.8 ± 4.6 min and concluded when at least one shrimp in each pair had recognised the presence of the anemone (**Figure 7A**). After 17 ± 8.2 min, 27 shrimps had found protection with the anemone, and only 3 individuals buried in the sand near an aquarium corner. All snapping shrimps were near the anemone and its shelter when 38.7 min had elapsed.

Den cleaning occurred 7.2 ± 10.5 min after anemone recognition and commenced by snapping shrimps clearing of the internal gallery as they made their way toward the shelter's entrance. This period was identified as an interlude and represented the latency of the first cleaning behaviours (**Figure 7A**). Shrimp mainly used "sweeping" to move most of the sediment and unearth the entrance to the den (**Figure 4A**). By removing the sediment in this manner, shrimp created a semi-circular depression in front of the

shelter's entrance that was surrounded by a slowly increasing mound of accumulated material. Second most frequently, they used "tamping" (**Figure 4C**), where they crawled toward the top of the mound and compacted the sediment with the second pair of chelated pereiopods. Shrimp occasionally used "dumping" to expel the larger particles away from the den's entrance (**Figure 4B**).

The frequency in which den cleaning behaviours were alternated with "resting" changed throughout the observation period (**Figure 7B**). Shrimp were most active during the first 2 h, when the frequency of "sweeping", "tamping" and "dumping" was 35.6, 17.6 and 1.5 times every 10 min. The external appearance of the shelter was transformed markedly during this period (**Figure 8**, 2 hours), but was thereafter almost unchanged (**Figure 8**, 5.5–12 hours) and (**Supplementary Material 4**). Correspondingly, the three main cleaning behaviours became more sporadic as shrimp advanced in the clearance of the shelter's
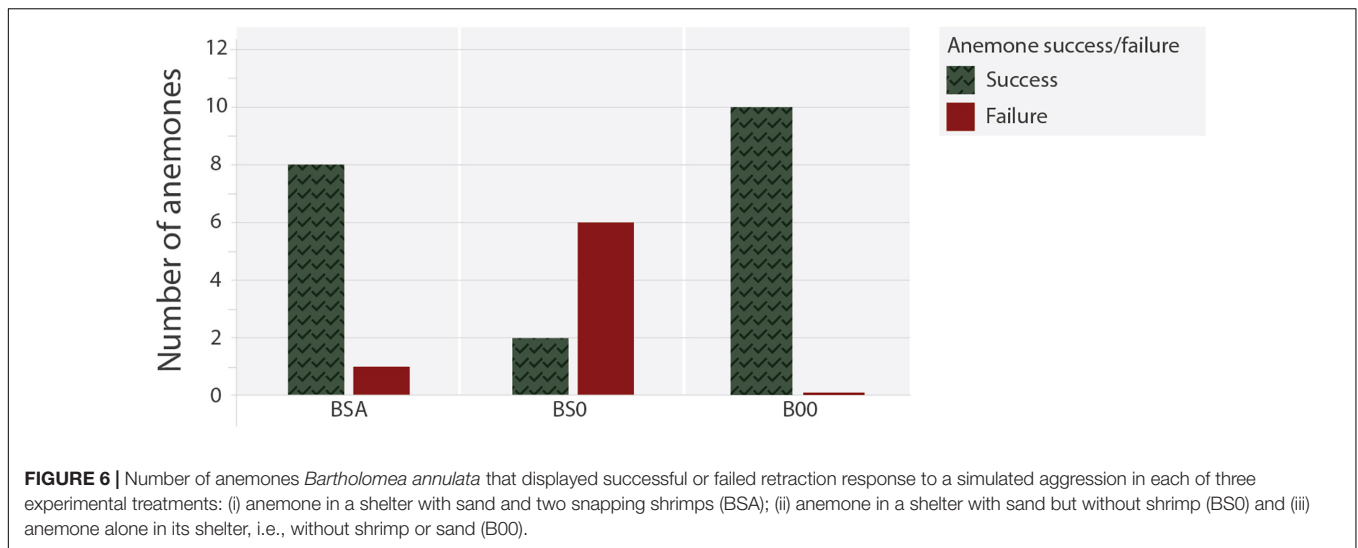
FIGURE 6 | Number of anemones *Bartholomea annulata* that displayed successful or failed retraction response to a simulated aggression in each of three experimental treatments: (i) anemone in a shelter with sand and two snapping shrimps (BSA); (ii) anemone in a shelter with sand but without shrimp (BS0) and (iii) anemone alone in its shelter, i.e., without shrimp or sand (B00).
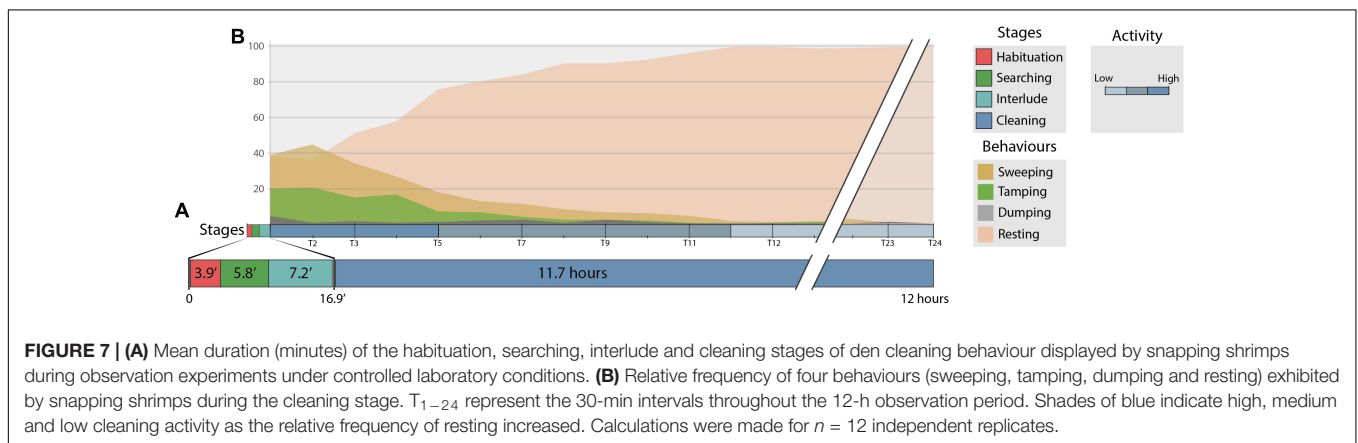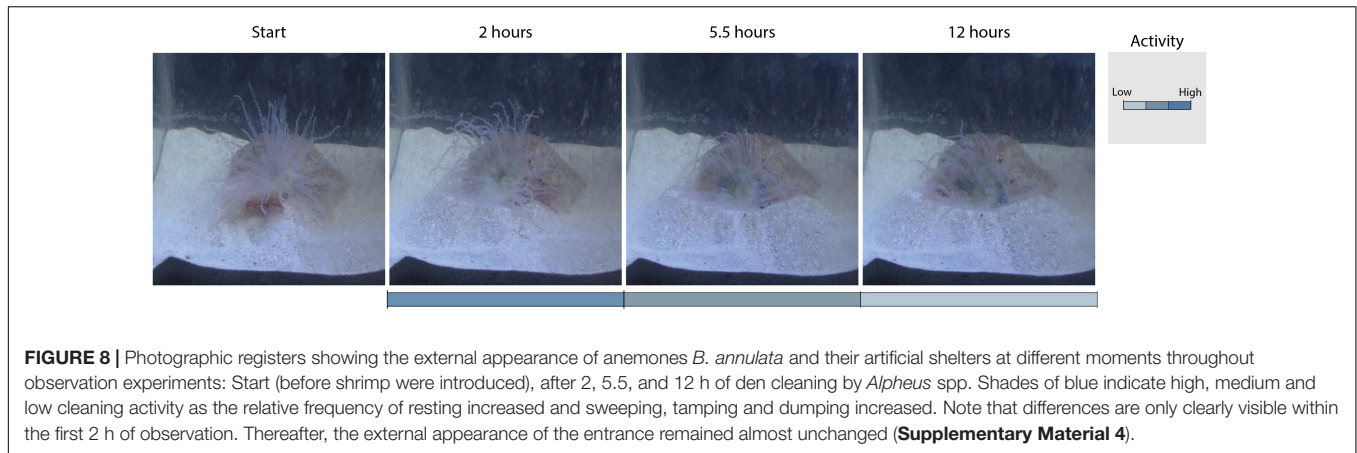

FIGURE 7 | (A) Mean duration (minutes) of the habituation, searching, interlude and cleaning stages of den cleaning behaviour displayed by snapping shrimps during observation experiments under controlled laboratory conditions. **(B)** Relative frequency of four behaviours (sweeping, tamping, dumping and resting) exhibited by snapping shrimps during the cleaning stage. T_{1-24} represent the 30-min intervals throughout the 12-h observation period. Shades of blue indicate high, medium and low cleaning activity as the relative frequency of resting increased. Calculations were made for $n = 12$ independent replicates.

gallery and entrance, and the anemone was free to expand its column and tentacles without obstruction. After 5.5 h an increase in the frequency of "resting" to 98.4 times every 10 min was observed (**Figure 8**, 12 hours). Thereafter, the frequency of "sweeping" and "tamping" decreased to 1 and 0.3 every 10 min, respectively. Despite its rareness, shrimps never stopped cleaning the den and intermittently displayed these behaviours throughout the 12-h observation period (**Figure 7B**). No differences in the frequency, duration or latency of behaviours were observed amongst naturally bonded pairs and those formed at their arrival at the laboratory.

Observations of snapping shrimp in the absence of an anemone showed a marked similarity in the body position and movement of shrimp during the habituation and searching stages. The habituation stage had less duration compared with previous observations (1 ± 0.1 min). The searching stage ended after 29.7 ± 40.8 min with shrimp full covered with sediment away from the artificial shelter, where they remained until the end of the observation period. It is important to note that despite that shrimps frequently encountered the empty shelters (25 times maximum), they never displayed any of the cleaning behaviours described previously.

DISCUSSION

The frequencies in which *Bartholomea annulata* is found in optimal and sub-optimal microhabitats within the reef is determined by their availability, as well as by the set of advantages and risks brought to the anemone from inhabiting such places. Whilst *B. annulata* does not need shrimp to survive, *A. armatus* complex is highly territorial and occurs only in association with this anemone species (Smith, 1977; Knowlton and Keller, 1983, 1985; McCammon and Brooks, 2014). This asymmetric interaction implies that the occurrence of *B. annulata* will depend on the availability of microhabitats with a varying degree of quality for anemone growth and survival, whereas shrimp occurrence will be primarily driven by the abundance of unoccupied anemones. The field data analysed herein showed variations in the abundance of both anemones and snapping shrimps amongst the seven regions in the Mexican Caribbean (**Table 1**), indicating a varying availability of optimal and suboptimal microhabitats for *B. annulata* throughout the study area. Previous studies on the distribution of *B. annulata* in the Caribbean have explained variations in the abundance within geographic areas in terms of the, exposure to wave dynamics,

FIGURE 8 | Photographic registers showing the external appearance of anemones *B. annulata* and their artificial shelters at different moments throughout observation experiments: Start (before shrimp were introduced), after 2, 5.5, and 12 h of den cleaning by *Alpheus* spp. Shades of blue indicate high, medium and low cleaning activity as the relative frequency of resting increased and sweeping, tamping and dumping increased. Note that differences are only clearly visible within the first 2 h of observation. Thereafter, the external appearance of the entrance remained almost unchanged (**Supplementary Material 4**).

microhabitat accessibility, photosynthetic efficiency and food availability (Cornell and Lawton, 1992; Amarasekare, 2002; Begon et al., 2009; Briones-Fourzán et al., 2012; Chesson, 2020).

Despite this environmental heterogeneity, the frequency of *B. annulata* hosting at least one alpheid shrimp was consistently higher when inhabiting interface microhabitats then in those distant from the sandy bottom (**Figure 5**), and these differences were statistically distinguishable from random noise. Caves and crevices located in the interface between hard coral or rock massifs and the soft sandy may constitute ideal places for anemones to attach because they can take advantage of both microhabitats. The hard substrate protects the anemone from ocean energy and natural predators (Knowlton and Keller, 1986; Barrios-Suárez et al., 2002), whilst the soft sediment is rich in a variety of food sources made easily available through nutrient re-suspension (Chapman and Tolhurst, 2004, 2007). Such sources of food for suspension feeders have a central role in oligotrophic environments (Hobson and Lorenzen, 1972; Aguirre and Salmerón, 2015) increasing the value of microhabitats where these sources are plentiful. In the present study, the odds of finding *B. annulata* in interface microhabitats compared to elsewhere varied from 0.58 to 3.08, suggesting anemones do not actively select the former. Whilst differences in anemone survival amongst different microhabitats were not examined the present study, if demonstrated such differences could offer a plausible explanation of the observed field patterns, particularly if survival was positively associated to the presence of shrimp.

The pattern revealed by the field data could be explained if the benefit of hosting a snapping shrimp varies amongst microhabitats and the interaction with *A. armatus* provides *B. annulata* an advantage in occupying interface microhabitats more frequently than others within the same reef. A mechanism explaining this advantage is provided by the results of the manipulation and observation experiments herein, in which full retraction by anemones into the den was successful only when snapping shrimps kept it clean. If den cleaning by alpheid shrimps is related to the effectiveness of the anemones' defence mechanism from predation, then this symbiotic interaction will prevail in those microhabitats where the benefits of such behaviour confer some ecological value; i.e., places where

sediment may obstruct the den and impede the anemone's full retraction, increasing the risk of predation. To choose habitats that are less preferred by other species, reduces interspecific competition and promotes positive interactions such as facilitation and mutualism (Bertness and Leonard, 1997; Chong et al., 2000; Levin et al., 2001; Halpern et al., 2007). Whilst the quality of the interface microhabitat by itself may not differ compared to others, it is the symbiotic interaction that improves the ability of anemones to withdraw before danger.

Proximity to the sediment can pose certain dangers to sessile animals or those with low mobility, since it increases the possibility of scoured tissue from abrasive erosion or accidentally being buried by sedimentation (Chapman and Tolhurst, 2007; Irving and Witman, 2009; Stewart et al., 2013). The Caribbean Sea has high sediment dynamics created by seasonal winds and waves, prevalent marine currents and occasional but recurrent pulses of high activity due to hurricanes and tropical storms (Vázquez-Lule and Díaz-Gallegos, 2009; Miloslavich et al., 2010). These hydrometeorological conditions result in a high and permanent risk of reef structures to be buried beneath the sand and animal shelters obstructed. The high occurrence of *B. annulata* in crevices located in the interface between hard structures and sand could be related to the symbiotic relation between this anemone and *A. armatus* complex. To protect themselves from predators, anemones need an obstacle-free, cavernous den into which they can retract the column and pedal disc whilst fully extending the tentacles toward the source of danger. When threatened, symbiotic *A. armatus* complex will benefit from the defensive display of anemones by hiding near the column or pedal disc behind the stinging nematocysts (the preferred zone anemone for the *Alpheus armatus* complex; Huebner et al., 2019). Thus, by keeping the sediment away, snapping shrimps provide the necessary conditions within the den that serve both the anemone's and the shrimp's higher chances of survival.

In the *B. annulata*—*A. armatus* complex. interaction, the anemone benefits from the snapping shrimp defence against benthic predators (Smith, 1977). This shrimp defence behaviour consists of a water jet capable of seriously harming the targeted animal (Lohse et al., 2001; McCammon and Brooks, 2014). Nonetheless, if the alpheid's first defence line breaks down, the

last resource for the anemone is to retract its hydraulic body into the shelter, block the den's entrance, and facilitate a quick snapping response from the shrimp to repel the attack from the predator. Our results of the manipulative experiment show clear evidence that the anemone's full retraction into the den is only possible when its interior is free of sediment (**Figure 7**). Moreover, only by maintaining the entrance to the den clear from occlusion, do shrimps enable the full expansion of the anemone's tentacles toward exterior perils. This defence position enhances the protection offered by the battery of nematocysts, whilst the shrimp seeks refuge close to the column and pedal disc.

Video recordings of the den cleaning behaviour displayed by *A. armatus* complex in the present study showed it constitutes a quick and effective mechanism to assure the anemone's full retraction. Despite being completely covered with sand, *A. armatus* complex were capable of finding the anemone and liberating the entrance of the artificial shelters in less than 2.5 h. In addition, the sweeping, tamping and dumping behaviours were displayed systematically by repeating them in a consistent order, duration and frequency amongst all experimental replicates. These features together with the velocity of this response suggest that den cleaning behaviour is common and effective under natural conditions and provides a true selective advantage for both the anemones and the shrimps. The mutual beneficial nature of this behaviour resides in that by keeping the den clean either through sweeping or otherwise moving sediment away from the entrance, *A. armatus* complex increases its chances of taking refuge when the anemone retracts. This idea is further supported by the fact that in in the two replicate trials where anemones were absent, shrimp did not delay to bury in the sediment.

After 5.5 h of the initial cleaning, shrimp intermittently removed particles and compacted the sediment surrounding the den's entrance throughout the duration of observations. It is interesting to note that shrimps continued to provide maintenance to their hosts' refuge in such a persistent manner, suggesting it constitutes a recurrent feature of their daily activity. Whilst snapping in *A. armatus* complex is a more conspicuous defence behaviour (Smith, 1977; McCammon and Brooks, 2014), den cleaning is more consistent and ubiquitous in the shrimp's behavioural repertoire. Furthermore, results herein demonstrate that anemones without shrimps will not be able to use full retraction as a last resource defence mechanism.

Overall, these findings describe a new, undervalued alpheid shrimp behaviour that contributes to cleaning the shelter's entry and internal passage, and experimentally demonstrate its potential relation to enhanced predator avoidance, enabling the anemone to fully retract into its den. Such behaviour may constitute an important element of trade in the symbiotic interaction between *A. armatus* complex and its host anemone.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

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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.2021.677024/full#supplementary-material

Supplementary Material 1 | Video recordings examples of the behaviours observed on each of the treatments depicted in **Figure 6**. BOO, *Bartholomea annulata*, no *Alpheus* spp., no sediment/sand.

Supplementary Material 2 | Video recordings examples of the behaviours observed on each of the treatments depicted in **Figure 6**. BSO, *Bartholomea annulata*, sediment/sand, no shrimp.

Supplementary Material 3 | Video recordings examples of the behaviours observed on each of the treatments depicted in **Figure 6**. AOS, *Alpheus* spp., no anemone *Bartholomea annulata*, sediment/sand.

Supplementary Material 4 | Time-lapse of the den cleaning behaviour by *Alpheus* spp. showing the external appearance of artificial shelter of *Bartholomea annulata*.

Supplementary Material 5 | Photograph of the anemone and shrimp in the field for communication purposes.

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