Endobiotic fauna inhabiting the calcareous sponge *Clathrina* sp. in southwest Puerto Rico

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Running Head: Endobiotic fauna of the calcareous sponge, *Clathrina* sp.
Sponges provide an array of ecological services and benefits for Caribbean coral reefs. They function as habitats for a bewildering variety of species, however limited attention has been paid to the systematics and distribution of sponge-associated fauna in the class Calcarea or, for that matter, of most sponges in the Caribbean. The goal of this study was to characterize infaunal assemblages from a calcareous sponge, Clathrina sp., across multiple reefs from the La Parguera Natural Reserve, Puerto Rico. The associated fauna sampled from 43 Clathrina individuals yielded a total of 2,249 associated infauna specimens distributed over seven invertebrate phyla. Arthropoda was the most abundant phylum, accounting for 62.5% of total abundance, followed by Annelida (21.0%) and Nematoda (5.5%). Limited patterns of temporal or spatial variability were surmised due to the opportunistic sampling effort afforded to this investigation by the cryptic nature of the host species. A concordance between our data set and those for the class Demospongiae was observed, with the most abundant associated fauna being copepods and polychaetes. However, when compared to other Calcarea, the present study found a considerably higher diversity of associated fauna.

**Keywords:** Associated fauna, Calcarea, Caribbean, La Parguera
INTRODUCTION

Marine sponges (Porifera Grant, 1836) are perhaps the earliest metazoan phylum on the planet (Pisani et al., 2015), originating roughly 600 million years ago (Li et al., 1998; Yin et al., 2015). This evolutionary time has allowed sponges to develop complex biotic interactions with other marine organisms (Wulff, 1985; Wulff, 2012). Fossil evidence of associated phyla (Echinodermata, Bryozoa, Porifera, and Brachiopoda) in marine sponges from the early Ordovician Period has shed light on these ancient biotic interactions (Carrera, 2000). Like their ancient counterparts, modern sponges provide habitat for an array of taxonomically diverse populations of micro-invertebrates and fishes (Wulff, 2006; Marliave et al., 2009; Diaz & Rützler, 2012; Gloeckner et al., 2014). As filter feeder organisms, sponges provide their associates a continuous flow of water and food in the form of phytoplankton, organic and inorganic detritus and in some instances even sponge tissue (Pawlik, 1983; Corredor et al., 1987; de Goeij et al. 2013).

Sponges have been called ‘living hotels’ (Pearse, 1932; 1950; Gerovasileiou et al., 2016), ‘living islands’ (Villamizar & Laughlin, 1991) and ‘microcosms’ (Uriz et al., 1992); where different levels of interactions between host and colonizers are occurring simultaneously, often dependent upon the type of aquiferous system and the morphology of the sponge (Koukouras et al., 1992; 1996). Long (1968) put these relationships into four groups: (1) inquilinism, or lodging, within or upon the sponges, (2) co-existence of two organisms on the same substratum as a result of simultaneous growth, (3) predation or grazing; and (4) mutualism.

The majority of the studies regarding marine sponges and their associated fauna have focused on the class Demospongiae; perhaps because 86% of the extant Porifera are Demospongiae (van Soest et al., 2016). Among the most significant reports concerning demosponge-associated fauna
were those of Santucci (1922), Pearse (1932, 1950), Fishelson (1966), Long (1968), Pansini (1970), Sube (1970), Labate & D’Addaboo (1974), Rützler (1976), Peattie & Hoare (1981), Koukouras et al. (1985), Wendt et al. (1985), Voultsiadou-Koukoura et al. (1987), Koukouras et al. (1992), Klitgaard (1995), Koukouras et al. (1996), Duffy (1996), Magnino et al. (1999), Ribeiro et al. (2003), Skilleter et al. (2005), Abdo (2007), Palpandi et al. (2007), Huang et al. (2008), Greene (2008), Schejter et al. (2012), and Sivadas et al. (2014). Of the mentioned studies regarding the associated organisms of demosponges, the most represented phyla are Arthropoda, Annelida, Mollusca, Nematoda, and Echinodermata, and the most abundant classes are Crustacea, Polychaeta, and Ophiuroidea.

The remaining three sponge classes have received little attention worldwide, with six studies for Hexactinellida (Kunzmann, 1996; Beaulieu, 2001a, b; Schuchert & Reiswig, 2006; Fiore & Jutte, 2010; Kersken et al., 2014), three for Calcarea (Frith, 1976; Thomas & Klebba, 2007; Padua et al., 2013), and none for the class Homoscleromorpha. This lack of knowledge prohibits an understanding of how associated assemblages could differ among sponge classes. Since sponges act as bioengineers, in effect creating habitat for potentially thousands of organisms, studying the lesser known Porifera groups can shed light on the functional roles these sponges provide, especially since sponges are becoming the dominant benthic group in Caribbean reefs (Pawlik et al. 2013; Loh et al., 2015; Hammerman & García-Hernández, 2016). Even though research in the Caribbean region has yielded one of the most interesting associations between snapping shrimps and demosponges (eusociality; Duffy, 1996), relatively few investigations have quantified total sponge-associated fauna (e.g. Pearse, 1932; Westinga & Hoetjes, 1981; Villamizar & Laughlin, 1991; Chavarro et al., 2004).
The objectives of this study were to (1) describe the composition of the invertebrate assemblages associated with the calcareous sponge Clathrina sp. in La Parguera, southwest Puerto Rico, and (2) describe patterns of spatial and temporal variation of those infaunal assemblages. The calcareous sponge is a new species belonging to the genus Clathrina (Gray, 1867) (Fig. 1), which is formally being described by M. Klautau (pers. comm.). The genus Clathrina has the simplest organization among sponges: an asconoid aquiferous system, which means that all the cavities are lined by choanocytes (Klautau & Valentine, 2003). In Puerto Rico, Clathrina sp. inhabits sciophilous habitats, usually growing on cavern walls and ceilings, overhangs, small crevices, and sometimes exposed on reefs. Clathrina sp. can also be found growing at the base of gorgonians (Erythropodium caribaeorum and Eunicea flexuosa), zoanthids (Palythoa caribaeorum) and underneath scleractinian coral colonies (Montastraea cavernosa, Orbicella annularis, and O. faveolata) (García-Hernández, pers. obs.) (Fig. 1).

Only two previous studies (Frith, 1976; Padua et al., 2013) have looked in detail at the associated assemblages of calcareous sponges, both focusing on sponges belonging to the subclass Calcaronea. This study is the first to explore the associated organisms of a calcareous sponge belonging to the subclass Calcinea, and will set the foundation for future studies focusing on the associated fauna of calcareous sponges belonging to this subclass.

MATERIAL AND METHODS

Sampling and Laboratory Analysis

Clathrina sp. is a cryptic species, whose patterns of spatial distribution are vaguely known, with reports in Brazil and Puerto Rico. Consequently, a systematic sampling effort to ensure balanced
replicates per site and time was not possible. Despite these challenges, a total of 43 specimens were collected from eight coral patch reefs in La Parguera, off the southwest coast of Puerto Rico (Fig. 3) from April 2014 to April 2015 using SCUBA. La Parguera is characterized as a marine reserve, with a myriad of reef type stretching from inshore mangrove habitats to patch reefs, emergent linear reefs and a pronounced shelf edge habitat. Field collections consisted of wrapping a plastic bag around the sponge to reduce loss of fast moving associated fauna escapement (mostly crustaceans), then the sponge was placed into a large collection vial. In the laboratory, the seawater was discarded and the remaining sample was labeled, fixed and preserved in 95% ethanol in a 50 ml tube. All sponges were stored in a -20 °C freezer for further analysis. Before dissection, wet weight was calculated per sponge, followed by examination of the ethanol in which the host was preserved. Sponge specimens were then carefully inspected and dissected under a stereomicroscope to remove associated fauna from pores and canals, as in Ribeiro et al. (2003). The associated organisms, from both the ethanol and picked from the sponge, were separated and identified to the lowest taxonomic level possible. Associated fauna was then preserved in new ethanol and vouchers deposited in the Caribbean Laboratory of Marine Genomics in the Department of Marine Sciences at the University of Puerto Rico at Mayagüez. Taxonomic identification was performed down to the ordinal rank, with only few exceptions to the family level. This investigation describes the associated fauna of *Clathrina* sp. at a coarse taxonomic resolution; allowing for overall comparisons between this study and others like it.

**Data Analysis**

Extraction and sorting of specimens resulted in data matrices for abundance of different invertebrate taxa per sponge that were used to construct similarity matrices between samples.
using the Bray-Curtis index. Previous to this, data were squared-root transformed to down-weight the dominance of highly abundant taxa in the calculation of similarities, relative to the less-common taxa. Also, data were standardized by total counts per sponge because size and volume of each sampling unit (i.e., sponge specimen) was not the same. Decisions about transformations were done after visual inspections of Shade Plots (Clarke et al., 2013). Dissimilarity matrices were used to perform non-metric multivariate ordinations (nMDS) to illustrate patterns of spatial distribution of invertebrate assemblages associated with Clathrina sp. Given the nature of the sampling design used in this study, more detailed spatial-temporal analyses were done only for those sites that were sampled during at least two different seasons (i.e. the reefs Turrumote, San Cristobal and Pinnacles). In this particular case, principal coordinate ordinations (PCO) were done on centroids per site and sampling time, which allowed interpretations of magnitude and direction of change between sites and across seasons. All analyses were done using the software PRIMER V7 (Clarke & Gorley, 2015). Finally, correlations between sponge volume, number of individuals, and number of taxa of the infauna were calculated in relation to individual sponges.

RESULTS

Associated Infauna

The associated fauna from 43 specimens of the calcareous sponge Clathrina sp. from eight coral patch reefs in southwestern Puerto Rico yielded a total of 2,249 associated organisms in seven invertebrate phyla (Figs. 3 and 4, Table 1). Arthropoda was the most abundant phylum accounting for 62.5%, followed by Annelida and Nematoda, 21%, and, 5.5%, respectively.
Within the Arthropoda, the crustacean Copepoda (mostly siphonostomatoids) yielded 48.8% of all associated individuals within *Clathrina* sp. The next most abundant groups within Arthropoda were the Amphipoda and Isopoda. Tanaidacea, Decapoda, Ostracoda and Acari (halacarid mites) were also present (Table 1). The next largest group was the phylum Annelida, all worms within the class Polychaeta and accounting for roughly 21% of total species abundance. The remaining phyla accounted for roughly 16.5% of taxa abundance, including Nematoda (5.5%), Bryozoa in the class Stenolaemata (2.4%), Echinodermata, specifically the class Ophiuroidea (2.1%), Mollusca (gastropods) (1.6%) and the cnidarian class Hydrozoa (1.2%). Lastly, about 4% of total abundance was classified as unknowns (Fig. 4, Table 1).

**Reef site comparisons of infauna**

Among all eight sites, the copepods were the most abundant organisms found in *Clathrina* sp. (n=1,097; Table 1). Sponges from Laurel reef contained the highest number of associated copepods (n=300), while Enrique reef contained the least (n=6). Decapods (n=3) were found in three different sponges from reefs Enrique, Turrumote, and San Cristobal. Tanaids (n=7) were found in reefs Enrique, Turrumote, and Pinnacles. Halacarid mites (n=8) were recovered from sponges collected from reefs Enrique, Laurel, Turrumote, Margaritas, and San Cristobal. Hydrozoans belonging to the order Leptomedusae and Nematodes were found in all reefs, except in sponges from Margaritas reefs. Bryozoans belonging to the family Crisiidae, as well as gastropods and ophiuroids were found in sponges from all reefs, except at Enrique and Margaritas. Polychaetes were found in sponges collected from all eight reefs. Microscopic juvenile bivalves were found in all reefs except at Mario reef and Margaritas reef (Table 1).

Among all eight sampled reefs, twelve sponges from Turrumote reef contained the highest number of associated taxa (504 specimens belonging to 14 taxa), while two sponges from
Margaritas reef contained the lowest number of associated taxa (53 specimens belonging to 7 taxa). Sponges collected from Laurel reef contained a relatively high number of associated taxa (19 specimens belonging to 11 taxa), despite the fact that only two specimens were found in this site. Interestingly, sponges collected from Laurel reef had the lowest average volume 1.41 cm$^3$ (± 1.49, s), yet their average density of individuals was the second highest of all sites with 46.7 ind.cm$^{-3}$ (± 41.01, s), while sponges recovered from Margaritas reef had the third highest average volume (2.95 ± 2.58 cm$^3$), yet contained the lowest density of associated individuals with 13.3 ind.cm$^{-3}$ (± 11.1, s) (Table 2).

Overall, the average total volume of the sponges collected was 2.43 cm$^3$ (±1.62). With respect to sponge volume, regression analysis indicated that, despite a positive slope, the average total volume of the sponges dissected did not significantly correlate with the number of individuals (n) ($R^2 = 0.215$) and the number of taxa (S) ($R^2 = 0.230$) (Fig. 5).

**Temporal and Spatial Variation**

Multivariate ordinations for assemblages of invertebrates associated with *Clathrina* sp. showed no clear patterns of differences between reefs (Supplementary Fig. 1). This lack of patterns might be the result of important temporal variation as well as variation among sponges within a reef. In order to elucidate these patterns, multivariate ordinations (PCO) were done on centroids only for those sites that were sampled during at least two different seasons. These metric ordinations showed important temporal differences for San Cristobal reef, especially during the spring and fall of 2015, when polychaetes and ophiuroids increased compared to other seasons in San Cristobal reef (Fig. 6).
DISCUSSION

After examination of 43 specimens of the small cryptic sponge *Clathrina* sp., a great number of associated organisms was classified to higher taxonomic groups (Figs. 2 and 3), belonging to the phyla Arthropoda, Annelida, Bryozoa, Cnidaria, Echinodermata, Mollusca, and Nematoda with the most abundant classes represented by Crustacea and Polychaeta (Table 1). The taxonomic resolution to the ordinal rank yielded similar patterns and associated taxa compared between *Clathrina* sp. and sponges belonging to the class Demospongiae, with crustaceans being the most abundant taxon (Pearse, 1950; Pansini, 1970; Rützler, 1976; Koukouras et al., 1985; Voultsiadou-Koukoura et al., 1987; Villamizar and Laughlin, 1991; Koukouras et al., 1992; Ribeiro et al., 2003; Skilleter et al., 2005; Abdo, 2007; Palpani et al., 2007; Huang et al., 2008; Greene, 2008; Schejter et al., 2012; Kersken et al., 2014; Schönberg et al., 2015; Gerovasileiou et al., 2016). However, several other studies reported in Schejter et al. (2012) found Polychaeta to be the most abundant associated group. Comparisons among these studies should be made with caution since sample processing is not standardized. For example, use of different sieve sizes may bias the results towards more macrofauna or meiofauna taxa.

The only other publication focusing solely on associated fauna in calcareous sponges discovered two additional phyla (Porifera, Platyhelminthes) and the tunicate class Ascidiacea from *Paraleucilla magna*, in Brazil (Padua et al., 2013). Surprisingly, of the 54 sponges examined, only 349 associated organisms were found in total inside *P. magna*, on average, 6.5 individuals per specimen. In comparison, in the present study, seven phyla were discovered from 43 *Clathrina* sp. from southwestern Puerto Rico, with a total of 2,249 associated organisms, or 52.3 individuals per specimen. Both species of sponges are the most conspicuous Calcarea in their respective geographical regions and can be found in similar photophilous and sciophilous...
environments (Klautau et al., 2004; García-Hernández- unpublished data). The high number of associated fauna in the present study may be attributed to the differences in morphology between the two sponge species as well as their prevalence through seasons. *Paraleucilla magna* (subclass Calcaronea) exhibits strong seasonality throughout the year, being most abundant in summer and disappearing during autumn, only to reappear again in winter (Klautau et al., 2004). *Paraleucilla magna* is also comprised of a leuconoid aquiferous system with large atrial cavities and numerous canals (Padua et al., 2013). *Clathrina* sp. can be found readily year-round within the La Parguera Marine Reserve, southwest Puerto Rico. The species belongs to the subclass Calcinea and is composed of an asconoid aquiferous system, with a cormus of anastomosed tubes consisting of a series of interwoven canals lined by choanocytes (Klautau & Valentine, 2003; Cavalcanti & Klautau, 2011). In addition, Frith (1976) surprisingly observed no associated fauna in three other species of Calcarea, belonging to the subclass Calcaronea from England. Thus, of the five species of Calcarea examined (Frith, 1979; Padua et al., 2013; this study), *Clathrina* sp. had the most associated organisms, which might be due to its prevalence through seasons as well as the difference in sponge’s internal morphology, allowing more space and crevices, thus more niches for the associated fauna. Interestingly, analysis of associated specimens of the same species of *Clathrina* sp. collected from Brazil did not yield any associated organisms (M. Klautau pers. comm.). Although not tested in this study, this may be due to a difference in composition of allelochemicals between geographically distant populations of *Clathrina* sp. Changes in allelochemical composition might be related to differences in the environmental characteristics of the sampled sites (Betancourt-Lozano et al., 1998), as well as a difference in individual sponge antiparasitic activity capabilities (Martinez et al., 2001). Previous
studies have suggested that surrounding habitats may alter the structure of associated invertebrate assemblages (Koukouras et al., 1996; Gherardi et al., 2001; Gerovasileiou et al., 2016).

Regression analysis yielded a positive but non-significant correlation between sponge volume and the absolute abundance of associated individuals and taxa (Table 2 and Figure 5). Past studies have shown similar results with respect to volume and number of associated individuals (Pansini, 1970; Sube, 1970; Koukouras et al., 1985; Fiore & Jutte, 2010). Some specimens of *Clathrina* sp. were particularly small but contained many copepods, whereas other, larger sponges often contained a few organisms, such as polychaetes and ophiuroids. In this case, the lack of a significant correlation between volume and total number of inhabitants might be due to predation and competition. Previous literature has shown ophiuroids feeding on larvae of the Caribbean sponge *Callyspongia vaginalis* (Henkel & Pawlik, 2014). Similarly, polychaetes have been documented as feeding upon sponge tissue and other smaller crustaceans (Pawlik, 1983).

Since many individuals of *Clathrina* sp. contained both brittle stars and polychaetes, they could limit the number of copepods, isopods, mites, and amphipods, if they were preying on them. However, in this study, gut content of associated fauna was not examined to study trophic links among inhabitants of sponges.

In *Clathrina* sp., and in *Paraleucilla magna* (Padua et al., 2013), Crustacea was the most abundantly represented group of associated organisms, 63% and 54%, respectively. Padua et al. (2013) found that amphipods of the family Stenothoidae were the most abundant crustaceans in the calcareous sponge *P. magna*. In comparison, specimens of *Clathrina* sp. were dominated by copepods, with a total of 78% fauna abundance. Rützler (1976) also reported crustaceans (67.8%) as the most abundant group within six different species of sponges from the Mediterranean Sea. Interestingly, only within the sponge *Ircinia variabilis* were copepods the
most abundant group, the remaining five were dominated by polychaetes, caridean shrimps, and
gammarid amphipods (Rützler, 1976). In the Aegean Sea, Koukouras et al. (1992) found that
Crustacea was the most abundant taxon in four demosponges: Agelas oroides, Petrosia
ficiformis, Ircinia variabilis, and Aplysina aerophoba. In Brazil, Ribeiro et al. (2003) also
reported crustaceans as the most abundant group (83%) within the sponge Mycale
microsigmatosa. In the Mediterranean, Pansini (1970) found that copepods were the most
abundant group within the demosponges Spongia (Spongia) officinalis, Sarcotragus fasciculatus,
and Petrosia (Petrosia) ficiformis, 40.7%, 36.7%, and 36.1% respectively. Similarly, Westinga
& Hoetjes (1981) reported the presence of copepods in large quantities from 35 individuals of
the demosponge Spheciospongia vesparium, however, these were not considered in their study
due to difficulty in identification. Westinga & Hoetjes (1981) noted that most of their copepods
were siphonostomatoids, of the genus Asterocheres, similar to the findings of the present study.
In deeper environments, Kersken et al. (2014) also found copepods to be the most abundant
organisms associated with the hexactinellid sponge Rossella antarctica. New species of
amphipods have also been recorded from calcareous sponges. Thomas and Klebba (2007)
described several new species of commensal leucothoid amphipods, from Florida and Belize,
which were in association with the calcareous sponges Leucetta imberbis and Leucosolenia sp.,
and concluded that further investigations of sponges will yield the discovery of new cryptic
species of amphipods.

When observing changes in associated assemblages across temporal or spatial variation, few
clear patterns emerged, possibly due to the opportunistic sampling design, which did not allow
for equal collections across reefs through time (Supplementary Fig. 1). Of the reefs which were
sampled fairly evenly across seasons (i.e. Turrumote, San Cristobal and Pinnacles), only San
Cristobal reef showed a transition from harboring predominately ophiuroids and polychaetes in the fall and spring, to copepods and gastropods in the summer and winter (Fig. 6). It is possible that these temporal patterns of variation are cyclical due to the predator/prey dynamics described above. Nevertheless, further studies on the trophic dynamics of assemblages associated with Clathrina sp. will have to be conducted to test this hypothesis.

It would be difficult to partition associated fauna as either being facultative (i.e., actively select Clathrina sp.) or ephemeral (i.e., transitory associates). However, the fact that multiple life stages of polychaetes were found in sponges across all eight reefs suggests that some of the associated fauna could be facultative. Especially for polychaetes, which was the second most abundant group living in Clathrina sp., with a total abundance of 21%. Klitgaard (1995) reports a similar percent abundance of polychaetes (26%) observed in 11 demosponges. Polychaetes take advantage of the micro-niches found in sponge tissue, such as those that occur exclusively inside the canals of the aquiferous system of the demosponge Anomoianthella lamella (Magnino et al., 1999). Although collections were not done for every season in every reef, a similar faunal composition was found across an entire year of sampling among all reefs. Thus, Clathrina sp. offers suitable habitat year round for a mildly diverse group of facultative and ephemeral associated fauna.

Along with the infauna analysis, several other ecological and natural history observations were made. An unknown species of juvenile fish was observed taking shelter within the folds of Clathrina sp. (Fig. 1b). Sponges providing shelter and refuge for fishes have been reported (Tyler et al., 1972) before, but never included a calcareous sponge. Frith (1976) observed on several occasions the fishes Pholis gunnellus and Labrus bergylta, laying in the folds of the sponge Halichondria (Halichondria) panicea. Also, Gerovasileiou et al. (2015) made note of
rarely reported fish species in the sponge *Agelas oroides*, in eastern Mediterranean marine caves.

During collections, isopods were also observed scattering through the pores, as the sponge was lightly disturbed prior to sampling.

A noteworthy observation was that of a sea anemone inhabiting the surface of *Clathrina* sp. (Fig. 1c). This represents the first record of an anemone, (order Actiniaria) in association with a calcareous sponge (Fig. 2b). This anemone more than likely belongs to the genus *Bunodeopsis* (pers. comm. E. Rodriguez & R. González-Muñoz), and has been previously found throughout the Caribbean growing on the leaves of the sea grasses *Thalassia testudinum* and *Syringodium filiforme* (Day, 1994; González-Muñoz et al., 2012), as well as on the demosponge *Aplysina cauliformis* (pers. comm. D. Gochfeld). Pearse (1950) also found anemones attached to the Caribbean demosponges *Ircinia strobilina* and *Amphimedon compressa*. All other published reports of sea anemones have been in association with deep-water hexactinellid sponges (Reiswig et al., 2011; Sanamyan et al., 2012; pers. comm. C. Morrow, C. Kelley).

Despite their cryptic nature, the high number of endobiotic associates of *Clathrina* sp. emphasizes the key role and importance that marine sponges play in the ecosystem, especially since they are predicted to become the most dominant taxa on Caribbean reefs in the near future (Pawlik et al., 2013; Loh et al., 2015). Within the phylum Porifera, calcareous sponges tend to be, for various reasons, the most neglected group by sponge biologists (van Soest et al., 2012; van Soest and de Voogd, 2015; Cóndor-Luján and Klautau, 2016). We believe that further investigations of sponges in the class Calcarea will yield greater insights into reef biodiversity and the complex species interactions that unravel among the benthos. These biotic interactions are especially important to observe and characterize now, since Caribbean and worldwide coral
reefs are experiencing drastic community phase shifts due to a myriad of natural and anthropogenic stressors.

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Table 1. Total Counts of Associated fauna living within the sponge, *Clathrina* sp.,
Art=Arthropoda, Cni=Cnidaria, Ann=Annelida, Ech=Echinodermata, Nem=Nematoda,
Bry=Bryozoa and Mol=Mollusca.

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<th>Enrique</th>
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<th>Media Luna</th>
<th>Turumote</th>
<th>Pinnacles</th>
<th>Margaritas</th>
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## Table 2. Summary of ecological data collected at each site.

<table>
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<tr>
<th>Site</th>
<th>Number of Taxa (S)</th>
<th>Number of Specimens (N)</th>
<th>Average Volume +/- SD (cm(^3))</th>
<th>Average Density +/- SD (ind.cm(^{-3}))</th>
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<tbody>
<tr>
<td>San Cristobal (n=9)</td>
<td>13</td>
<td>285</td>
<td>2.49 ± 1.87</td>
<td>16.8 ± 13.7</td>
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<tr>
<td>Margaritas (n=2)</td>
<td>7</td>
<td>53</td>
<td>2.95 ± 2.57</td>
<td>13.3 ± 11.1</td>
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<tr>
<td>Media Luna (n=3)</td>
<td>11</td>
<td>195</td>
<td>3.80 ± 0.70</td>
<td>17.0 ± 0.90</td>
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<tr>
<td>Mario (n=4)</td>
<td>11</td>
<td>229</td>
<td>3.35 ± 0.46</td>
<td>17.2 ± 2.04</td>
</tr>
<tr>
<td>Pinnacles (n=9)</td>
<td>12</td>
<td>409</td>
<td>1.41 ± 1.49</td>
<td>49.1 ± 38.1</td>
</tr>
<tr>
<td>Turrumote (n=12)</td>
<td>14</td>
<td>504</td>
<td>2.50 ± 1.41</td>
<td>16.6 ± 15.3</td>
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<tr>
<td>Laurel (n=2)</td>
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<td>419</td>
<td>3.18 ± 2.97</td>
<td>46.7 ± 41.01</td>
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<tr>
<td>Enrique (n=2)</td>
<td>10</td>
<td>67</td>
<td>1.24 ± 1.58</td>
<td>33.9 ± 10.9</td>
</tr>
</tbody>
</table>
Figure 1. The Calcareous sponge *Clathrina* sp. A. *In situ* photograph of *Clathrina* sp. growing in a cavern overhang, B. Juvenile fish taking refuge in the folds of the sponge, C. Sea-anemone, *Bunodeopsis* sp. attached to the sponge, D. *Clathrina* sp. growing at the base of the octocoral, *Briareum asbestinum*, E. *Clathrina* sp. growing underneath the scleractinian coral, *Orbicella faveolata*, and F. *Clathrina* sp. growing underneath the white encrusting zoanthid, *Palythoa caribaeorum*. 
Figure 2. Associated infauna of the calcareous sponge, *Clathrina* sp., A. Polychaete within the sponge, B. Sea Anemone, *Bunodeopsis* sp., with tentacles partially extended, C. Brittle star attached to the exposed internal canal, D. Polycheate found through a cross-section of the sponge, and E. Copepod found within the interior cavity of *Clathrina* sp.
Figure 3. Collection sites across southwestern Puerto Rico, La Parguera Natural Reserve. A. Mario reef (17°95′N, -67°05′W), B. Enrique reef (17°57′N, -67°02′W), C. Laurel reef (17°56′N, -67°03′W), D. Media Luna reef (17°95′N, -67°05′W), E. Turrumote reef (17°93′N, -67°02′W), F. Pinnacles reef (17°90′N, -67°00′W), G. San Cristobal reef (17°93′N, -67°11′W), and H. Margarita reef (17°95′N, -67°11′W).
Figure 4. Invertebrate phyla of associated fauna found within the calcareous sponge *Clathrina* sp.
Figure 5. Linear regression between sponge volume and (top) number of individuals and (bottom) number of species of the infauna associated with *Clathrina* sp.
Figure 6. Principal Coordinate Ordinations (PCO) of centroids (site and season) of Bray-Curtis similarities calculated from data of invertebrate assemblages associated with *Clathrina* sp. in three different sites (▲ = Turrumote reef, □ = San Cristobal reef, ● = Pinnacles reef) during four different seasons (SP = Spring, SU = Summer, FA = Fall, WI = Winter).
Supplementary Table 1. Ecological data for *Clathrina* sp. collected within this investigation, all reef sites are located within the Marine Reserve of La Parguera, southwest Puerto Rico.

<table>
<thead>
<tr>
<th>Sponge ID</th>
<th>Reef Site</th>
<th>Month</th>
<th>Depth (m)</th>
<th>Wet weight (g)</th>
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Supplementary Figure 1. nMDS of Bray-Curtis similarities of square-rooted transformed data of assemblages associated with *Clathrina* sp. from eight different reefs at La Parguera Natural Reserve, PR (▲ = Turrumote, △ = Mario, ■ = Media Luna, □ = San Cristobal, ● = Pinnacles, ○ = Margaritas, ◆ = Enrique, ◇ = Laurel); sampled across different seasons (SP = Spring, SU = Summer, FA = Fall, WI = Winter).