

Article

Evaluation of the Use of Autonomous Reef Monitoring Structures (ARMS) for Describing the Species Diversity of Two Coral Reefs in the Yucatan Peninsula, Mexico

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Abstract: Autonomous reef monitoring structures (ARMS) have been proposed as a standardized, passive, nondestructive sampling tool. This study assessed the ability of ARMS to capture the cryptic species diversity of two coral reefs by recording species richness and taxonomic representativeness using conventional taxonomy. The capacity of ARMS, as artificial substrates, to favor the establishment of nonindigenous species over native species was also evaluated. The use of ARMS allowed the detection of 370 species morphotypes from nine phyla, yielding 13 new records of geographic distribution expansion, one exotic species for the Gulf of México and the Caribbean Sea, and six newly described species. It was also possible to make spatial comparisons of species richness between both reefs. ARMS captured cryptic diversity exceptionally well, with the exception of echinoderms. Furthermore, these artificial structures did not hinder the colonization ability of native species; in fact, the colonization patterns on the structures themselves represented the spatial differences in the structure of benthic assemblages. This study represents the first effort to make a conventional taxonomic description of the cryptic fauna of the Yucatan Peninsula using ARMS. It is recommended to assess coral reef species diversity, but more taxonomists specialized in marine invertebrates are needed.

Keywords: benthic fauna; cryptobiome; coral reef biodiversity; artificial substrates; Gulf of Mexico; Mesoamerican reef; taxonomic expertise

1. Introduction

Coral reef cavities represent up to 75% of the total volume of a reef [1]. The organisms that inhabit these cavities represent, in turn, a significant proportion of the reef biodiversity [2], similar to or greater than that of the exposed area of the reef [3]. These cryptic fauna of the reefs, mainly invertebrates [4,5], play ecologically important roles as predators [4,5], detritivores [6], and removers of suspended organic matter [7]. However, most efforts to describe and monitor the diversity of fauna in coral reefs have focused primarily on conspicuous organisms found on the surface, such as macroalgae, reef-building corals, massive sponges, fish, and megabenthos. These organisms represent only a fraction of the known biomass and diversity of coral reefs [8], and, consequently, the total biodiversity of coral reefs tend to be underestimated [2].

The current knowledge of the diversity of cryptic organisms inhabiting coral reefs has been obtained through procedures and techniques that, for the most part, involve damage to the reef. Some of the methods that have been used include the application of electric current, dredging, chemical dumping [9], and even the intentional fracture of the reef substrate [10]. Furthermore, the diversity estimates thus obtained are difficult to compare quantitatively due to the lack of common and standardized sampling procedures [11]. This makes it very difficult to detect diversity patterns, and even harder to propose models and test hypotheses of ecological theories on the role of the diversity of reef cryptic fauna and the potential link with the state of reef health [2].

Autonomous reef monitoring structures (ARMS) have been proposed as a standardized, passive, nondestructive sampling tool that can help overcome the known limitations in the description of reef cryptic fauna [12]. More than 2000 ARMS have been deployed around the world, many of these through international cooperation networks, and others through local projects [13]. However, despite the advantages of ARMS as a standardized sampling tool, their effectiveness in representing such biological diversity of cryptic fauna has not been quantitatively evaluated. It is widely recognized that the artificial habitats units used in ecological experiments tend to overestimate the presence and abundance of nonindigenous species [14–16]. In general, the diversity of species found on hard substrates (such as coral and rocky reefs) is strongly influenced by physical, chemical, and microbiological properties of the surface that change with time, affecting the ecological

succession [17,18], which is difficult to imitate in experimental artificial substrates. This is particularly important when dealing with smooth substrates, such as the material with which ARMS are built (PVC). Despite the three-dimensional complexity of the ARMS (with open and closed cavities that can serve as habitats for different species), the potential negative effect that the nature of the material could have on the recruitment, establishment, and dominance of nonnative species and/or inhibiting the establishment of native species, could imply that the biological diversity found on these structures cannot be considered representative of the cryptic diversity of coral reefs.

Measuring the effectiveness of artificial substrates in mimicking the characteristics of natural substrates usually requires complex experiments, involving more than two treatments, reference sites, and a large number of experimental replicates [14,15]. Evaluating the efficiency of ARMS as a substitute for natural substrates would require experiments with coral rocks. However, controlling the volume, area, and complexity between both treatments would involve challenges (of design, extraction of natural material, processing, assembly, and disposal) that may compromise the comparison. Alternatively, the suitability and biological representativeness of the organisms captured with ARMS could be evaluated by estimating the taxonomic distinctness and testing for deviations from expectations considering the species already registered in the region [19].

In general, the existing protocols for processing biological samples collected with ARMS focus on the use of molecular techniques such as DNA barcoding and metabarcoding to identify and quantify organisms [2,8,20–22]. However, the effectiveness of molecular analysis depends on the records available in databases and libraries of barcode sequences of known species [21], representing another analytical obstacle, because the taxonomic operational units (OTUs) vary according to the resolution of the genes analyzed [9]. Indeed, it is estimated that around 50% of the OTUs obtained with ARMS cannot be identified due to the lack of coincidences with database sequences, and only a small fraction (<12%) of these OTUs reach a high coincidence threshold [2,22]. As an alternative, organisms can be processed using classic taxonomy. Techniques based on the observation of morphoanatomical characters have established a sound basis for the quantification, evaluation, and conservation of species diversity [23]. Most importantly, the available taxonomic records are more accessible and comparable than the molecular ones, which would make it easier to assess the suitability of ARMS as a sampling method. However, due to the large number of organisms collected with ARMS, processing and identification would be limited without the collaboration of several specialists and many hours of laboratory work.

Our research focused on assessing the ability of ARMS to capture the cryptic biodiversity of two coral reefs from two reef systems with different environmental conditions. To determine this, we analyzed the species richness, taxonomic representativeness, and relative abundance of sessile benthic groups. The first null hypothesis was that average taxonomic distinctness index of the assemblages captured by ARMS reflects the taxonomic diversity of the known species in each region, using Ocean Biodiversity Information System (OBIS) and Felder and Camp (2009) [24] as a baseline. Rejecting this hypothesis suggests that the diversity of taxonomic categories collected with ARMS does not represent what was expected for the region. The second null hypothesis was that local conditions do not affect the structure of sessile biota, which depends on ARMS properties. Rejecting this hypothesis suggests that regardless of the artificial and smooth surface of ARMS, the availability of larvae and recruits, along with local environmental conditions, drive the structures of sessile biota. The present study represents the first effort to describe the cryptic fauna of the Yucatan Peninsula through autonomous reef monitoring structures (ARMS) using conventional taxonomy.

2. Materials and Methods

2.1. Study Area

The Yucatan Peninsula is located in the southeast of Mexico, bordered on the east by the Caribbean Sea and on the north and west by the Gulf of Mexico [25]. There are two

main reef systems around the Yucatan Peninsula. An important part of the Mesoamerican Reef System extends along the Caribbean coast, where up to 153 reef areas have been recorded; these are mainly barrier and fringing reefs [26,27]. The second reef system is found in the southeast of the Gulf of Mexico (Campeche and Yucatan Bank) and contains patch reefs and submerged banks away from the coast (up to 200 km) [28], surrounded by Caribbean waters from the Yucatan Channel current, with no influence of continental runoff [29].

2.2. Sampling

A total of eight ARMS were deployed: four in a shallow reef in the Campeche Bank (Bajo de 10, 21°20'53.82" N, 90°08'45.48" W) at seven meters depth, and four ARMS in a shallow reef of the Mesoamerican Reef System (Mahahual, 18°37'24" N, 87°43'32" W) at four meters depth (Figure 1). All ARMS were placed 3–5 m apart and fixed over carbonate substrates. The ARMS were deployed in February 2018 (20 and 27, respectively), left undisturbed for one year, and recovered using the standard method for ARMS [30]. The collected organisms were grouped by phylum, labeled, and preserved for identification by conventional taxonomy according to the existing literature. Detailed procedures are available in Palomino-Alvarez et al. [31].

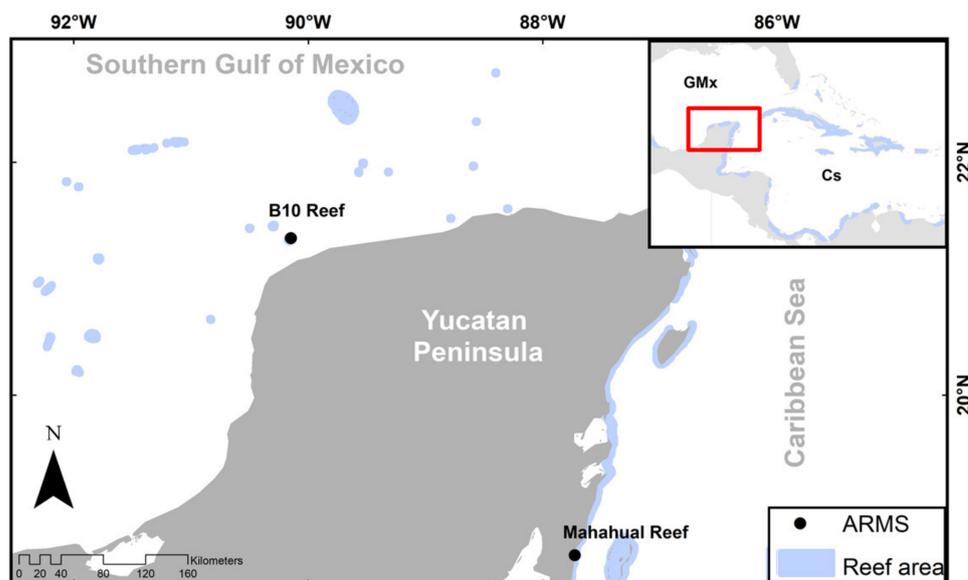


Figure 1. Studied reefs in the Mexican Caribbean sea (Cs) and the southern Gulf of Mexico (GMx) [32], where autonomous reef monitoring structures (ARMS) were deployed.

2.3. Statistical Analyses

The diversity of faunal assemblages was evaluated by estimating the average taxonomic distinctness index (Δ^+) [19]. This measure has the advantage of being independent of sampling effort, a desirable feature in studies with a low sample size (four ARMS per site) [33]. Any value of Δ^+ can be assumed to be representative if falls within the expected range of Δ^+ values for each region (Gulf of Mexico and Caribbean Sea) according to the richness observed. On the other hand, any deviation below the lower limit will indicate overrepresentation of some taxonomic groups, typical of assemblages of opportunistic organisms, such as nonindigenous species. The Δ^+ were tested using the taxonomic distinctness test—TAXDTEST [34]. The expectations were constructed using 999 simulated sublists for each richness value. The Δ^+ value was estimated for each region, and the 5% of extreme values in both tails of the distribution served as a reference to rule out the null hypothesis of taxonomic representativeness for the recorded value of Δ^+ . The tests were applied independently for each phylum, as recommended by Warwick and Somerfield [35], and the regional species lists (Gulf of Mexico and Caribbean Sea) were used as taxonomic

aggregation matrices. These lists were based on information obtained from specialized literature of each phylum [24] and from the Ocean Biodiversity Information System (OBIS) [36], using Caribbean Sea area (ID 34287) and Gulf of Mexico area (ID 34287) as geographic filters. These subsets of data were selected using filters to constrain the expectations of diversity for the cryptic fauna. The filters used were as follows: depth range from 0 to 50 m, coral reef habitat, exclusion of synonyms, taxonomic resolution to the species level, and only return records of species with preserved specimens and material samples held in collections with a catalog number and available for reference. The nomenclature and hierarchical classification used for each phylum were species, genus, family, superfamily, suborder, order, class, and subphylum of each phylum, according to the World Register of Marine Species (WoRMS) [37]. To avoid misleading statistical results (i.e., false representativeness), the taxonomic distinctness tests were performed for taxonomic groups that have been historically evaluated by several authors in these regions: these are Annelida, Arthropoda, Echinodermata, and Mollusca. The taxonomic lists used for these analyses are provided in Palomino-Alvarez et al. [38], and the data matrix of species from ARMS identified in each reef is provided in Palomino-Alvarez et al. [39].

To test the null hypothesis that local conditions do not affect the structure of sessile taxa on ARMS, both sides of each plate were photographed, and the difference in the relative abundance of the organisms was analyzed using taxonomic resolution at the Phylum category. Abundance was estimated as relative coverage using the point intersection method (400 points per plate on each side) [40], with the CPCe v4.1 software (Coral Point Count with Excel extension v4.1) [41]. Relative abundance counts were organized in an $N \times P$ matrix, with N being the total number of samples and P being the total number of phyla. The dataset with abundance per phylum and side-plate is provided in Palomino-Alvarez et al. [42]. The Bray–Curtis dissimilarity coefficient between each pair of plate sides was estimated, generating a triangular matrix of dissimilarities that was used for statistical analysis and sorting. The differences in relative abundance of phylum between reefs and ARMS within each reefs were analyzed using a two-way nested ANOSIM with 9999 permutations [43], with the plates representing the replicates of each ARMS and the ARMS nested within each reef. The spatial patterns of dissimilarities were represented using a nonmetric multidimensional scale (nMDS). All statistical analyses were performed using PRIMER v7 software [44].

3. Results

3.1. Diversity of the Cryptofauna Assemblage

370 species were identified in nine phyla (Figure 2). The taxonomic resolution at which these morphotypes were identified is as follows: 244 species, 95 genera, 23 families, and eight classes. The highest richness was recorded in Bajo de 10 reef (Figure 2). The phyla with the highest number of species in both reefs were Mollusca, Arthropoda (Amphipoda, Decapoda, Pantopoda, and Stomatopoda), and Annelida (Polychaeta), followed by Chordata (Ascidiacea), Echinodermata, Porifera, and Platyhelminthes. The phyla Bryozoa and Cnidaria (Hydrozoa) were not recorded in Mahahual reef (Figure 2). Of the species identified, 13 are new records: 11 for shallow reefs of Campeche and Yucatan Bank (Gulf of Mexico), and four in the Mesoamerican Reef System (Caribbean Sea). The genus *Geminella* (Hydrozoa) was recorded for the first time, as well as the nonindigenous species *Sillys bella* (Polychaeta: Annelida) (Table 1), and six possible new species: *Leucothoe* sp. (Arthropoda: Amphipoda), *Plesiocleidochasma* sp. (Bryozoa), *Botrylloides* sp. 1, *Botrylloides* sp. 2, *Botrylloides* sp. 3, and *Botryllus* sp. 1 (Chordata: Ascidiacea). The taxonomic list is available in Palomino-Alvarez et al. [45], and the dataset, in Darwin Core format, can also be found in the Caribbean OBIS Node [46].

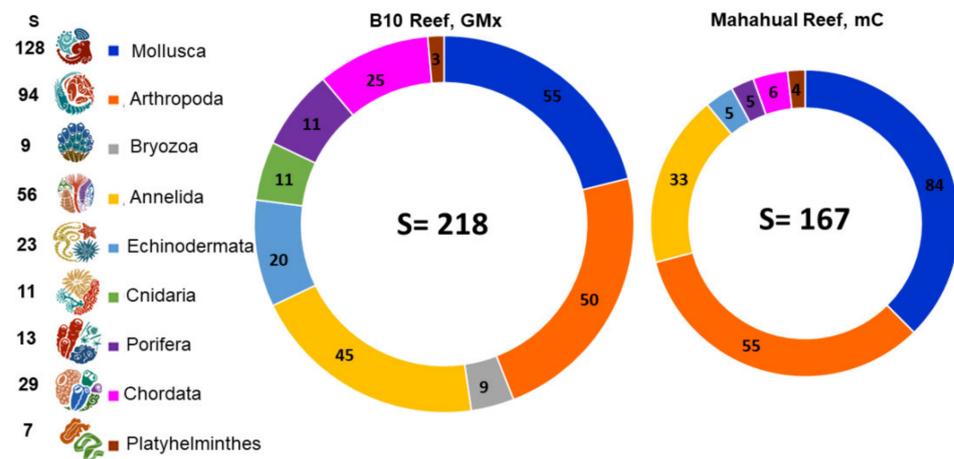


Figure 2. Number of species morphotypes (S) identified by phylum of the cryptofauna in autonomous reef monitoring structures (ARMS) during one year of recruitment in two reefs: Bajo de 10 reef (Campeche and Yucatan Bank Reefs, GMx = Gulf of Mexico) and Mahahual Reef (Mesoamerican Reef System, mC = Caribbean Sea).

Table 1. New records per region of cryptic fauna species recorded in autonomous reef monitoring structures (ARMS) during a year of recruitment in Bajo de 10 reef and Mahahual Reef of the Yucatan Peninsula. GMx = Gulf of Mexico; mC = Caribbean Sea.

Species	GMx	Cs	Phylum	Documented Distribution
<i>Treptopale rudolphi</i> Perkins, 1985	✓		Annelida (Polychaeta)	Miami, USA [47], Caribbean [48]
<i>Rullierinereis</i> cf. <i>bahamensis</i> (Hartmann-Schröder, 1958)	✓		Annelida (Polychaeta)	Bimini Islands, Bahamas [48], gulf of Cariaco, Venezuela [49]
<i>Pseudobranchiomma perkinsi</i> Knight-Jones and Giangrande, 2003	✓		Annelida (Polychaeta)	Florida, USA [50]
<i>Syllis bella</i> (Chamberlin, 1919)	✓	✓	Annelida (Polychaeta)	California, USA [48], Mediterranean [51], recorded as an invasive species [52,53] Pacific Ocean [54], Santa Marta and Cabo Arrecifes, Colombia [55], Morro Sao Paolo, Brasil [56]
<i>Ammothella spinifera</i> Cole, 1904	✓		Arthropoda (Chelicerata)	Gulf of Mexico [24], northern coast of Yucatan [57,58]
<i>Aruga holmesi</i> J.L. Barnard, 1955		✓	Arthropoda (Amphipoda)	Penambuco, Brazil [59]
<i>Chevalia caetes</i> Souza-Filho, Souza and Valério-Berardo, 2010	✓	✓	Arthropoda (Amphipoda)	Bermuda, West Florida, USA [60,61]
<i>Clessidra tinkerenis</i> (Kunkel, 1910)	✓	✓	Arthropoda (Amphipoda)	Burma, Myanmar [62]
<i>Parasmittina bimucronata</i> (Hincks, 1884)	✓		Bryozoa	Villefranche-sur-Mer, France [63], Northwestern Atlantic [64]
<i>Plumularia obliqua</i> (Johnston, 1847)	✓		Cnidaria (Hydrozoa)	Philippines, Kei islands, Indonesia [65] and New Caledonia [66], Siboga [67], Western Central Pacific [68]
<i>Geminella ceramensis</i> (Billard, 1925)	✓		Cnidaria (Hydrozoa)	Australia [69]
<i>Cycloporus variegatus</i> Kato, 1934	✓		Platyhelminthes	Curacao, Venezuela [70], Colombia [71]
<i>Pericelis cata</i> Marcus and Marcus, 1968	✓		Platyhelminthes	

3.2. Taxonomic Distinctness

The average taxonomic distinctness index estimated in ARMS per reef for Annelida (Bajo de 10 reef: $S = 29$, $\Delta^+ = 85.22$, $p = 0.22$; Mahahual reef: $S = 18$, $\Delta^+ = 86.27$, $p = 0.97$) and Mollusca (Bajo de 10 reef: $S = 35$, $\Delta^+ = 84.3$, $p = 0.52$; Mahahual reef: $S = 50$, $\Delta^+ = 86.35$, $p = 0.89\%$) were within the expected frequency distribution regarding species registered in OBIS and by Felder and Camp [24]. In the phylum Arthropoda, the average taxonomic distinctness in Bajo de 10 reef was above the average estimated from the expected frequency distribution ($S = 42$, $\Delta^+ = 70.81$, $p = 0.002$). Similarly, the frequency of phylum Echinodermata in Mahahual reef ($S = 5$, $\Delta^+ = 51.66$, $p = 0.0012$) was below the expected range (Figure 3).

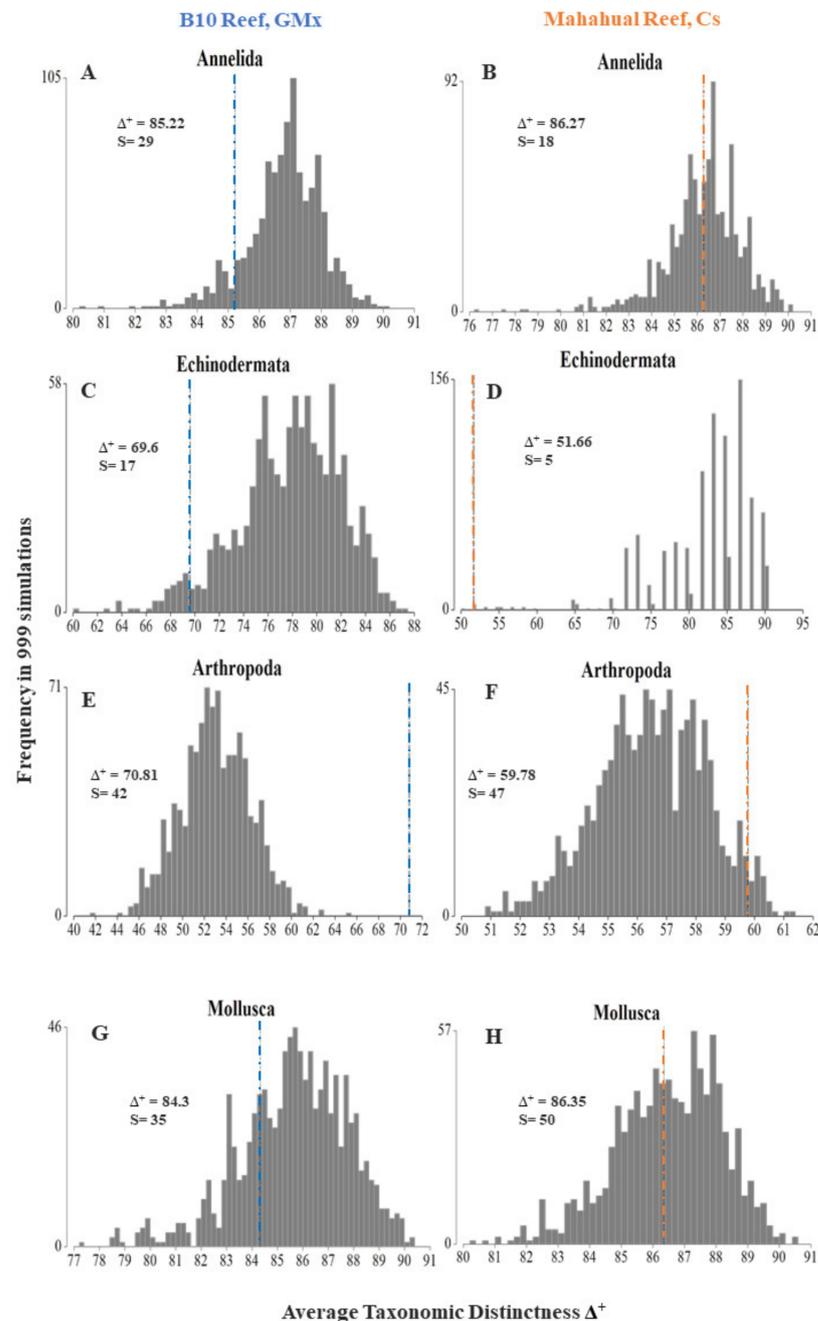


Figure 3. Frequency distribution of simulated Δ^+ values generated from 999 sublists drawn randomly from the master list of species of each phylum by region: Gulf of Mexico (A,C,E,G) and Caribbean Sea (B,D,F,H) of the Yucatan Peninsula. Taxonomic aggregation matrices from OBIS and Felder and Camp [24]. The vertical colored lines are the estimation of Δ^+ found in autonomous reef monitoring structures (ARMS) deployed during one year in each reef.

3.3. Abundance of Sessile Biota

The sessile organisms belonged in six phyla: Chordata (Ascidiacea), Porifera, Bryozoa, Cnidaria (Hydrozoa), Annelida (Polychaeta), and Chlorophyta. Chlorophyta, Chordata, and Bryozoa were the most dominant phyla in Bajo de 10 reef, accounting for more than 80% of the abundance on the plates. In Mahahual reef, Chlorophyta, Chordata, and Annelida were the most dominant phyla on the plates. Bryozoa and Hydrozoa were absent in Mahahual Reef (Figure 4). There is statistically significant evidence to reject the null hypothesis that local ecological conditions do not affect the structure of sessile species on

ARMS (two-way nested ANOSIM, $R_{\text{reef}} = 0.927$, $p = 0.029$). The phyla Bryozoa, Chordata, and Chlorophyta accounted for most (75%) of the differences between reefs (47.5% Bray–Curtis similarity). In addition, the dissimilarity between ARMS within the same reef was very low, but statistically significant (two-way nested ANOSIM, $R_{\text{ARMS(reefs)}} = 0.12$, $p = 0.01$).

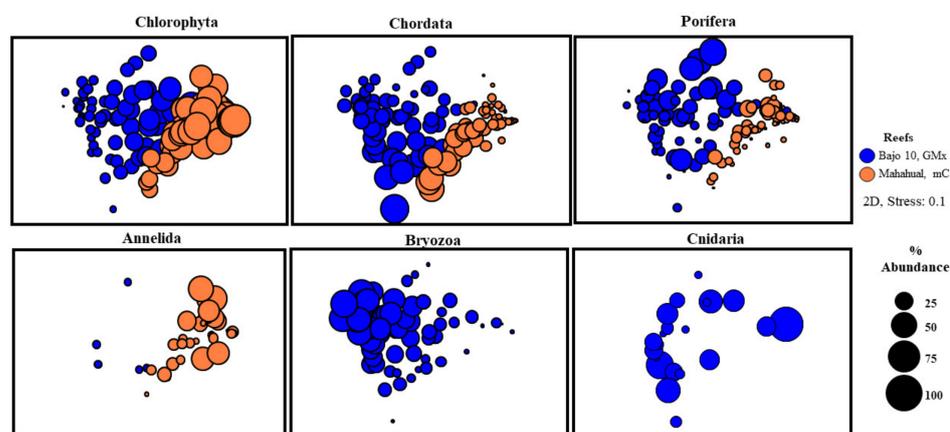


Figure 4. Patterns of Bray–Curtis similarities between plate-sides of each ARMS in both reefs, considering the relative abundance (coverage) of six phyla, represented in a nonmetric multidimensional scaling ordination (nMDS). The size of each symbol represents the relative abundance of the phyla.

4. Discussion

The use of ARMS facilitated a significant contribution to the knowledge of the cryptic diversity of two reefs of the Yucatan Peninsula. With only eight of these structures, 370 species morphotypes were simultaneously recorded, corresponding to nine phyla which are difficult to collect in normal sampling, owing to their cryptic nature. This number represents 16% of the species listed in OBIS for those nine phyla for the Gulf of Mexico and Caribbean Sea region [36]. The effectiveness of ARMS in representing the biological diversity of the region was not consistent for all phyla and, contrary to predictions regarding the nature of the material (PVC), ARMS harbored mainly native species. It can be assumed that the ability of the identified species to colonize ARMS depended mainly on local processes rather than on the artificial and smooth nature of the surface of the ARMS. Nevertheless, any potential effect of these structures in the ecological succession must be experimentally evaluated. Despite this, the results show that the use of ARMS as a standardized method would allow for comparisons of species richness between reefs in environmentally different regions.

For example, in Veracruz (also in the Gulf of Mexico), García [72] used seven ARMS for up to eight months and detected around 100 species morphotypes belonging to six phyla (Mollusca, Porifera, Annelida, Arthropoda, and Echinodermata). A preliminary comparison revealed that the taxa recorded in Bajo de 10 and Mahahual reefs differ by 95% from the taxa recorded in Veracruz, but also that the richness recorded in the present study was substantially greater than that recorded by García [72]. Only three species were shared with Bajo de 10 and Mahahual reefs: an arthropod (*Mithraculus forceps*) and two mollusks (*Arca imbricata* and *Columbella mercatoria*). These differences could be attributed to the influence of local ecological processes rather than to the use of different sampling methods, which would favor the hypotheses that suggest that ecological processes are the key to understanding the diversity of cryptic reef organisms even across different regions.

In general, the species of Annelida and Mollusca that were collected by ARMS did not show a reduction in the expected taxonomic distinctness for each region. This result suggests that the use of ARMS can help to detect the diversity of these phyla in the reefs under study. The same can be inferred for arthropods, which were one of the phyla with the highest number of species in the ARMS, despite showing a greater taxonomic

distinctness than expected for the richness recorded. Although statistically the average taxonomic distinctness index (Δ^+) of arthropods exceeded 2.5% of the maximum expected values under a true null hypothesis, this value indicates that all the taxonomic branches of this phylum known to be present in the region were recorded by the ARMS. This, in fact, is a very good result. However, the species of the phylum Echinodermata that were collected with ARMS in the Mahahual reef showed a reduction in the expected taxonomic distinctness in both regions. As this method is limited fauna from hard substrate, it should be complemented with another type of sampling technique to record the diversity of echinoderms with cryptic habits [73], and other vagile organisms.

The use of conventional taxonomic methods to identify the species collected with ARMS required extensive collaborative work, which highlighted the need to train new specialists in such diverse groups as crustaceans, mollusks, bryozoans, ascidians, and sponges. This collaborative work made it possible to record, for the first time in the Gulf of Mexico, nine species that had been previously recorded only in adjacent ecoregions such as the Western Caribbean, Greater Antilles, and Bahamian [74]. These nine species include three polychaetes (*T. rudolphi*, *R. cf. bahamensis*, and *P. perkinsi*), one pycnogonid (*A. spinifera*), one bryozoan (*P. bimucronata*), two hydrozoans (*P. obliqua* and *G. ceramensis*), and two flatworms (*C. variegatus* and *P. cata*). Two species of amphipods that had been previously recorded only in the Gulf of Mexico (*A. holmesi* and *C. tinkerensis*) were recorded in the Caribbean. In addition, two species were recorded for the first time in both regions: *C. caetes*, described in Brazil, and *S. bella*. *Sillys bella* was originally recorded in the Pacific [75] and has been classified as an invasive species of the Mediterranean Sea [52,53]. Above all, collaborative work allowed the identification of 91% of the found morphospecies at a fine taxonomic resolution (67% at the species level and 24% at the genus level). As with molecular techniques [20,22,76–78], the most diverse groups according to conventional taxonomic techniques were Arthropoda and Mollusca [20,76,77]. Unfortunately, the possibility of having an available team of taxonomists as diverse as the one orchestrated for this study is low. Hence, considering that it is widely recognized that the shortage of taxonomists is critical for addressing the current biodiversity crisis [79], and molecular technology is still imprecise for cryptic organism in reefs [2,22], more people need to be trained in reef invertebrate taxonomy, but simultaneously, larger molecular databases necessary for effective metabarcoding should be built.

Aside from the patterns related to taxonomic distinctness, there were differences in abundance and composition of sessile biota between sites. Species typically recognized as members of fouling assemblages on artificial substrates were not registered in either of the sites. These results imply that the artificial nature of ARMS does not favor colonization by nonindigenous species over local species, as is typical of marine fouling on hard artificial substrate. In addition, with these results, we can infer that ARMS are a useful tool to detect changes in the structure of sessile communities. For example, high macroalgal coverage was detected in both reefs, but the coverage was substantially higher in Mahahual. This phenomenon is consistent with what has been observed in recent decades in the coral reefs of the western Caribbean [80], due to changes in ecological conditions and a decrease in the herbivore population [81]. The use of ARMS yields effective results in the detection and comparison of changes in the cryptic reef biota. Using ARMS as a long-term diversity assessment tool to complement other survey methods (e.g., Atlantic and Gulf Rapid Reef Assessment: AGRRA) will improve our understanding of the dynamics, conservation, and degradation of species diversity on reefs.

5. Conclusions

This study represents the first effort to describe the cryptic fauna of the Yucatan Peninsula through autonomous reef monitoring structures (ARMS) using conventional taxonomy. ARMS showed a great capacity for recording the diversity of native cryptic organisms in two coral reefs of the Yucatan Peninsula during a year. They also enhanced colonization by local species and were able to capture changes in the structure of sessile

communities. However, they were not suitable for the study of the diversity of the echinoderm assemblage. The use of ARMS allows preliminary geographic comparisons of species diversity with other studies without causing confusion due to differences in sampling methods. Finally, the training of new specialists on highly diverse taxa (e.g., arthropods, mollusks, bryozoans, ascidians, annelids, and sponges) is necessary in order to effectively estimate reef biodiversity.

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