

Article

Form Meets Function: Role of Macroalgal Morphology in Structuring Mobile Epifaunal Assemblages in Selected Algae from the Central Mediterranean

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Abstract

Quantitative measurement of macroalgal thallus morphology and its link with epifaunal communities remains largely unexplored in the central Mediterranean. This paper explores the link between thallus morphology and the associated mobile epifaunal assemblages of three common macroalgae native to the Maltese Islands (central Mediterranean Sea): *Ellisolandia elongata*, *Jania rubens*, and *Dictyota* spp. (*D. dichotoma*, *D. mediterranea*, and *D. fasciola* in mixed assemblages). We hypothesised that macroalgal species exhibiting distinct morphological characteristics support mobile epifaunal assemblages that differ significantly in structure and composition. Using a full factorial framework, macroalgal beds dominated by each target species were sampled from shallow water in three localities on the northeastern coast of Malta, using SCUBA diving. Dry biomass and fractal dimensions were used to characterise the distinct morphological traits of the three macroalgal species. A total of 85 epifaunal taxa were associated with *Dictyota* spp., 98 taxa with *E. elongata*, and 112 taxa with *J. rubens*. Excluding the disproportionately abundant invasive foraminiferan *Amphistegina lobifera*, 911 individuals were recorded on *E. elongata*, 1224 on *Dictyota* spp., and 2233 on *J. rubens*. Of the 152 taxa identified across all macroalgae studied, Arthropoda (44 taxa) and Mollusca (39 taxa) dominated, with individuals occupying the small inter-frond spaces as shelters that provide refuge from predators and hydrodynamic stress. Multivariate analysis revealed that the structure and composition of mobile epifaunal assemblages were associated with the interaction between macroalgal morphology and site-specific environmental conditions. The more structurally complex, articulated, calcified, and densely branched forms of *E. elongata* and *J. rubens* supported more varied epifaunal communities that differed from those associated with the compressed and leafier thalli of *Dictyota* spp., which were instead dominated by surface-associated taxa. Notably higher richness and diversity values were recorded in sheltered sites, especially in the case of *J. rubens*, showing that the effect of habitat architecture may be modulated by wave exposure.



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1. Introduction

Foundation species shape biodiversity by providing structural complexity, which enhances habitat availability and resource heterogeneity for associated organisms [1,2]. In

marine ecosystems, macroalgae act as key foundation species and ecosystem engineers, forming complex three-dimensional habitats that promote species diversity and maintain ecosystem functioning [3]. Vegetated habitats generally support greater epifaunal diversity and abundance than unvegetated substrata [4,5]. Macroalgae range in morphology from filamentous and foliose to leathery and calcareous forms and drive ecological interactions by modulating refuge availability, food accessibility, hydrodynamics, and predator–prey dynamics [6–8]. Consequently, structural changes within algal communities can lead to shifts in epifaunal composition and trophic networks [9,10]. Despite their ecological significance, marine macro-epifauna are relatively understudied compared to more conspicuous megafauna like fish and echinoderms. Their cryptic behaviour and small size can make them difficult to investigate, along with limited taxonomic information to achieve high-resolution identifications [10]. Furthermore, research in this field has historically been hindered by inconsistent terminology and the difficulty of quantitatively sampling epifauna within the structurally diverse habitats of rocky reefs [10,11].

While numerous studies have attempted to relate macroalgal structure to mobile epifaunal diversity, the objective quantification of ‘habitat complexity’—rather than its qualitative description—remains a developing field, particularly within the Mediterranean Sea. Qualitative approaches, such as categorical classification of thallus form, branching, and flexibility, are often subjective [12]. In contrast, quantitative metrics such as frond density, surface area, and canopy height offer more reproducible measures [6,13]. However, traditional proxies of habitat complexity such as biomass or thallus length have been criticised for overlooking fine-scale interstitial spaces that are ecologically important for epifauna [4,14].

Fractal analysis has emerged as a particularly robust tool for quantifying macroalgal structural complexity [14]. Fractal measures, including fractal area (D_a) and perimeter (D_p), capture surface roughness and branching at ecologically relevant scales, thus providing a biologically meaningful way of objectively quantifying macroalgal morphology through image analysis [15]. Studies applying this approach have shown that higher fractal dimensions correspond to greater structural complexity and in turn often result in higher epifaunal abundance and diversity [4,16]. Although macroalgae are not true fractals since they lack self-similarity across all scales, fractal metrics remain superior proxies for the habitat complexity experienced by macrofauna [15]. Combining fractal dimensions with traditional proxies like biomass allows for a comprehensive characterisation of macroalgal architecture [4]. Despite these advantages, coarse structural metrics still prevail in recent Mediterranean literature (e.g., [17]). This reveals a persistent methodological gap in Mediterranean epifaunal ecology, where integrating advanced quantitative descriptors of habitat complexity could substantially improve our understanding of organism–habitat relationships.

Understanding the link between foundation species and their associated epifauna is critical for assessing the reliability of using marine benthic habitats defined by the presence of dominant algal species as proxies for the distribution and spatial patterns of epifaunal biodiversity. Such knowledge also provides insights into how shifts in algal communities and their structural complexity, whether resulting from environmental change, disturbance, or invasive species, may lead to alterations in associated macrofaunal assemblages, with cascading impacts on higher trophic levels. Addressing this gap is therefore essential for advancing habitat-based biodiversity assessments and predicting ecological responses to ongoing changes in Mediterranean coastal ecosystems.

The present study evaluates the structural differences in dominant habitat-forming macroalgae and the relationship between host macroalgal morphology and the diversity and composition of the associated epifaunal assemblages in shallow rocky habitats within

the central Mediterranean region (Maltese Islands). Macroalgal morphology is assessed both qualitatively (physical description) and using objective quantitative measures (biomass and fractal dimensions). We tested the hypothesis that macroalgal species exhibiting distinct morphological characteristics support mobile epifaunal assemblages that differ significantly in structure and composition.

2. Materials and Methods

2.1. Study Sites and Target Macroalgae

Snorkelling and SCUBA surveys were conducted between October and November 2023 at various locations on the Maltese coast to identify gently sloping rocky bottoms with macroalgal-dominated ‘reefs’ (rocky shoals) at depths of 1–5 m. Sites were selected based on topographical similarity, extensive macroalgal cover, and the presence of common dominant species. The selected sites were Baħar iċ-Ċagħaq, Westin Rock Beach in St. Julian’s, and Exiles Beach in Sliema, all situated along the northeastern coast of Malta (Figure 1). This regional concentration was a result of these locations being the only surveyed sites that met all inclusion criteria. Wave exposure at each site, estimated with the Exposure Index (EI) proposed by Thomas [18] as adapted for Maltese shores [19], indicated that Baħar iċ-Ċagħaq was slightly more exposed to wind and wave action (EI = 8.01) than Sliema (EI = 3.59) and St. Julian’s (EI = 2.13). Nonetheless all three sites are relatively sheltered by local standards, where the most exposed coasts have EI values > 30 [20].

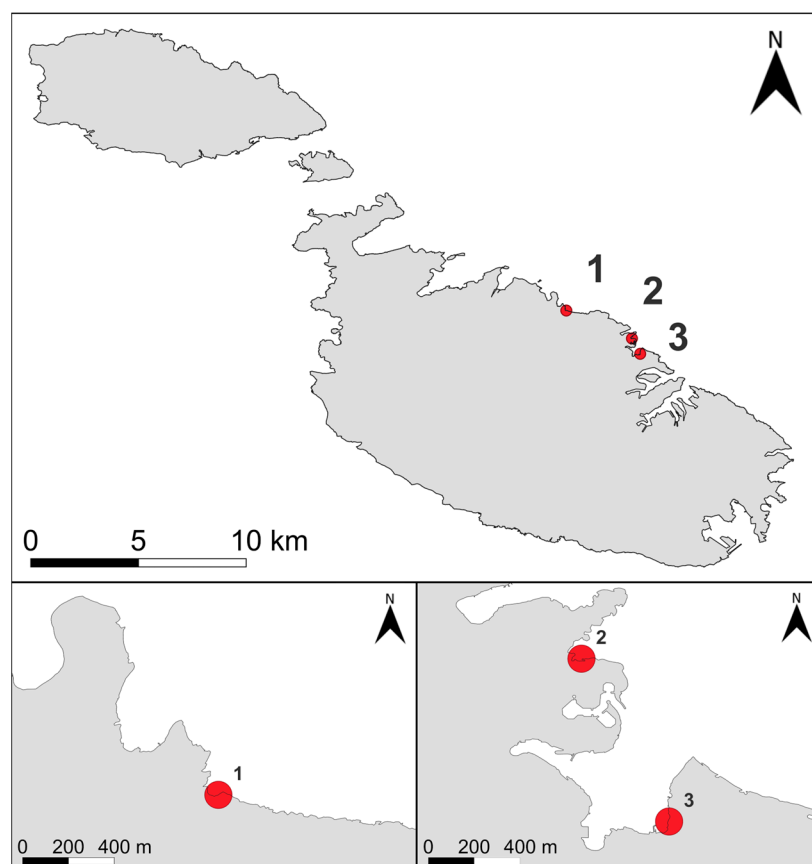


Figure 1. Map of the Maltese Islands showing the three sampling locations (1 = Baħar iċ-Ċagħaq [35.938098° N, 14.459584° E]; 2 = Westin Rock Beach, St. Julian’s [35.923760° N, 14.493298° E]; 3 = Exiles Beach, Sliema [35.915887° N, 14.497539° E]).

Assemblages at the three sites were dominated by *Jania rubens* J.V.Lamouroux (Rhodophyta, Corallinaceae), *Dictyota* spp. (Ochrophyta, Dictyotaceae) (mainly *D. di-*

chotoma (Hudson) J.V.Lamouroux, but including also *D. mediterranea* (Schiffner) G.Furnari, and *D. fasciola* (Roth) J.V.Lamouroux), and *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders (Rhodophyta, Corallinaceae), providing a full factorial design where all three target taxa occurred at each sampling site. The *Dictyota* species were grouped together due to their mixed occurrence and similar morphologies. Although this grouping may mask intra-generic differences among the three species of *Dictyota*, their similar foliose morphology, particularly in terms of blade architecture and branching pattern, makes them functionally comparable with respect to the habitat they provide for associated epifaunal assemblages. The grouping was therefore considered appropriate for the objectives of the present study.

2.2. Sample Collection and Processing

Sampling was conducted over a two-month period (December 2023 to January 2024) to minimise temporal variability in community composition, although this also represents a limitation for broader generalisation. Mean sea temperatures ranged between 17 and 18 °C at the time of sampling. At each site, five replicate samples were collected from patches dominated by each target alga ($n = 45$). Haphazard sampling at ~2 m depth was used, with individual replicates maintained at a horizontal distance of >5 m from one another. Care was taken to avoid sampling from the same algal patch, ensuring independence among replicates. Sampling was carried out using a 20 × 20 cm quadrat (0.04 m²). All algae and associated fauna within the quadrat were detached from the substratum and transferred to 0.5 mm mesh bags, which were immediately sealed to retain mobile organisms.

Samples were transferred to the laboratory in seawater-filled containers, where they were preserved in 70% ethanol. Each sample was subsequently rinsed through a 0.5 mm sieve to remove fine debris. Under white light, mobile epifauna were manually separated from the macroalgae, sorted by phylum, and stored in 70% ethanol. Epifauna were identified to the lowest possible taxonomic level under a stereomicroscope and enumerated to construct a species-abundance matrix.

As recommended by Torres et al. [4], two aspects were considered to characterise the morphology of the macroalgal species. Biomass was used as an indication of the habitat quantity, whilst fractal dimensions (fractal area and fractal perimeter) were used as a proxy of structural complexity. To determine the biomass, the algal content of each sample was first air-dried (after extracting the epifauna), then oven-dried at ~75 °C for 24 h and finally weighed to an accuracy of 1 mg. For assessment of fractal dimensions, fresh fronds of each of the macroalgae were collected via snorkelling from Baħar iċ-Ċagħaq. In the case of *Dictyota* spp., *D. dichotoma* was used since it was typically the dominant species present.

Three fronds per species were extended in a film of water in a shallow white tray, separating the branches such that any interstitial spaces were clearly visible in a two-dimensional plane. The fronds were dabbed dry, carefully pressed flat, and photographed [4] at high resolution (12 MP) with an adjacent scale. Compression may introduce artefacts and reduce or homogenise fine-scale structural complexity, potentially influencing fractal dimension estimates. However, this approach is widely used in structural complexity studies [4,14–16] as it enables standardised, high-resolution, and repeatable measurements under controlled conditions, reducing variability associated with image acquisition. Photographs were taken of whole fronds (“low magnification”) and as close-up sections (“high magnification”); the two scales are both important in a biological context, with the latter being especially relevant to epifauna associated with macroalgae. Low magnification was used to capture differences in overall thallus architecture among algal species, while high magnification was used to resolve fine-scale structural features not

readily visible at the whole-thallus scale but likely relevant to smaller epifaunal organisms exploiting microhabitat complexity [15]. Images were processed in Fiji/ImageJ v1.54f [21] via scale calibration (0.02 mm px^{-1} for low, 0.009 mm px^{-1} for high magnification), conversion to an 8-bit greyscale and thresholding to binary. Manual thresholding was applied to produce binary images that best represented thallus structure while minimising artefacts such as background noise and edge distortion. Finally, the binary images were analysed using the 'Fractal Box Count' tool (grid sizes 2–64 px; [15]).

Fractal area (D_a) and fractal perimeter (D_p) were derived from the absolute slope of log–log plots of count versus box size [22]. Mean D_a and D_p were calculated for each species at both magnifications. For two-dimensional structures, like the frond silhouettes created for this procedure (Figure 2), the values of D_a and D_p typically fall between 1 and 2. A higher fractal area closer to 2 indicates a denser structure that occupies more space, whereas a lower value closer to 1 implies a sparser or more perforated structure [23]. Fractal perimeter describes the contour of the object, where a higher value implies a more irregular boundary and a higher degree of convolutions, whilst a lower value indicates a smoother boundary [15].

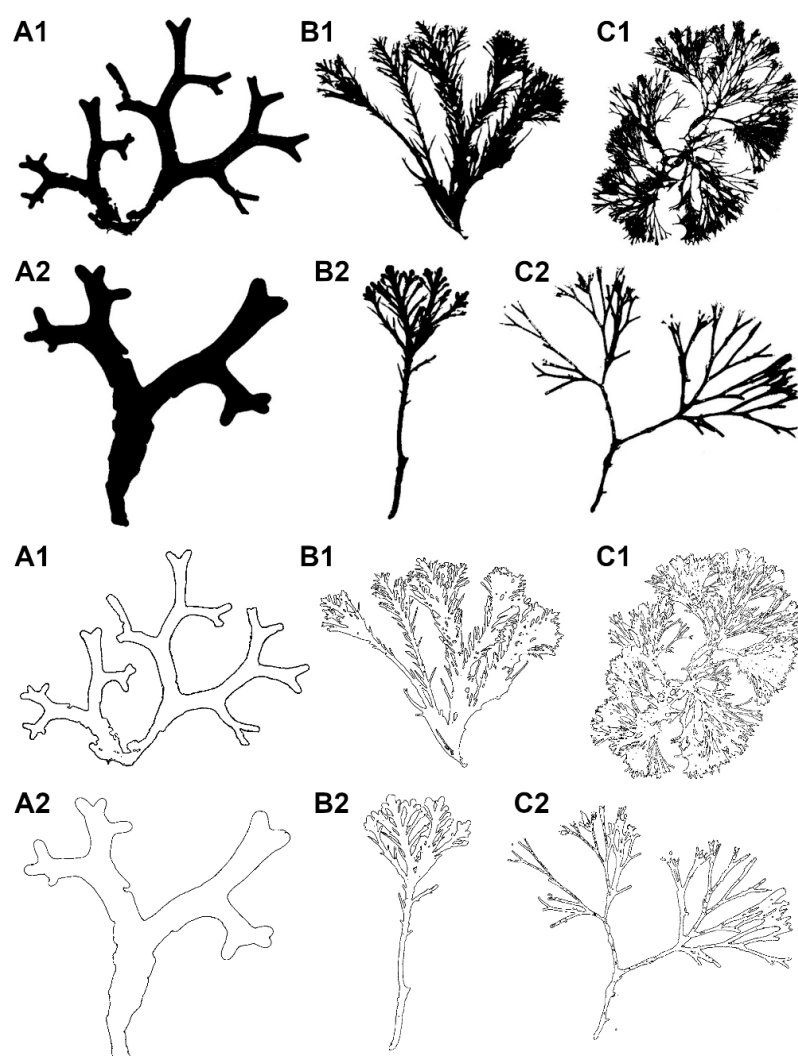


Figure 2. Thresholded images created for fractal area (**Top**) and outline images created for fractal perimeter (**Bottom**) of *Dictyota dichotoma* (A), *Ellisolandia elongata* (B), and *Jania rubens* (C) at 'low magnification' (1) and 'high magnification' (2). Images generated using Fiji by ImageJ.

2.3. Data Analysis

Statistical analyses were performed using PRIMER v7 with the PERMANOVA+ add-on [24,25]. Due to its extremely high abundance relative to other taxa, the invasive alien foraminifer *Amphistegina lobifera* was treated separately and excluded from most analyses to avoid its disproportionate influence on abundance-weighted measures of community structure. It was included in species richness calculations as a presence-based metric but excluded from diversity and evenness analyses due to its extreme numerical dominance, which would obscure variation in the remaining epifaunal assemblage. Differences in the abundance of this species were analysed separately.

The following community descriptors were calculated per sample: total abundance (total number of individuals, excluding *A. lobifera*), abundance of *A. lobifera* (total number of individuals), species richness (total number of taxa, including *A. lobifera*), Pielou's evenness (J' , excluding *A. lobifera*; [26]), and the Shannon diversity index (H' , excluding *A. lobifera*; [27,28]). To test for differences in these parameters in relation to the dominant algal species, a two-way factorial PERMANOVA based on Euclidean distance [29] was carried out for each descriptor, with 'Alga' as a fixed factor and 'Site' as a random factor. Significant differences ($p < 0.05$) were further investigated using pairwise permutational t -tests.

For community structure, species-abundance data (excluding *A. lobifera*) were square-root-transformed and analysed using Bray–Curtis similarity [30]. A two-way factorial PERMANOVA [29] was used to test the effects of algal species and site on epifaunal community structure. Significance was assessed using a maximum of 9999 possible permutations. Monte Carlo p -values were applied for pairwise comparisons due to the limited number of unique permutations (<50). Community structure was visualised through unconstrained non-metric multidimensional scaling (nMDS) ordination, while Canonical Analysis of Principal Coordinates (CAP) [31] was used as a constrained ordination to maximise group separation. Finally, SIMPER analysis identified taxa contributing most to within-group similarity and between-group dissimilarity [24].

3. Results

3.1. Macroalgal Morphology, Dry Biomass and Structural Complexity

The three target taxa exhibited distinct structural characteristics. The thallus of *Jania rubens* (Rhodophyta, Corallinaceae) was composed of several fine, cylindrical, articulated, partially calcified, and dichotomously branched fronds which together create a 'bushy' growth form. In contrast, *Ellisolandia elongata* (Rhodophyta, Corallinaceae) displayed pinnately branched, thicker, and more robust cylindrical fronds with a higher degree of calcification. As in *J. rubens*, the fronds of *E. elongata* are also somewhat flexible as they are articulated with non-calcified joints, but with larger, more rigid segments. *Dictyota* species (Ochrophyta, Dictyotaceae) were characterised by non-calcified, flattened, 'ribbon-like', and dichotomously branched fronds. The surface area varied within *Dictyota* species: *D. dichotoma* featured relatively wide branches with bilobed ends, whilst *D. fasciola* and *D. mediterranea* had narrower fronds tapering at the tips.

Mean dry biomass (per 0.04 m² quadrat) was consistently highest in *E. elongata*, followed by *J. rubens* and *Dictyota* spp. Spatially, Baħar iĉ-Ĉagħaq supported the highest overall biomass, where *E. elongata* (15.79 g $\pm$ 7.14 SD) recorded the highest mean value. Conversely, the lowest biomass values for all macroalgae were recorded at Exiles Beach (Figure 3).

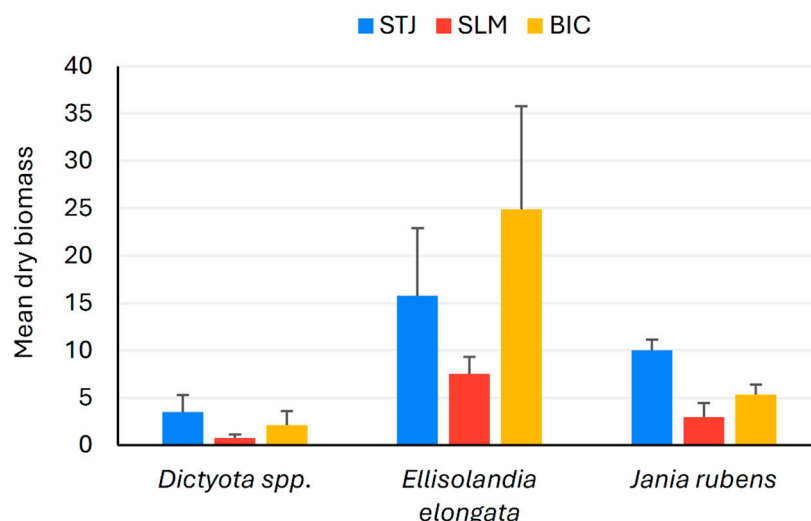


Figure 3. Mean dry biomass (g) per 0.04 m² quadrat of *Dictyota* spp., *Ellisolandia elongata* and *Jania rubens* at St. Julian's (STJ), Sliema (SLM), and Baħar iċ-Ċagħaq (BIC). Error bars represent one standard deviation above the mean.

At the whole-thallus scale (low magnification), fractal area (D_a) showed minimal variability between the three macroalgal species (Table 1). *Ellisolandia elongata* exhibited the lowest mean D_a , followed by *J. rubens* and *D. dichotoma*. At the frond-part scale (high magnification), mean D_a values showed slightly greater variation. From low to high magnification, *D. dichotoma* and *E. elongata* had marginal increases in mean D_a (+0.014 and +0.027, respectively), whereas *J. rubens* showed a notable decrease (−0.189). Consequently, at high magnification, mean D_a was highest for *D. dichotoma* and lowest for *J. rubens*. Mean fractal perimeter (D_p) varied more markedly across species and scales. At both magnifications, mean D_p was highest in *J. rubens*, intermediate in *E. elongata*, and lowest in *D. dichotoma*. When moving from low to high magnification, mean D_p increased slightly in *D. dichotoma* (+0.020) but decreased in *E. elongata* (−0.066) and *J. rubens* (−0.174). Taken together, these values imply that *J. rubens* was characterised by a more complex thallus morphology, while *Dictyota* spp. had the lowest structural complexity.

Table 1. Mean fractal area (D_a) and fractal perimeter (D_p) of macroalgae ($\pm$ standard deviation) based on 3 replicate fronds at 'low magnification' and 'high magnification'.

		<i>Dictyota dichotoma</i>	<i>Ellisolandia elongata</i>	<i>Jania rubens</i>
Mean $D_a \pm$ SD	'Low magnification'	1.848 $\pm$ 0.015	1.793 $\pm$ 0.038	1.805 $\pm$ 0.024
	'High magnification'	1.862 $\pm$ 0.017	1.820 $\pm$ 0.016	1.616 $\pm$ 0.023
Mean $D_p \pm$ SD	'Low magnification'	1.109 $\pm$ 0.080	1.363 $\pm$ 0.017	1.480 $\pm$ 0.049
	'High magnification'	1.129 $\pm$ 0.013	1.297 $\pm$ 0.013	1.306 $\pm$ 0.064

3.2. Associated Epifauna

A total of 56,402 epifaunal individuals belonging to 152 taxa from five different phyla were identified and enumerated (full list of taxa provided in the Supplementary Materials). Of these, 44 taxa were Arthropoda, 39 Mollusca, 34 Foraminifera, another 34 Annelida, and one Echinodermata. The foraminiferan *Amphistegina lobifera* heavily dominated the assemblages, accounting for 52,034 individuals. When *A. lobifera* was excluded, the most abundant phyla were Foraminifera (1788 individuals), followed by Arthropoda (1081 individuals) and Mollusca (982 individuals).

Epifaunal taxa from all five phyla were recorded from all three macroalgal species (Figure 4). Arthropoda consistently exhibited the highest species richness, while Echin-

odermata was the least diverse. Besides Arthropoda, *E. elongata* also supported a high number of Molluscan taxa, whereas *J. rubens* hosted similar numbers of taxa for Arthropoda, Foraminifera, Mollusca and Annelida. *Dictyota* spp. had the lowest overall taxonomic richness for most phyla.

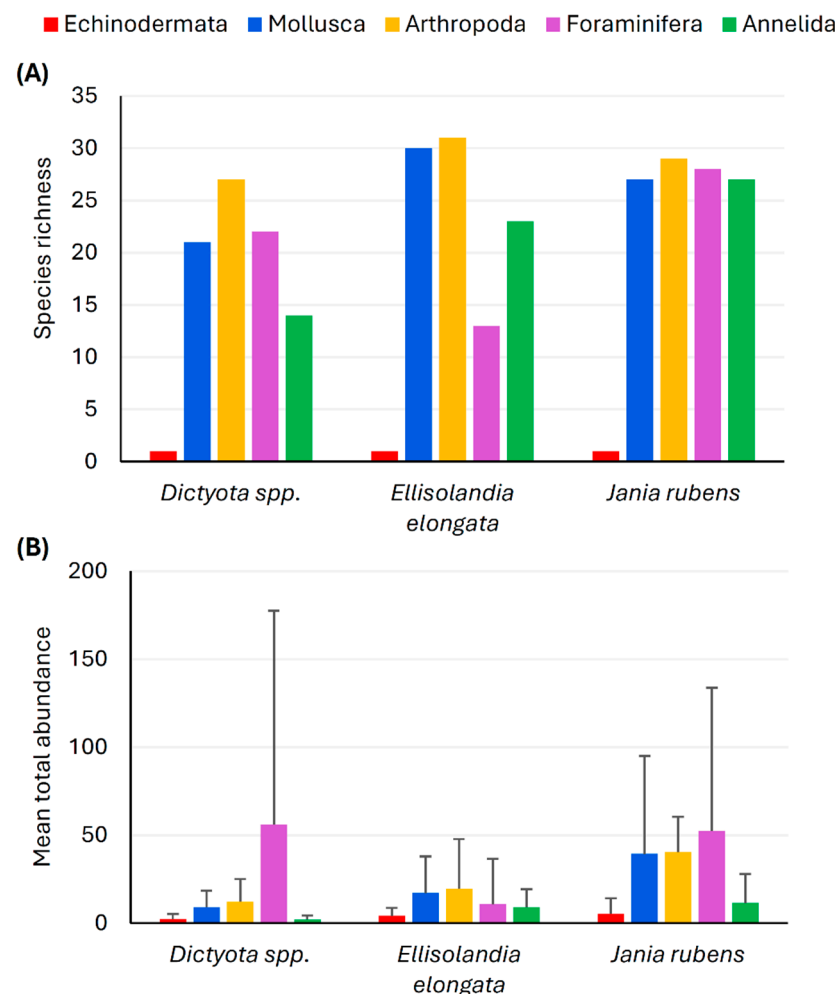


Figure 4. Total species richness (no. of different taxa) (A) and mean + SD total abundance (no. of individuals per 0.04 m² quadrat, excluding *Amphistegina lobifera*) (B) by phylum recorded from *Dictyota* spp., *Ellisolandia elongata* and *Jania rubens* across all samples from the three different sampling sites.

In terms of abundance (excluding *A. lobifera*), foraminiferans dominated on *Dictyota* spp., accounting for 68.6% of all individuals. Foraminifera was also the most abundant phylum on *J. rubens*, but Arthropoda and Mollusca had a relatively similar abundance, such that *J. rubens* had the highest overall mean total abundance. In contrast, *E. elongata* exhibited a more even distribution of individuals among phyla.

3.3. Community Descriptors

Mean species richness (Figure 5) was highest for *J. rubens* at St. Julian's, whereas values recorded from Baħar iċ-Ċagħaq and Sliema did not differ appreciably between the three macroalgae, with Sliema having the lowest richness values. Similarly, all assemblages showed the lowest mean total abundance at Sliema, while the overall highest value was recorded from *J. rubens* at St. Julian's. In the case of *E. elongata* and *Dictyota* spp., Baħar iċ-Ċagħaq supported the highest mean epifaunal abundances; the values recorded from *Dictyota* spp. at this site exceeded that recorded from *J. rubens*. All three macroalgal species

exhibited relatively high and similar mean Pielou's Evenness overall, although values associated with *Jania rubens* at Baħar iċ-Ċagħaq were slightly lower than those of *E. elongata* and *Dictyota* spp. at the same site. The Sliema site consistently showed the highest evenness values for all algae species, while St. Julian's generally showed the lowest values. Lastly, mean Shannon Diversity (H') was generally highest at St. Julian's and Baħar iċ-Ċagħaq, while Sliema generally showed lower values. *Jania rubens* at St. Julian's had the highest overall diversity, followed by *Dictyota* spp. and *Ellisolandia elongata* at Baħar iċ-Ċagħaq, whereas *Dictyota* spp. at Sliema had the lowest value. Therefore, there was no consistent trend when comparing the three algae, with different patterns at the different sites.

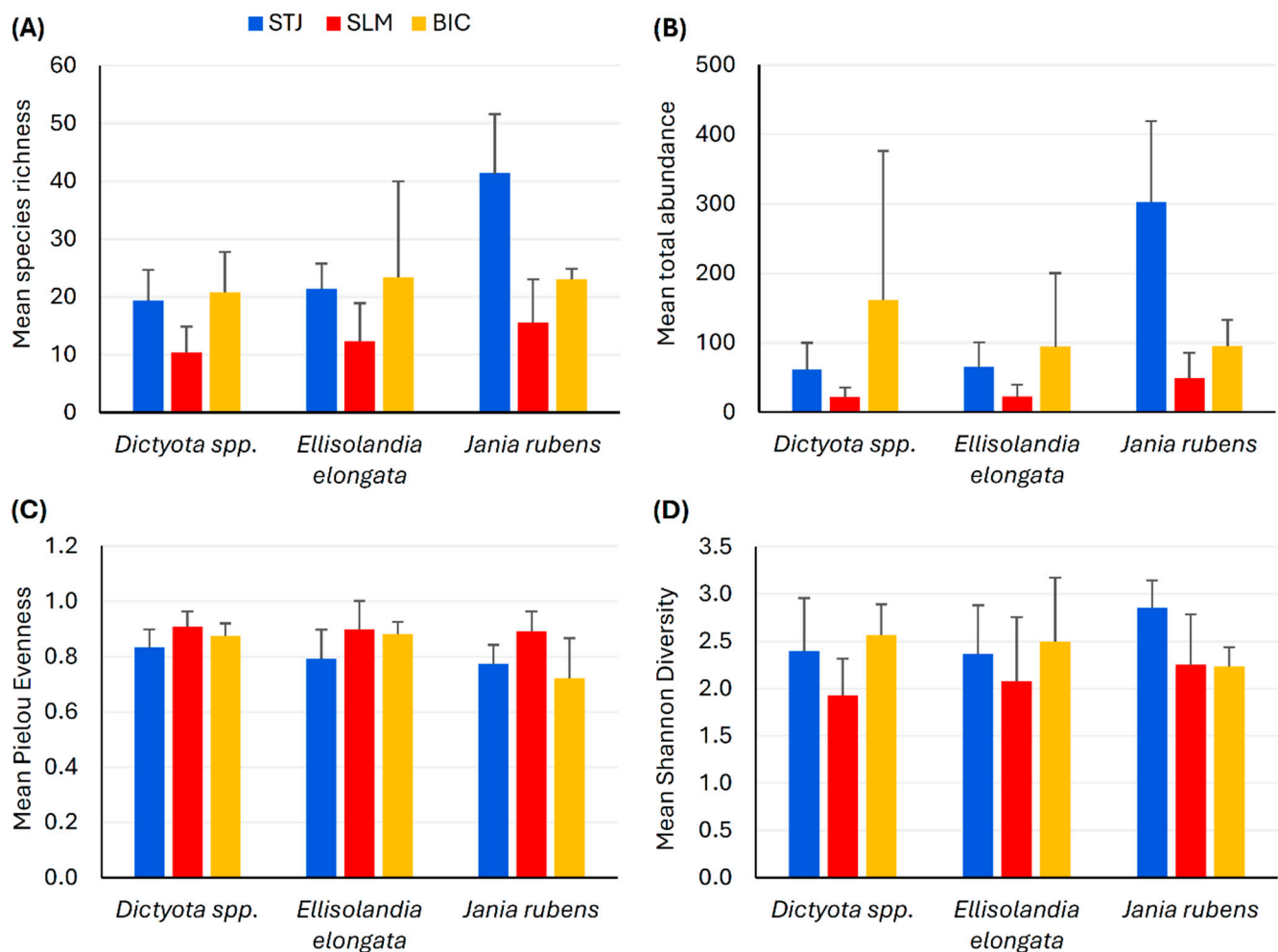


Figure 5. Community descriptors of epifaunal assemblages associated with each of *Dictyota* spp., *Ellisolandia elongata* and *Jania rubens* at St. Julian's (STJ), Sliema (SLM), and Baħar iċ-Ċagħaq (BIC), recorded from five replicate quadrat samples per algal species sampled at each site. (A) Mean species richness (no. of taxa); (B) Mean total abundance; (C) Mean Pielou's Evenness (J'); (D) Mean Shannon Diversity (H'). All values are expressed per 0.04 m² quadrat. Calculations of abundance, evenness and diversity excluded the extremely highly abundant invasive foraminiferan *Amphistegina lobifera*.

The two-way PERMANOVA based on Euclidean distance revealed a significant Alga $\times$ Site interaction for total abundance and species richness, with the strongest effect observed for the former, indicating that the influence of macroalgal identity varied across sites (Table 2). In contrast, sampling site had a significant effect on all four community metrics, whereas macroalgal species alone showed no significant effect. The significant interaction term was reflected in site-specific patterns of abundance and richness. For instance, *Dictyota* spp. supported the highest abundance at Baħar iċ-Ċagħaq, whereas in

the case of St. Julian's the highest abundance was recorded from *J. rubens*. Likewise, *J. rubens* had a noticeably higher richness than the other two algae at St. Julian's, but not at the other two sites.

Table 2. Two-way PERMANOVA test of differences in community descriptors (as single response variables) based on Euclidean distance. Significant *p*-values (<0.05) are indicated in bold.

Community Descriptor	Alga (Fixed Factor)		Site (Random Factor)		Alga × Site (Interaction)	
	Pseudo-F	p(Perm)	Pseudo-F	p(Perm)	Pseudo-F	p(Perm)
Total abundance *	0.882	0.492	6.005	0.003	4.221	0.004
Abundance of <i>Amphistegina lobifera</i>	4.110	0.096	5.919	0.005	2.168	0.078
Species richness	2.046	0.241	12.433	<0.001	2.918	0.032
Pielou's evenness (J') *	2.322	0.193	5.707	0.006	1.491	0.227
Shannon Diversity Index (H') *	0.364	0.712	4.054	0.026	1.340	0.271

* Parameter calculated based on dataset excluding *Amphistegina lobifera*.

3.4. Community Structure

The unconstrained nMDS ordination showed replicates clustering by macroalgal species, but also a high degree of overlap between *J. rubens* and *E. elongata* (Figure 6). This suggests similar community structure among replicates within species groups, as well as a higher similarity in the assemblages associated with *J. rubens* and *E. elongata* compared to those found on *Dictyota* spp. However, the relatively high stress value (0.25) indicates that the ordination should be interpreted cautiously [24]. The constrained CAP ordination provided clearer separation between the three algal species, where *Dictyota* spp. formed a distinct cluster, while *J. rubens* and *E. elongata* showed only very minor overlap.

Two-way PERMANOVA supported these findings, confirming that both independent variables 'Alga' and 'Site' significantly influenced epifaunal community structure, with the random factor 'Site' exerting the strongest influence (higher pseudo-F, lower *p*-value) (Table 3). A significant interaction term was also obtained, indicating that the differences between the three algae were not consistent across all sites. Despite the overlap seen between *J. rubens* and *E. elongata* in the NMDS ordination, PERMANOVA pairwise comparisons indicated significant differences in epifaunal community structure between all three species.

The SIMPER analysis identified taxa contributing most to intra-group similarity and inter-group variation (see the Supplementary Materials for detailed results). Within-group similarities were highest for *J. rubens* (28.25%), dominated by *Hyale* sp., *Ampithoe* cf. *ramondi*, and *Caprella hirsuta*. *Dictyota* spp. (20.06%) were characterised mainly by *Peneroplis* spp., *Amphipholis squamata*, and *Dynamene bidentata*, while *E. elongata* (19.40%) was dominated by *A. squamata*, *Caprella grandimana*, and a syllid polychaete. The greatest average dissimilarity (85.92) occurred between *Dictyota* spp. and *E. elongata*, primarily driven by *Peneroplis* spp., *Barleeia unifasciata*, and *A. squamata*. Dissimilarities between *J. rubens* and *Dictyota* spp. (82.78) were attributed mainly to *C. hirsuta*, *Rosalina* spp., and *Hyale* sp., while *J. rubens* and *E. elongata* (81.82) were differentiated chiefly by *Rosalina* spp., *B. unifasciata*, and *C. hirsuta*.

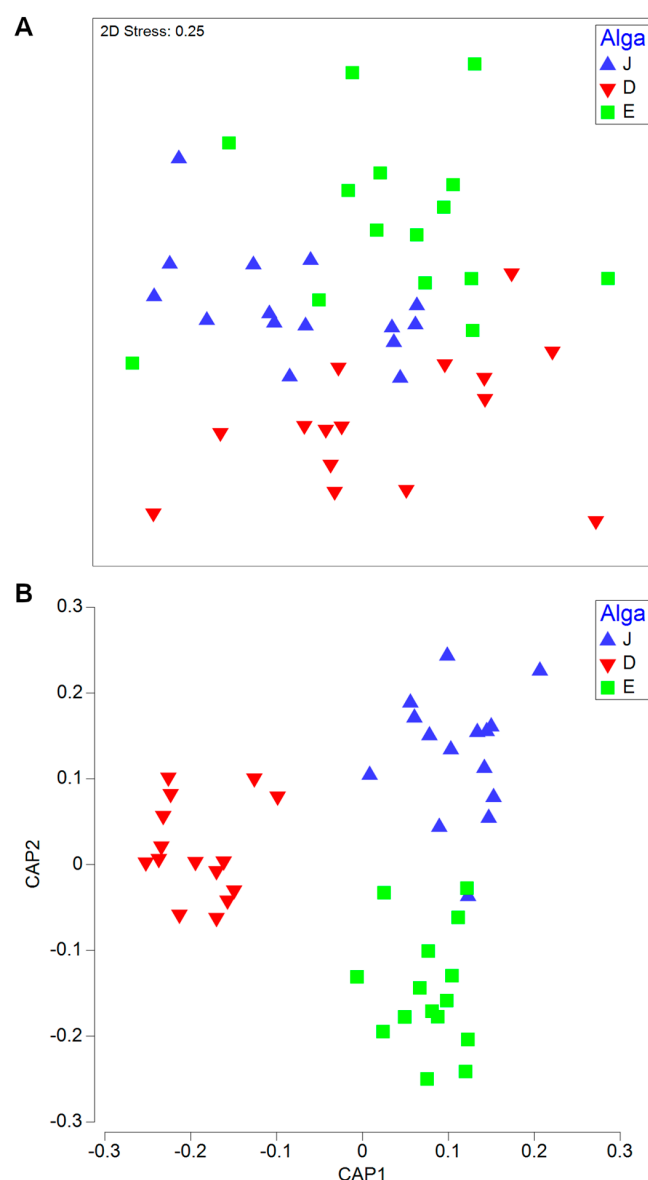


Figure 6. Non-metric multidimensional scaling (nMDS) ordination (A) and Canonical analysis of principal coordinates (CAP) ordination (B). Both analyses carried out using Bray–Curtis similarity based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. J = *Jania rubens*, D = *Dictyota* spp., and E = *Ellisolandia elongata*.

Table 3. Two-way PERMANOVA and post hoc tests of differences in community structure based on Bray–Curtis similarity, calculated using the species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Monte Carlo *p*-values [p(MC)] are shown for pairwise tests due to the low number of unique permutations (see the Section 2). Significant *p*-values (<0.05) are indicated in bold.

Main Test	Pseudo-F	p(Perm)
Alga (Fixed factor)	2.3675	0.0133
Site (Random factor)	5.1355	0.0001
Alga × Site (Interaction)	1.7220	0.0001
Pairwise tests		p(MC)
<i>Jania rubens</i> vs. <i>Dictyota</i> spp.		0.0431
<i>Jania rubens</i> vs. <i>Ellisolandia elongata</i>		0.0303
<i>Dictyota</i> spp. vs. <i>Ellisolandia elongata</i>		0.0225

4. Discussion

This study is correlational in design; therefore, interpretations focus on associations rather than causal relationships. Differences in the structure of epifaunal assemblages were linked to variation in macroalgal morphology, particularly biomass, calcification, and fractal complexity, modulated by site-specific factors such as wave exposure. While there were differences between the assemblages associated with all three algal species, the greatest variation was observed between the articulated, calcified rhodophytes *E. elongata* and *J. rubens* and the non-calcified brown algae (Phaeophyceae) *Dictyota* spp., reflecting the influence of habitat complexity on faunal assemblages [32–34]. Multivariate analyses showed the strongest dissimilarity between *E. elongata* and *Dictyota* spp. (85.9%) and the weakest between *J. rubens* and *E. elongata* (81.8%), with key discriminating taxa, such as the amphipods *Caprella hirsuta* and *Hyale* sp. and the foraminiferan *Rosalina* spp., being more abundant on the structurally complex corallines [32,33].

Ellisolandia elongata exhibited the highest mean dry biomass across sites, attributed to its coarse, highly calcified and branched thallus, which provides high resistance to dislodgement in high-energy environments [12]. This morphology creates a dense three-dimensional structure with numerous crevices and refuges, supporting diverse molluscan and arthropod assemblages [35]. Such structural complexity, as evidenced by the comparatively high fractal area and fractal perimeter values recorded in the present work (Table 1), likely provides protection from predators and hydrodynamic stress, maintaining relatively high community evenness and diversity across sites, even at the most exposed locations [6,13,36].

Jania rubens showed slightly lower biomass than *E. elongata* but the highest fractal perimeter, with its fine cylindrical fronds creating numerous interstitial spaces. This alga hosted assemblages of amphipods, molluscs and foraminiferans indicating that its intermediate calcification and intricate branching promote habitat heterogeneity, facilitating coexistence of diverse feeding guilds such as detritivores, herbivores, and filter-feeders [32,37,38]. However, this effect appears to be dependent on sheltered conditions. Indeed, *J. rubens* had the highest mean species richness, abundance, and Shannon Diversity values of all three algae at the most sheltered sites (St. Julian's, EI = 2.13; and to a lesser extent at Sliema, EI = 3.59). In contrast, it had lower mean abundance and Shannon Diversity values at Baħar iċ-Ċagħaq (EI = 8.01), which was likely due to the effects of a four-fold increase in wave exposure relative to the most sheltered site (St. Julian's). This pattern may reflect the susceptibility of *J. rubens* to dislodgement under strong wave action due to its narrow, brittle branches and small holdfast compared to *E. elongata*. Even within generally sheltered conditions, this four-fold variation in exposure may still represent an ecologically meaningful gradient, particularly for small mobile epifauna that are sensitive to fine-scale differences in hydrodynamic stress and dislodgement risk. On the other hand, the higher richness and abundance values on *J. rubens* relative to its biomass in sheltered areas emphasise the role of fine-scale structural complexity over sheer biomass in supporting biodiversity.

In contrast, the *Dictyota* spp., with their non-calcified, foliose thalli and lowest biomass, supported communities dominated by crawling taxa, particularly foraminiferans such as *Peneroplis* spp. The low fractal perimeter values indicate simpler, flatter morphologies offering fewer microhabitats [23]. Such structure does not facilitate colonisation by clinging or sessile forms, and potentially limits vertical stratification of fauna, leading to lower Shannon diversity [33,39], as observed in certain sites like Sliema.

The invasive foraminifer *Amphistegina lobifera*, which was disproportionately abundant across several samples, has been reported to increase in abundance in the Mediterranean in association with rising sea surface temperatures, reflecting its ongoing range expansion

and tolerance of warmer, relatively variable environmental conditions [40]. Although its distribution and environmental drivers were not explicitly investigated in the present study, no significant differences in its abundance between the three different algae were recorded. Previous studies have reported its occurrence on macroalgal hosts with contrasting morphologies, including *Caulerpa cylindracea*, *Halopteris scoparia* and *Padina pavonica* [41,42].

Taken together, the present results show that both macroalgal morphology and site characteristics significantly affected community structure. Site-specific conditions such as wave exposure were associated with broad assemblage patterns, while the fine-scale morphological attributes of each alga, particularly branching complexity, calcification, and surface heterogeneity, were critical in determining local epifaunal diversity and composition. Overall, algae with more complex structures such as articulated corallines act as microhabitat engineers, sustaining more varied communities, whereas simpler algal morphologies support assemblages dominated by surface-associated taxa. Nonetheless, the effects of algal morphology are modulated by the hydrodynamic regime—in sites with a higher wave exposure, more delicate algae such as *J. rubens* may not support assemblages with an appreciably higher diversity despite their more complex morphology.

While this study successfully utilised fractal dimensions to quantify habitat complexity, other variables remain unexplored. Physical and chemical attributes, such as thallus thickness, branch rugosity, or the presence of surface mucus and exudates, may also influence settlement, attachment, and grazing behaviour [14]. Two macroalgae with similar fractal dimensions may therefore provide different microhabitat qualities for epifauna, highlighting the need for complementary morphological and biochemical analyses in future research. Furthermore, the winter sampling period did not capture temporal shifts in macroalgal cover and biomass or in epifaunal abundances [43,44]. Nonetheless, this research contributes valuable baseline data on mobile epifaunal assemblages associated with native Mediterranean macroalgae and demonstrates the applicability of fractal measures for quantifying habitat complexity. The findings support existing evidence linking algal morphology to faunal diversity [32,33] and enhance understanding of how structural traits shape epifaunal communities within Mediterranean shallow subtidal systems.

Future studies should aim to investigate seasonal variation in both macroalgal structure and associated epifaunal assemblages to provide a more comprehensive understanding of temporal dynamics, include additional macroalgal morphologies such as filamentous, lobed, or crustose forms, and incorporate other quantitative descriptors, such as thallus length, volume, and surface rugosity, alongside biomass and fractal dimensions, to better represent three-dimensional habitat complexity [15,16].

5. Conclusions

This study demonstrated that the structure and composition of mobile epifaunal assemblages were associated with the interaction between macroalgal morphology and site-specific environmental conditions. Variations in biomass, fractal complexity, and wave exposure collectively influenced epifaunal diversity and relative abundance. Arthropods were the most species-rich and abundant phylum across all macroalgae, followed by molluscs, reflecting these groups' versatility and dependence on algal habitats. *Ellisolandia elongata* and *Jania rubens*, with their articulated, calcified, and densely branched thalli, supported communities that differed significantly from those associated with the more foliose *Dictyota* spp., with *J. rubens* having the highest abundance and diversity values in sheltered sites, emphasising the importance of structural complexity in providing varied microhabitats. Overall, the findings highlight the combined role of macroalgal architecture and environmental factors in shaping mobile epifaunal communities. This study enhances understanding of macroalgae–epifauna relationships in the central Mediterranean and pro-

vides a foundation for future research on seasonal dynamics, additional algal morphologies, and refined measures of habitat complexity.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/oceans7040063/s1>, Table S1: Full list of epifaunal taxa identified from all of the macroalgal samples; Table S2: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Jania rubens* (J) vs. *Dictyota* spp. (D) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average dissimilarity and contribution percentage; Table S3: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Jania rubens* (J) vs. *Ellisolandia elongata* (E) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average dissimilarity and contribution percentage; Table S4: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Dictyota* spp. (D) vs. *Ellisolandia elongata* (E) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average dissimilarity and contribution percentage; Table S5: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Jania rubens* (J) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average similarity and contribution percentage; Table S6: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Dictyota* spp. (D) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average similarity and contribution percentage; Table S7: Similarity percentages routine (SIMPER) with Bray–Curtis similarity for *Ellisolandia elongata* (E) based on species-abundance matrix (excluding *Amphistegina lobifera*) with a square root transformation. Taxa are listed in descending order according to the average similarity and contribution percentage.

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