RESEARCH ARTICLE

Biodiversity of coastal polyclad flatworm assemblages in the wider Caribbean

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Abstract Polyclads are mobile predators and possibly an important functional component of hard substrate marine environments globally. To understand the natural patterns of spatial differences, polyclad assemblages were sampled in seven coastal regions across the wider Caribbean spanning 15° latitude and 24° longitude between May and June 2005 and May and September 2006. In total, 67 species in 28 genera and 17 families were recorded from 62 sites. Only two species were found in all surveyed regions, *Melloplana ferruginea* and *Pseudoceros bicolor*. Conversely, 41 species were restricted to one or two sites, and 34 species were represented by one or two individuals. The distribution and abundance of species varied between the two suborders. Cotyleans were most species rich and had a higher number of species of restricted range, while Acotylea showed a higher proportion of rare species; however, two species were highly abundant comprising over half of the individuals counted. In most habitats, polyclads were rare, but in some intertidal habitats two species, *Styloplanocera fasciata* and *Boninia divae* were densely aggregated and dominant members of the benthic epifauna. Alpha diversity was variable but showed no evidence of a relationship with latitude, longitude or depth. Beta diversity increased with the number of habitats sampled and was highest for cotyleans. Highest gamma diversity was recorded in Jamaica and the US Virgin Islands and was not significantly correlated to alpha diversity. Overall assemblages from the seven regions were similar, revealing faunal homogeneity across the wider region. Reef assemblages were distinct from other habitats dominated by species of the Cotylea. Reefs from Panama and the US Virgin Islands were the most species rich.

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Introduction

Little is known about how marine benthic diversity is spatially distributed, especially among lesser-studied invertebrate taxa. Spatial patterns of assemblages can be due to the spatial arrangement of environmental conditions such as depth, temperature and substrate, as well as dispersal-limiting factors (Mora et al. 2003). Marine systems are considered more "open" with barriers to dispersal relatively weak, and unlike most terrestrial systems, species can disperse over much broader ranges. In geographically compact regions with no obvious oceanic barriers, such as the Caribbean Sea, there is potential for pelagic larvae to disperse long distances on currents. The Caribbean region is a natural biogeographic province, harboring a diverse array of species (Briggs 1974; Robertson 1998) and encompassing broad faunal homogeneity (Avise 2000). Despite the ubiquity of many species across the Caribbean region, the presence of regional endemism (e.g. Williams and Mounts 2003; Morrison et al. 2004) and restricted distributions of some species (Domeier 1994) suggest that biogeographic breaks may be present.

The distribution of polyclads in tropical waters of the western Atlantic indicates that there is a faunistic area extending from the Gulf of Mexico to southern Brazil including the Antilles and the Bermudas, constituting a West Indian province in polyclad diversity (Prudhoe 1985). I investigated spatial patterns of diversity within this province, including sites in the Florida Keys, Panama, Honduras, Belize, Jamaica, the US Virgin Islands and Curaçao, an area extending across more than 24° longitude and about 15° latitude. The Polycladida are a diverse order of free-living Platyhelminthes, globally distributed in marine environments from the intertidal (Newman and Cannon 2003) to the deep sea (Quiroga et al. 2006). They are important mobile predators on hard substrate environments and many species appear to have close associations with sessile invertebrates on which they feed (Newman et al. 2000). These hard substrate communities are common in coastal environments and are often subject to disturbances both natural and anthropogenic. As such, understanding polyclad diversity may act as a useful indicator of overall diversity and habitat health.

In total, over 1,000 species of polyclad have been described, more than half of which were found between latitudes 30°N and 30°S and about two-thirds of these in the Indo-Pacific region (Prudhoe 1985). Like land planarians (Sluys 1998), polyclads are sensitive to the temperature of their environment and it appears that many species are restricted in their latitudinal range. The Polycladida are divided into two suborders, Acotylea and Cotylea, based on the absence or presence of a sucker (Lang 1884), and the Cotylea are more species rich in lower latitudes than the Acotylea (Prudhoe 1985).

Due to their size, cryptic behavior and delicate nature polyclads are difficult to collect and identify, consequently very little is known about their interactions with either their biotic or abiotic environments. The published literature consists predominantly of alpha taxonomic descriptions and species lists from Colombia (Quiroga et al. 2004a), the tropical Western Atlantic (Marcus and Marcus 1968; Quiroga et al. 2004b), the Indo-Pacific (Newman and Cannon 1994, 1996; Newman et al. 2003) and east Africa (Prudhoe 1989). A few studies have addressed their biology, such as life histories (e.g. Ballarin and Galleni 1984a, b, 1987; Newman et al. 2000), toxicology (e.g. Ritson-Williams et al. 2006), and phylogenetics (Litvaitis and Newman 2001; Rawlinson and Litvaitis (2007). To date none have quantitatively addressed polyclad distribution and diversity over different spatial scales making it difficult to interpret their ecological interactions. Studies on the distribution of other turbellarian groups have shown limited numbers of species in any given locality (α-diversity) due to narrow ecological niches. However, diversity across habitats has been high with distinct faunas (Boaden 1995).

In this study, I present the first quantitative baseline estimation of polyclad species diversity, distribution of species as well as assemblage differences. Coastal polyclad assemblages in the wider Caribbean were investigated using univariate and multivariate techniques. The main objectives of this study were to (1) determine the patterns of alpha, beta and gamma species richness and diversity, and (2) examine if suites of species form distinct clusters according to habitat or region using multivariate analyses.

Materials and methods

Study sites

The study was carried out over two consecutive summers (May–June 2005, and May–September 2006) spanning an area of 15° latitude and 24° longitude at the following locations (from west to east): Belize (BEL) (South Water Caye 16°N, 88°W), Honduras (HON) (Cayos Cochinos 15°N, 86°W), Panama (PAN) (Bocas del Toro, 9°N, 82°W), Florida Keys (FLO) (Long Key, 24°N, 80°W), Jamaica (JAM) (St. Ann's Bay, 18°N, 77°W), Curaçao (CUR) (12°N, 68°W), and the United States Virgin Islands (UVI) (St. John, 18°N, 64°W) (Fig. 1). The Caribbean Sea has a microtidal range, generally less than 50 cm and the tide is predominantly diurnal (Kjerfve 1981). Polyclads were collected from shallow subtidal habitats ranging in depth from intertidal to a maximum of 15 m. Worms were collected from all major coastal marine habitats accessible by shore work, snorkelling and SCUBA. This included (from shallowest to deepest) rocky shore (rs), rock wall (rw), limestone pavement (ls), coral rubble intertidal (cri), fouling communities, (e.g. mangrove roots, pier pilings) (fc), seagrass beds (sg), coral rubble subtidal (crs) and coral reef (r). Most regions were only represented by a subset of the habitats (Appendix 1).

Collecting methods

Polyclads are generally negatively phototactic and are therefore found under rocks and coral rubble during daylight hours. Collectors worked in pairs and due to the patchy distribution of polyclads and to standardize the sampling effort across different habitats, each sample represented 1 h of active, continuous collecting (i.e., including time required to transfer the catch to a vial, but excluding time due to interruptions). Sixty-two sites over the seven regions were sampled; at 38 of these sites two or more replicated samples were taken (Appendix 2). However, after an hour of collecting at 24 sites the entire area of the habitat had been covered and therefore only one sample was taken. Additional information on each site (geographic coordinates and habitat type) is recorded in Appendix 2.
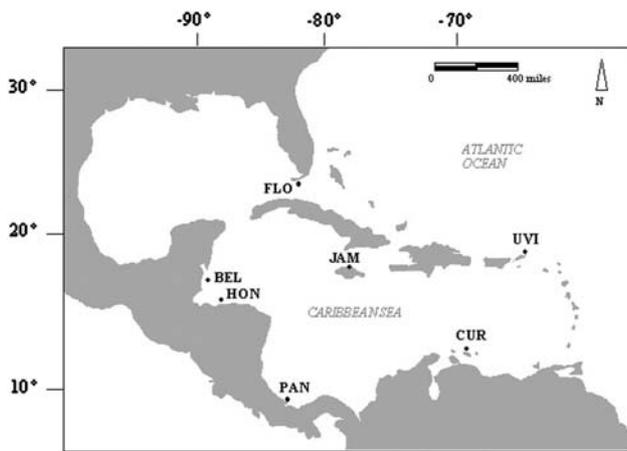
Fig. 1 The geographic distribution of the sampling regions in the wider Caribbean. Belize (*BEL*), Honduras (*HON*), Panama (*PAN*), Florida (*FLO*), Jamaica (*JAM*), Curacao (*CUR*), US Virgin Islands (*UVI*)

All polyclads encountered were collected, except for highly abundant species, where the first five individuals per sample were collected and the remainder counted.

Each polyclad was lifted off the substrate with a paintbrush and placed into a small pot with seawater. Once back in the laboratory specimens were numbered, measured, photographed live and a small tissue sample from the tail or flank was placed in 95% undenatured ethanol for DNA analysis. The rest of the specimen was preserved according to Newman and Cannon (1995) for histology or whole mounts. Individuals were identified to the lowest possible taxonomic level (typically species) by analysis of morphology (using species descriptions and the classifications of Faubel (1983, 1984) and Prudhoe (1985) and DNA. Voucher specimens of each species identified in this study will be deposited at the Smithsonian Institution, Washington DC (USNM).

Statistical analysis

As univariate measures of diversity, species richness (*S*) and the exponentiated form of the Shannon–Wiener index (*ExpH'*) (log base 2) were used. *S* is the number of species regardless of abundance, whereas *ExpH'* is most affected by species in the middle of the species rank sequence. The mean of these parameters (and SD) were plotted versus longitude, latitude and depth/habitat, and relationships and significance among them was evaluated using pair wise Spearman rank correlations (r_s).

In this study, β diversity was measured as $\beta_w = (\gamma/\alpha) - 1$. Here γ is the total number of species in each region (regional richness), α is the average number of species per site (local richness). This measures the proportion by which the whole area is richer than the average sites within it. Its formulation does not assume a gradient structure and is the measure of choice when samples cannot be arranged along a single gradient (Wilson and Shmida 1984), as was the case in the present study. β_w was also used to examine pairwise differences between sites. In pairwise β_w (as opposed to overall) γ is the number of species in the two sites combined. β diversity is the extent of change in species composition of communities among the samples of a dataset (Whittaker 1975). As a second measure of β diversity, the Bray–Curtis similarity between all pair wise permutations of sites was used by constructing a similarity matrix using square root transformation and the Bray–Curtis coefficient (Bray and Curtis 1957). The above measurements are included in the EstimateS software (Colwell 2005).

Sample sequences were randomized (without replacement) for the calculation of species accumulation curves for each region. Total species richness was assessed (using EstimateS, Colwell 2005) over all samples from the seven regions to examine how comprehensive the sampling of the wider Caribbean sites was. A sample-based rarefaction estimation of cumulative species richness, a *Chao2* (see Colwell and Coddington 1994) nonparametric estimation of cumulative species richness and the cumulative number of uniques and duplicates is presented. The *Chao2* estimate gave the least biased estimate of true species richness for small numbers of samples in a study of eight species richness estimators (Colwell and Coddington 1994) and has been previously used to assess marine benthic diversity (Ellingsen 2001). Uniques are species restricted to a single site, duplicates are species occurring exactly at two sites only, singletons are species represented by a single individual, and doubletons are species represented by only two individuals.

The composition and structure of the polyclad assemblages were assessed with multivariate analyses using the PRIMER software package (Clarke and Gorley 2001); following square root transformation of data, Bray–Curtis similarity measures were calculated (Bray and Curtis 1957). Sites were then classified into groups by hierarchical agglomerative clustering using group-average linking. The inter-relationships between sites were mapped using the ordination technique, nonmetric multidimensional scaling. (nMDS). The discrimination of assemblages based on region and habitat type was tested with one-way ANOSIM (Clarke 1993). The SIMPER routine was used to identify species that are representative of each habitat.

Results

Species richness and heterogeneity diversity

A total of 1,119 polyclad specimens belonging to 67 species, approximately 28 genera and 17 families were identified.

Acotylea was represented by 26 species and Cotylea by 41. Ninety-one percent of specimens could be assigned to known genera, and 87% could be identified as a previously named species, with just over 8% of individuals collected being undescribed taxonomically or unidentifiable. No polyclads were found at four sites: Florida: Tom's Harbour keys (sg), Old Sweat Bank (sg); Jamaica: St Anne's Bay mangrove (fc); Honduras: Cayos Menor Right Reef (r).

Alpha diversity (site species richness, SR_s and $ExpH'$) in 62 sites throughout the study area was highly variable (Fig. 2, Appendix 3). Mean site species richness ranged from 0 to 8 species (Fig. 2); there was no clear relation to latitude ($r_s = 0.004$, $P > 0.05$, $n = 62$) and a weakly positive relation to longitude ($r_s = 0.12$, $P = 0.04$, $n = 62$), with sites in the east (UVI) being more species rich. The Shannon–Weiner index also showed a weakly positive relation to longitude (Appendix 3) ($r_s = 0.05$, $P = 0.04$, $n = 62$), but not to latitude. Visualization of the data (Fig. 2; Appendix 3) showed the pattern to be dependent on the rich level of sites at USVI. On reanalysis excluding data from USVI there
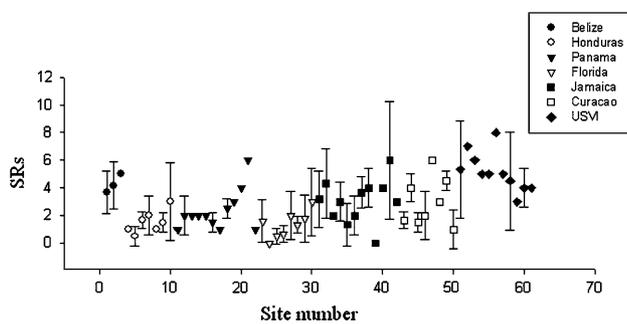
Fig. 2 Mean alpha diversity measures of Caribbean polyclads (SR_s site species richness) at sites ordered in sequence from west (88°W) to east (64°W). *Bars* indicate ±1 SD

was no correlation between species richness (and diversity) and longitude.

Although depth and habitat type varied between these sites, the relationship between site species richness (and diversity) and habitat type/depth was not significant ($r_s = 0.03$, $P = 0.07$, $n = 62$, for SR_s, and $ExpH'$). Species richness was closely positively correlated to $ExpH'$ ($r^2 = 0.86$, $P < 0.001$). Mean alpha diversity ($SR_{\check{S}}$) for each of the seven regions showed no systematic relation to latitude or longitude (Table 1). $SR_{\check{S}}$ was highest in Belize followed by USVI and the lowest in Panama.
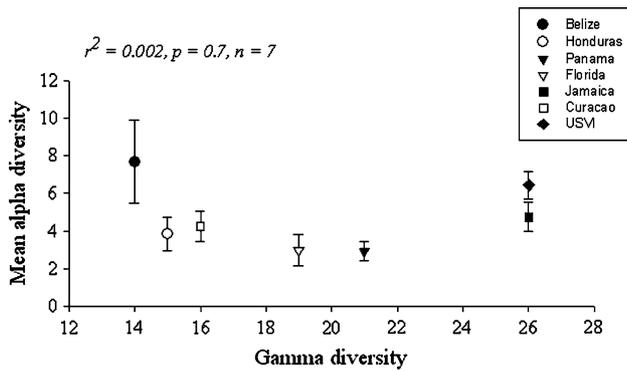
Gamma diversity (regional species richness, SR_R) was variable, ranging from 14 species in Belize to 26 species in USVI and Jamaica (Table 1). Thus gamma diversity showed an increase from west to east. There was no significant relationship between $SR_{\check{S}}$ (local or alpha diversity) and SR_R (gamma or regional diversity) ($r_s = 0.02$, $P = 0.07$, $n = 7$) (Fig. 3). However, low gamma diversity in Belize may be due to the lack of reef sites sampled. Reanalysis excluding Belize samples, showed a tendency for greater alpha diversity with higher gamma diversity but there was still no significant correlation ($r_s = 0.60$, $P = 0.22$, $n = 6$).

The sample-based rarefaction curves of observed species for each region showed decreased species accrual with increased sampling, but there was little sign of stabilizing towards asymptotic values (Fig. 4). The slopes of the curves varied, with the USVI samples having the greater rate of species accumulation and Florida samples the least. The data from the total area comprised 1,119 individuals and 67 species (SR_T) in 129 samples. The inventory of the region however was not complete, as the sample based rarefaction curve did not approach asymptote (Fig. 5). The *Chao2* species richness estimator appeared to stabilize at 94 ± 12 (±1 SD). Acotyleans (26 species) constituted 38%
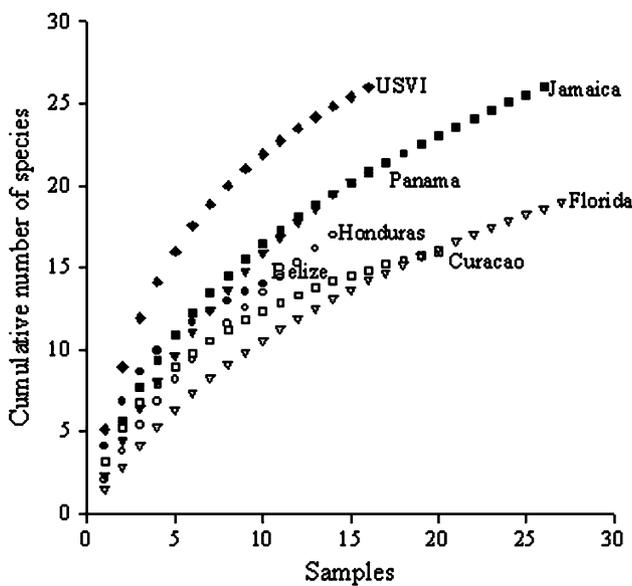
Table 1 Species richness (SR_S, site species richness = alpha diversity; $SR_{\check{S}}$, mean alpha diversity; SR_R, region = gamma diversity; SR_T, total area), the number of individuals (n), and the proportion of "rare" species.

Region	SR_s		SR_R	n	Uniques (%)	Duplicates (%)	Singletons (%)	Doubletons (%)
	Range	$SR_{\check{S}} \pm CI$						
Belize	5–12	7.67 ± 2.2	14	58	35.7	14.3	28.6	21.4
Honduras	1–7	3.86 ± 0.88	15	36	70.6	5.9	52.9	17.6
Panama	1–6	2.92 ± 0.5	21	60	57.1	19.0	42.9	14.3
Florida	0–7	3.0 ± 0.83	19	96	52.6	21.1	31.6	36.8
Jamaica	0–9	4.75 ± 0.78	26	206	46.2	11.5	42.3	7.7
Curaçao	2–9	4.25 ± 0.82	16	463	31.3	25.0	31.3	18.8
USVI	3–12	6.45 ± 0.73	26	202	34.6	19.2	34.6	19.2
Total	0–12	4.42 ± 0.34	67	1119	41.2	19.1	40.0	9.0
Acotylea	0–6	1.98 ± 0.21	26	690	54.0	11.5	46.2	15.4
Cotylea	0–6	2.34 ± 0.22	41	429	46.3	17.1	36.6	4.9

Uniques, species restricted to a single site; duplicates, species occurring in exactly two sites; singletons, species represented by a single individual; doubletons, species represented by only two individuals. CI 95% confidence intervals
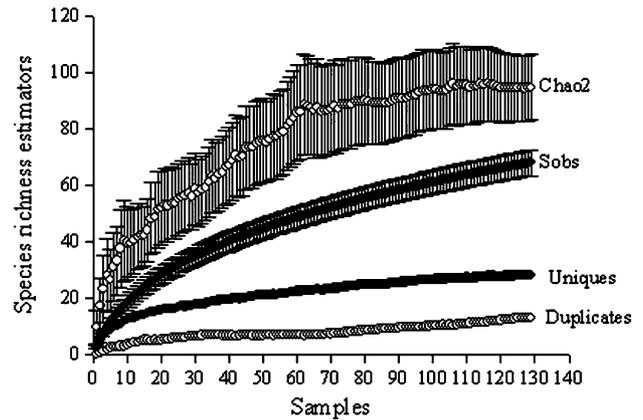
Fig. 3 Relationship between mean alpha diversity ($SR_{\check{s}}$) and gamma diversity (SR_R, regional species richness) of Caribbean polyclads. *Bars* indicate ±1 SE


Fig. 5 Sample-based rarefaction curves of Caribbean polyclads for all samples taken from the entire study area. Estimators of species richness are the total number of all species (*Sobs*), the *Chao2* estimator of true richness, the number of species restricted to a single sample (*uniques*) and the number of species found in exactly two samples (*duplicates*). *Bars* indicate ±SD. Plotted values are means of 50 estimates based on 50 randomizations of sample accumulation order (without replacement)

Fig. 4 Sample-based rarefaction curves of regional (gamma) polyclad diversity in the wider Caribbean. Plotted values are means of 50 estimates based on 50 randomizations of sample accumulation order (without replacement)

of the total number of species, whereas cotyleans (41 species) constituted the remaining 62%. Neither suborder reached an asymptotic value (Appendix 4).

Sample-based rarefaction curves for each habitat (not shown) within each region revealed that reef and sea grass habitats were the most species rich except in Honduras, where rocky shores had the highest *S* and in Belize (where reefs were not sampled due to adverse weather conditions) limestone pavement showed the highest number of species. In reef habitats throughout the region, cotyleans were significantly more species rich than acotyleans (one-factor ANOVA; $F_{1,10} = 6.45$, $P < 0.05$). Other habitats showed no significant difference in suborder species richness. Species accumulation curves for reef habitats across the six regions

(excluding Belize) showed greatest species richness in Panama and the USVI.

Abundance and distribution of species

The cumulative dominance of the ten most abundant species in the study area was 81% (Appendix 1). Of the 1,119 individuals counted, 62% were acotyleans. There was a significant relationship between the number of individuals per sample and depth/ habitat ($r_s = 0.06$, $P = 0.004$, $n = 129$). Greater abundances of polyclads were found in intertidal sites; in Curacao over 100 individuals per sample of the acotylean *Styloplanocera fasciata* were found in intertidal coral rubble habitats. Rocky shore habitats had the second greatest abundance per sample and were dominated by a pink acotylean species (Acotylea sp.1) in Florida, *Boninia divae* in Jamaica and USVI, and *Enchiridium periommatum* in Belize. Only two species were found in all seven regions across the Caribbean; the acotylean *Melloplana ferruginea* and cotylean *Pseudoceros bicolor*, but not in all habitats (Appendix 1).

Conversely, the Caribbean assemblage has a large number of species that are represented by few individuals (rare species) (Appendix 1) and of restricted range (Fig. 6). Twenty-eight species, or 41% of the total number of species, were restricted to a single site (uniques) and 13 species (19%) were restricted to only two sites (duplicates) (Table 1). Forty per cent (27 species) of the total species were singletons (represented by a single individual), and 9% (6 species) were doubletons (two individuals). At the scale of regions, the unique species comprised between 31 and 71% and the singletons between 29 and 53% (Table 1).
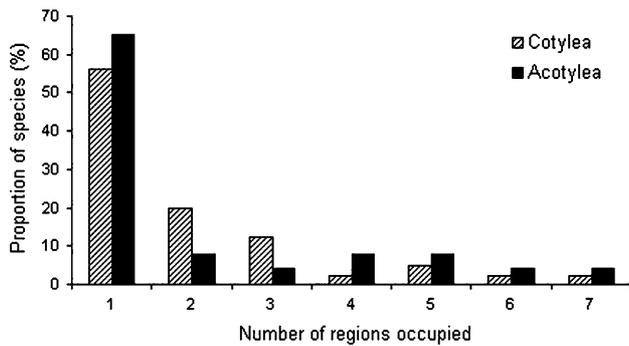
Fig. 6 Distribution of polyclad species range sizes in the wider Caribbean. Range size is the number of regions occupied by a species out of seven total regions

In total, uniques had low abundances, where 23 species were singletons, 3 species were doubletons and the remaining two species had three individuals. Species with restricted ranges (uniques and duplicates) were still being found with increasing sampling coverage as neither reached asymptote (Fig. 5). Species range size was positively correlated with local abundance for both suborders (Acotylea, $r_s = 0.48$, $P = 0.01$, $n = 26$; Cotylea, $r_s = 0.39$, $P = 0.01$, $n = 41$). Thus common species were widely distributed spatially, while species of low abundance had strongly compressed range sizes.

There was a clear positive correlation between the total number of species and the number of singletons ($r_s = 0.81$, $P = 0.01$, $n = 7$). Number of uniques was not related to total number of species and there was no correlation between the number of uniques and the number of singletons per region.
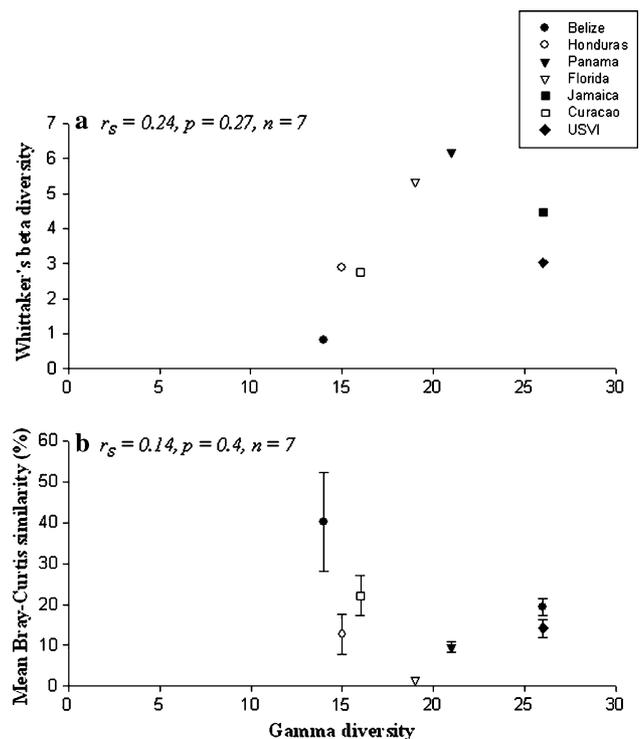
Beta diversity

Total β_w varied between suborders and was highest overall for cotyleans (16.75) in all regions except Honduras, where acotyleans showed higher species turnover between sites. Table 2 shows that Whittaker's β_w was greatest at the scale of the total area ($SR_T/SR_{\hat{S}}$) than at the region scale. The highest β_w was found in Panama for all acotylean species, and in Florida for cotylean species. The lowest β_w was found in Belize for all species pooled.

At the scale of regions, mean Bray–Curtis similarity showed that species turnover was lowest in Belize and highest in Florida (Table 2). β_w ($SR_c/SR_{\hat{S}}$), and mean Bray–Curtis similarity were not significantly correlated to gamma diversity (SR_C) (Fig. 7a, b), although there was a tendency for less biotic distinctness and greater similarity with lower regional diversity. There was also a tendency for greater similarity with a greater number of habitat types sampled (although these were not significantly correlated). For all combinations of sites in the study area, the Bray–Curtis

Table 2 Measures of beta diversity: Whittaker's diversity for regions $[\beta_w = (SR_R/SR_{\hat{S}}) - 1]$ and for the total area $[\beta_w = (SR_T/SR_{\hat{S}}) - 1]$ for all species pooled and for each suborder; Bray–Curtis similarities for all pair wise combinations of sites intervals

Region	β_w			Bray–Curtis similarity (%)	
	All species	Acotylea	Cotylea	Range	Mean ± CI
Belize	0.83	0.4	0.80	24.4–64	40.1 ± 12.1
Honduras	2.89	4.0	3.21	0–44.4	12.6 ± 4.9
Panama	6.19	6.20	6.59	0–66	9.62 ± 2.3
Florida	5.33	3.86	6.74	0–44.4	3.7 ± 1.6
Jamaica	4.47	4.15	4.58	0–74.0	14.2± 2.2
Curaçao	2.76	0.74	1.96	0–84.8	22.1 ± 4.9
USVI	3.03	2.19	3.67	0–66.7	19.4 ± 2.1
Total	14.15	12.13	16.75	0–100	8.6 ± 0.3

CI 95% confidence

Fig. 7 Relations between gamma diversity (SR_R) and measures of beta diversity of Caribbean polyclads. **a** Whittaker's beta diversity ($\beta_w = SR_R/SR_s$). **b** Mean Bray–Curtis similarity (%). *Bars* indicates ±95% CI

similarities were highly variable and ranged between 0 and 100% (mean Bray–Curtis: 8.6%).

Faunal assemblages in space

Clusters of the mean of replicate samples for the 58 sites where polyclads were found covered a range of similarities

(0–83%; Fig. 8a) with faunal assemblages from each region showing little distinction (ANOSIM region: $R = 0.19$, $P < 0.1\%$). Multiple pair wise comparisons between pairs of regions showed that assemblages from Honduras and Belize, Florida and Belize, and Curaçao and Jamaica were most similar, whereas faunal differences were most significant between Curaçao and Panama, and Honduras and USVI assemblages.

A multidimensional ordination of the mean of replicates per site (Fig. 8b) showed that assemblages from different habitats overlapped but that there was some separation (ANOSIM habitat: $R = 0.34$, $P < 0.1\%$), especially between reef and intertidal habitats.

Pair wise comparisons between habitats showed greatest similarities amongst sea grass and limestone pavement assemblages, which in turn were significantly distinct from fouling community assemblages. The results of the SIMPER analysis determined the following species as most representative of each habitat type: reef, *Pseudoceros bicolor, Phrikoceros mopsus* and *Diposthus popeae*; seagrass,

Enchiridium evelinae; fouling communities, *Maritigrella crozieri*; rocky shores, *Styloplanocera fasciata, Melloplana ferruginea, Boninia divae, Phaenocelis medvedica*; limestone, *Melloplana ferruginea, Armatoplana leptalea* and *Boninia divae*.

Discussion

A checklist of the Tropical Western Atlantic (Quiroga et al. 2004b) compiled from the literature lists 124 species from an area spanning from North Carolina to Trinidad and including the Gulf of Mexico. A second checklist restricted to the Caribbean coast of Colombia (Quiroga et al. 2004a) lists 25 species. In this study, 67 putative species were found, 40 of which could be identified to the species level. Twenty-nine of these were common to both checklists. Three species were first records for the Caribbean *Pseudoceros bolool, Pseudobiceros hymanae* and *Cycloporus papillosus*. Descriptions of new species and a taxonomic

Fig. 8 Multivariate analyses showing similarities in polyclad assemblages between 58 sites in the Caribbean. **a** Hierarchical agglomerative clustering of square root transformed data using group average linking on Bray–Curtis similarities (%). *B* Belize, *H* Honduras, *P* Panama, *Fl* Florida, *J* Jamaica, *C* Curaçao, *U* USVI. *rw* rock wall, *rs* rocky shore, *cri* coral rubble intertidal, *ls* limestone, *fc* fouling community, *sg* seagrass, *crs* coral rubble subtidal, *r* reef. **b** Multidimensional scaling ordination for square root transformed data based on Bray–Curtis similarities. Outlying sites from fouling communities have been excluded. Reef sites are delineated

inventory are the focus of separate work (Bolaños et al. (2007), Litvaitis et al. in prep).

In contrast to previous checklists, this study showed a higher number of species belonging to Cotylea compared to Acotylea. This is perhaps a reflection of habitats sampled. Two-thirds of the collecting sites in this study were subtidal, compared to predominantly intertidal collections in past studies. These data showed that cotyleans were significantly more species rich in reef habitats throughout the region, and that with seven species, the most species rich genus was *Pseudoceros*. Considering Pseudocerotidae as a whole, 14 species belonging to 4 genera were recorded during this study. This is in agreement with findings in the Indo-Pacific and on the Great Barrier Reef (Newman and Cannon 1994, 1996). Whether this pattern of cotylean species dominance in reef habitats is due to actual differences in the biology and ecology of the two sub-orders or whether it is due to a sampling bias introduced by a collector's familiarity with these species and their relative ease of detection needs further examination.

The actual number of species in a given sampling unit is usually not measurable, and a central problem for any sampling based study is to estimate to what extent values obtained represent reality. The species accumulation curve for the whole area did not reach asymptote, indicating that not all potential species had been collected. However, the *Chao2* estimator leveled out, suggesting that the number of samples collected was adequate for a reliable estimate of polyclad species richness in these habitats. Application of the *Chao2* method in marine studies is increasing (e.g. Patterson et al. 1998; Gray 2000; Ellingsen 2001; Witman et al. 2004) however; most suggest that *Chao2* underestimates the actual species richness. For invertebrate and microbial assemblages everywhere and for many taxa in tropical habitats, asymptotes may never be reached (e.g. Fisher 1999; Novotony and Basset 2000). With respect to acotylean polyclads, their cryptic behavior and dull brown coloration are the most likely reasons for under collection. Despite standardizing collection methods across different habitats, potential sources of error in this study include unequal replication. Fortunately species accumulation curves allow for comparisons between sites despite unequal sampling and if the curves fail to reach asymptote the curves themselves can be compared (Gotelli and Colwell 2001).

Studies of geographical clines in diversity in marine systems have focused on latitudinal variations at a range of scales, with the central and debated paradigm being that species richness decreases with increasing latitude, especially in the northern hemisphere (e.g. Stehli and Wells 1971; Clarke 1992; Roy et al.1998). Recent studies on benthic community diversity have shown that smooth latitudinal clines (Kuklinski et al. 2006) and gradients at smaller scales (e.g. 15° of latitude) do not exist (Ellingsen and Gray

2002). In this study, there was no evidence of a latitudinal cline in SR_s or in mean alpha diversity ($SR_{\check{s}}$) over the seven regions. This may simply be due to the relatively restricted latitudinal range (9°–24°N) and primarily tropical environment encompassed. Instead, the data revealed a weak easterly increase in species richness and diversity. For gamma diversity, the data also provided evidence of an increase from west to east. However, in order to conclude whether polyclad diversity conforms to the norm of decreasing diversity with increasing latitude and whether a longitudinal gradient in diversity occurs across the Caribbean, further sampling incorporating a wider geographic scope, and more extensive sampling in general, is needed.

Rareness is a dominant characteristic of polyclad assemblages. As with most ecological data sets, the majority of polyclad species were represented by a small number of individuals. Most individuals belong to a few abundant species. At larger scales the frequency distribution of species' range sizes are typically strongly right-skewed (Gaston 1994) (Fig. 6). Common species were widely spatially distributed, while species of low abundance had strongly compressed range sizes. However, any correlation between these two parameters should be treated cautiously because a possible explanation for this pattern is under sampling of rare species. Whilst most species were rare, a few species were relatively abundant. Two intertidal specialists, the acotylean *Styloplanocera fasciata* and the cotylean *Bonina divae* were dominant members of the benthic epifauna on intertidal coral rubble sites, and rocky shores and limestone pavements, respectively. Intertidal specialists are indicative of low levels of disturbance (Kuklinski et al. 2006), and dense aggregations (>20 individuals boulder^{-1} and a maximum of 144 individuals sample^{-1}) show adaptation to this ecological niche. *K*-selection may also explain the aggregations and probably occurs in more habitat-specialized turbellarian species (Hoxhold 1974). However the life histories of most polyclad species (including these species) is unknown. Consequently, relating distribution range to dispersal ability cannot be assessed for the majority of species found in this study.

Many ecological factors influence the occurrence and distribution of land planarians, depth, temperature, substrate texture, presence of refugia, prey abundance and habitat disturbance (e.g. Sluys 1998; Winsor 1998; Fick et al. 2006). These same factors must also influence the turnover of marine polyclads between the habitats. Species turnover measured using a univariate index (Whittaker's diversity, β_w) showed all species and both suborders had greatest turnover between sites in Florida, Jamaica and Panama, and lowest turnover in Belize. The low species turnover and high similarity amongst sites in Belize was due to sampling being restricted to intertidal and shallow sub-tidal sites. Cotyleans were more over dispersed than acotyleans and showed higher differentiation between sites over the entire region and also in

most individual regions. The multivariate measure of beta diversity (Bray–Curtis similarity) showed that intra-regional similarity was generally higher than over the total area (but this was not the case for Florida). The lack of clear distinction between assemblages from the seven regions was confirmed by visualization of the Bray–Curtis similarities using cluster analysis. This indicated that some species have a pan-Caribbean distribution and many others were found in more than one region, implying a level of faunal homogeneity across the Caribbean region. This homogeneity has also been shown for coral reef fish (Acero 1985) and for the dinoflagellate symbionts of coral (LaJeunesse 2004). However, faunal distributions of other marine taxa in the Caribbean have revealed discrete subprovinces; for example, caenogastropods show an abrupt shift at Santa Marta, Colombia caused possibly by the combined barrier effect of an extremely narrow shelf and upwelling induced by cold waters (Diaz 1995). This phenomenon may explain the distinction in polyclad assemblages between Curaçao and Panama.

Greater variation in assemblages was explained by habitat type. Fouling community assemblages in Florida were the most distinct; the euryleptid *Maritigrella crozieri* was exclusively found in this habitat, and is known to live in close association with its prey the mangrove ascidian, *Ecteinascidia turbinate* (Newman et al. 2000). Reef assemblages were different from shallower intertidal habitats and were dominated by cotylean species. However, overlap with intertidal sites, and overlap in general between the habitats and regions, can be explained by three factors: abundant generalists, habitat heterogeneity, and very patchy assemblages. First, the majority of the ten dominant species were found in more than one habitat type, e. g., *Pseudoceros bicolor* was found on reefs, limestone pavements, rocky shores and in sea grass beds, implying tolerance of different substrate type, refugia and possibly prey. Secondly, defining what is a habitat is not a simple task as no habitat is truly homogeneous (Colwell and Coddington 1994). Many marine habitats in close proximity form gradients and clear distinctions between them rarely exist. As a result, faunal mixing occurs, as is illustrated by similar assemblages found in sea grass beds and limestone pavements, although my sampling design controlled for this as much as possible. Finally, polyclads are very patchily distribution. Some sites had no worms, whilst others had dense aggregations of up to 144 individuals of the same species, and replicated samples within sites showed the same spatial heterogeneity. Consequently, variation in abundance and diversity were frequently greater at smallest scale. High variability in numbers and low species frequency calls for many replicate samples for ecological studies. The next step in understanding the ecology of polyclads is to relate spatial heterogeneity to the distribution of prey, environmental processes, biogenic structures and biogeographic histories.

A major ecological phase shift has occurred in Caribbean coastal environments over the past 50 years. There has been a loss of macrofauna, reduced fish stocks, a shift from fish dominated to echinoid dominated herbivory, destructive overgrazing and bioerosion by food-limited sea urchins and reduced coral recruitment (Bellwood et al. 2004) All this has led to the widespread loss of coral cover, erosion of the three dimensional environment, and, with the die-off of *Diadema antillarum*, a rapid phase shift to dominance of macroalgae. The effect of this environmental shift on polyclad diversity is unknown. It is predicted that reduction in coral cover will limit the availability of suitable habitat for reef specialists, such as many of the pseudocerotids. This family, and cotyleans in general, showed narrower ranges and greater species turnover than acotylean species and may therefore be the most vulnerable to environmental change. Quantitative studies on the diversity of neglected taxa whose functional roles in ecosystems have not yet been investigated are important, especially in low diversity reefs, such as in the Caribbean Basin. These reefs show low functional redundancy (Bellwood et al. 2004) and are rendered particularly vulnerable as minor changes in biodiversity can have major impacts on ecosystem processes.

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