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Reef Location and Client Diversity Influence the Skin Microbiome of the Caribbean Cleaner Goby *Elacatinus evelynae*

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Abstract

Fish-associated microorganisms are known to be affected by the environment and other external factors, such as microbial transfer between interacting partners. One of the most iconic mutualistic interactions on coral reefs is the cleaning interactions between cleaner fishes and their clients, during which direct physical contact occurs. Here, we characterized the skin bacteria of the Caribbean cleaner sharknose goby, *Elacatinus evelynae*, in four coral reefs of the US Virgin Islands using sequencing of the V4 region of the 16S rRNA gene. We specifically tested the relationship between gobies' level of interaction with clients and skin microbiota diversity and composition. Our results showed differences in microbial alpha- and beta-diversity in the skin of gobies from different reef habitats and high inter-individual variation in microbiota diversity and structure. Overall, the results showed that fish-to-fish direct contact and specifically, access to a diverse clientele, influences the bacterial diversity and structure of cleaner gobies' skin. Because of their frequent contact with clients, and therefore, high potential for microbial exchange, cleaner fish may serve as models in future studies aiming to understand the role of social microbial transfer in reef fish communities.

Keywords Skin microbiota · Microbial diversity · Cleaner fish · *Elacatinus evelynae* · Coral reefs

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Introduction

Coral reefs are highly complex marine ecosystems that have been the focus of numerous studies examining the drivers of biodiversity and community dynamics [1]. As in other ecosystems, coral reef microorganisms are emerging as key members to maintaining reef health and resilience in the face of large-scale degradation due to climate change, human impacts, and emerging diseases of corals (reviewed by [2]). This has resulted in increased efforts to characterize diseases of reef microbial communities and to identify which organisms influence resilience and recovery (e.g., [3]). Recent studies have shown the importance of interactions between the coral microbiome and the larger reef community (e.g., between reef-building coral and benthic algae, [4]). However, relatively few studies have examined the microbiome associated with the most mobile members of the reef community, such as fishes (e.g., [5, 6]).

Fish microbial communities are known to be affected by multiple biotic and abiotic variables. There is evidence that fish-associated bacteria are organ-specific, species-specific, and individual specific, thus comprising highly diverse

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communities [7–9]. While host factors seem to be the major drivers of fish gut bacterial diversity (e.g., feeding habit [10] and taxonomy [11]), the physicochemical properties of the water exert a considerable effect on the diversity of fish skin-associated microbes (e.g., temperature and salinity, [12]). Despite the impact of the surrounding environment, the contribution of the water microbiota to the fish skin microbiota composition seems negligible, with fish mucosae being a highly selective environment (e.g., [13]). However, other external factors, such as direct transfer of microorganisms between fishes, might also play a major and still unexplored role [14–16]. Microbial transfer between interacting partners has been shown to be common in nature, shaping microbial consortia in humans and other animal groups (reviewed in [17, 18]), including fishes [14]. Although social microbial transmission could ultimately increase microbiome complexity, which may reduce the abundance of opportunistic and/or pathogenic taxa, as seen in bees [19] or chimpanzees [18], social interactions may also facilitate pathogen transmission and consequently increase levels of infection and disease (reviewed by [20]).

One of the most iconic mutualistic interactions on coral reefs is the relationship between cleaner fishes and clients. Cleaners attract individuals from multiple species (clients) to their "cleaning stations," which are usually fixed territories where they inspect the body of multiple client fishes per day to remove parasites, dead tissue, and mucus [21]. Although many fish species are facultative cleaners as juveniles, members of two genera are dedicated cleaners [22]. These include the cleaner wrasses (*Labroides* spp.) in the Indo-Pacific and the cleaner gobies (*Elacatinus* spp.) in the Caribbean region. Cleaner gobies of the genus *Elacatinus* reside on benthic substrate, moving only to make contact with client fishes [21, 23], which travel and interrupt other activities to visit cleaner gobies [24, 25]. Visits to cleaner goby stations can be influenced by multiple factors, including location relative to territorial client fish [24–26], local fish abundance [27], structural complexity [28], and parasite activity and abundance [26, 29]. Consequently, the abundance and diversity of client fishes can vary widely among cleaning stations. Because of their frequent contact with heterospecifics, and therefore, high potential for microbial exchange, cleaner fish may serve as a useful animal model system to understand the role of social microbial transfer in ecological communities [16]. Indeed, a recent study comparing "cleaner" vs "non-cleaner" ecotypes of *Elacatinus prochilos* from Barbados found that bacterial diversity was significantly increased in "cleaner" ecotypes [15].

Here, we characterized the skin bacteria of the most ubiquitous Caribbean cleaner goby species, the sharknose goby *Elacatinus evelynae*, in several reefs within the US Virgin Islands, using 16S rRNA gene (V4 hypervariable region) amplicon sequencing. We specifically studied the relationship between gobies' level of interaction with clients, and skin microbiota diversity and composition. We expected to find a relationship between microbial diversity and client diversity, and geographical differences in the skin microbiota among reefs due to putative socio-environmental differences.

Methods

Study species, sites, and behavioral observations

This study was conducted in the US Virgin Islands on July 2017. All behavioral observations were performed in four sampling localities: two sites on the Island of St. Thomas, Brewers Bay (18°20′23.6"N 64°58′38.6"W; $n = 7$ cleaner gobies) and Hull Bay (18°22′09.5"N 64°57′10.4"W; $n = 13$); and two on the island of St. John, one along the west rim of Greater Lameshur Bay at West Lameshur (18°19′04.4"N 64°43′26.0"W; $n = 12$) and another along the east rim at Donkey Bight (18°18′50.4"N 64°43′15.2"W; $n = 12$) (Fig. 1). Donkey Bight is dominated by a mix of rocky reef, live and dead coral, sand, and seagrass, while West Lameshur is highly degraded with almost no live coral, and it is located near a mangrove swamp [30]. Both Hull and Brewers are shallow bays (< 15 m depth) with a rocky perimeter that supports isolated coral heads. Hull Bay has patches of live and dead coral in the center, while Brewers has coral reefs surrounded by extensive seagrass beds. Hull Bay faces the Atlantic Ocean on the north side of St. Thomas and is more wave-exposed than Brewers, which faces the Caribbean Sea on the south side of the island.

We focused on the sharknose goby *Elacatinus evelynae*, which are small fish (1.2–3.5 cm total length) with a prominent lateral blue and yellow stripe running from the snout to the base of the tail. This species is common across the study reef sites, inhabiting the surface of living coral, usually *Siderastrea* spp., *Orbicella* spp., *Montastrea* spp., *Diploria* spp., and *Pseudodiploria* spp. [23]. Cleaning stations were identified, and a subset was randomly selected for this study. In the degraded reef of West Lameshur, some coral colonies were still found with cleaner gobies. Observations of cleaning interactions were made by two snorkelers positioned as far from the station as possible while still being able to see cleaning interactions (at least 2 m), in shallow waters (< 4 m depth). Individual gobies were observed for 30 min at each location three times a day (at dawn, midday and dusk, in a total of 1 h 30 min for each cleaner) encompassing the hours during which *E. evelynae* is more active [25]. Observations were registered after a 2–5 min delay to allow the fish to become accustomed to the presence of the observer. During each observation period, the number of clients visiting each cleaning station, number of clients inspected, number

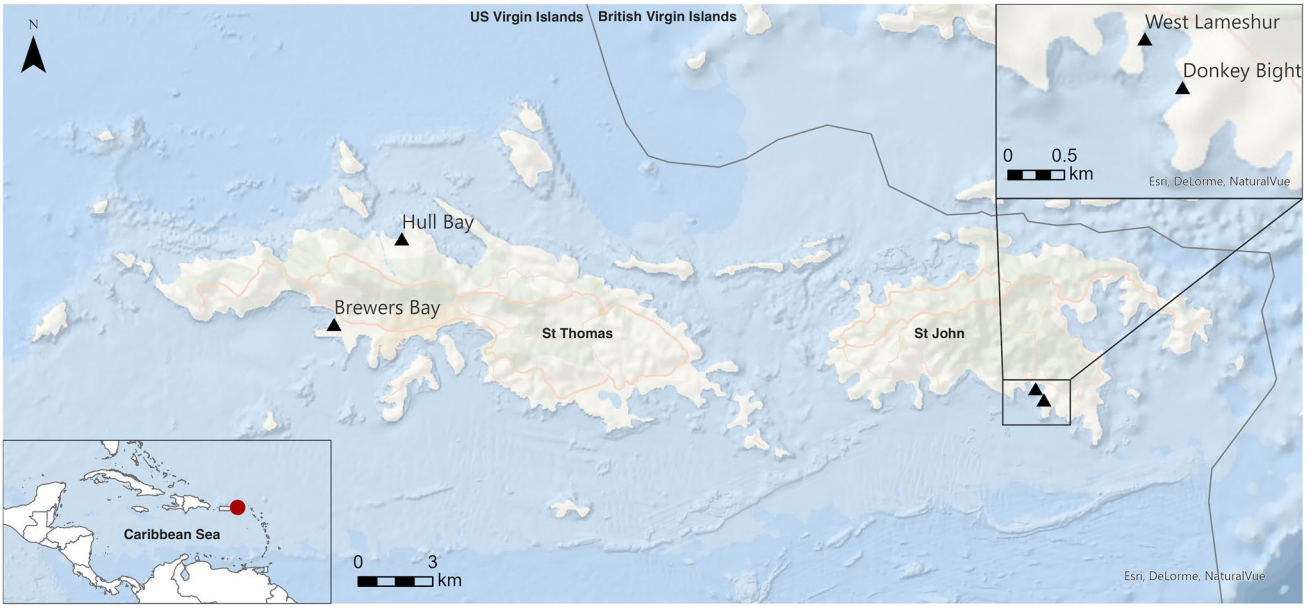

Fig. 1 Sampling sites located in the US Virgin Islands. Map created on ArcGIS software by Esri

of client genera visiting, number of client genera inspected, and average inspection time were recorded.

Following the final observation, cleaner gobies were captured using individual hand nets and transferred to individual sealed plastic bags. Immediately upon capture, fish were taken to the lab and swabbed in both sides of the body with tubed sterile cotton swabs (MedicalWire). Sampling was performed using gloves, and nets were submerged in a 30% bleach solution and rinsed with fresh water prior to each use. Samples were stored at − 80 °C until DNA extraction.

Laboratory procedures

The total DNA was extracted using the DNeasy PowerSoil DNA isolation kit (QIAGEN) following manufacturer's protocol. Extracted DNA concentration and quality were measured in a NanoDrop^{TM} 2000 Spectrophotometer (ThermoFisher Scientific). DNA was shipped in dry ice to the Centre for Microbial Systems at the University of Michigan Medical School (USA) where the V4 hypervariable region of the 16S rRNA gene (~ 250 bp) was amplified for each sample and controls (i.e., extraction and PCR blanks) with primers 515F/806R [31]. PCR amplification was performed using an initial denaturation cycle at 95 °C for 2 min, followed by a total of 40 cycles of a denaturation step at 95 °C for 20 s, an annealing lasting 15 s, with temperature decreasing 0.3 °C from an initial 60 °C until 55 °C, where it was kept for an additional 20 cycles, and elongation at 72 °C for 5 min. A final elongation step at 72 °C was performed for 10 min. Libraries were prepared using a dual indexing strategy in a single PCR (see [32] for additional details on library preparation), pooled and sequenced in a single Illumina MiSeq sequencing run.

Data analysis

Raw FASTQ files were analyzed using the DADA2 pipeline [33] for merging paired end reads, filtering, and sequencing error correction using the following parameters: trimLeft = 20, truncLen = c(220, 200), maxN = 0, maxEE = c(2,2), truncQ = 2. Singletons were discarded and reads were collapsed into amplicon sequence variants (ASVs). Taxonomy was then determined against the SILVA reference database (release 132) using the assignTaxonomy function [34]. ASVs present in PCR and extraction blanks that remained unclassified or were classified as Mitochondria (identified as Family) and Chloroplast (identified as Class) were removed from the dataset. Archaea were also excluded because the primers used are known to discriminate against this group in the marine environment [35]. An ASV frequency table was constructed with the R package *phyloseq* [36] and read-normalized counts were obtained using the negative binomial distribution implemented in DESeq2 [37]. Raw sequence reads were deposited into NCBI's Short Read Archive under accession PRJNA756005.

Alpha-diversity (intra-sample) was estimated using Shannon, Fisher and Faith's Phylogenetic Diversity (PD) indices using the R package phyloseq [36]. Alpha-diversity differences among localities were tested using generalized linear models (GLMs) and post-hoc comparisons were evaluated with Tukey's HSD test. To test the effect of each cleaning behavior parameter on microbial alpha-diversity, linear

mixed models (LME) were performed using the number of clients visiting each cleaning station, number of clients inspected, number of client genera visiting, number of client genera inspected, and average inspection time for each model as fixed factors (predictors). Locality was included as a random factor and models were built using the R package *lmer* (lme(Alpha-diversity ~ cleaning behavior parameter + 1/locality). The significance of GLM and LME models was estimated using ANOVA of type III with Satterthwaite approximation for degrees of freedom. Beta-diversity (inter-samples) was estimated using phylogenetic informed weighted and unweighted UniFrac [38] and Bray Curtis (BC) indices using the R package phyloseq [36]. Dissimilarity in microbial structure among reef locations was visualized using principal coordinates analysis (PCoA). The homogeneity of beta-diversity dispersion among localities was also assessed by first calculating the average distance to the sample group centroid using the *betadisper* function of the *vegan* R package [39] and then compared using a permutation test. Tukey's HSD test was used for post-hoc comparisons. Differences in microbial structure among reefs were then tested with a PERMANOVA with the strata option for locality and 9999 permutations, as implemented in the *adonis* function of the vegan package. Post-hoc comparisons were performed using a pairwise PERMANOVA with the Bonferroni *p*-value correction for multiple testing. Additionally, the differences in the abundances of phyla and genera represented by ≥ 3% on average of all sequences were also assessed among reef locations using the same GLM and LME structure described above. A Venn diagram was used to depict the number of ASVs shared among reef locations.

A dissimilarity matrix with the client genera inspected by each goby was constructed and differences among localities were also tested with a PERMANOVA using the BC index. To test the correlation between cleaning activity and the microbial community of each locality, Mantel statistical correlations based on Spearman's rank were performed between the number of clients visiting the cleaning station, number of clients inspected, number of client genera visiting and inspected, and the beta-diversity distance matrices using the vegan R package. For all tests, differences were considered significant when $p < 0.05$.

Results

Client composition and cleaning activity

A total of 44 fish species belonging to 29 genera and 14 families visited the observed cleaning stations (Supplementary Table S1). The striped parrotfish (*Scarus iseri*) was the most common client fish in Brewers Bay (22% of the total visits in that reef), the yellow goatfish (*Mulloidichthys martinicus*) in Hull Bay (33%), the ocean surgeonfish (*Acanthurus bahianus*) in Donkey Bight (20%), and the longfin damselfish (*Stegastes diencaeus*) in West Lameshur (19%) (Supplementary Table S1). Client fish genera composition was significantly different among reefs (PERMANOVA, $F = 2.11$, $p = 0.001$), and the pairwise differences were significant between Donkey Bight and West Lameshur (both located in St. John) ($F = 3.72$, $p = 0.03$) and also between West Lameshur and Hull Bay ($F = 2.78$, $p = 0.03$). Cleaner gobies from Donkey Bight and West Lameshur inspected higher number of client genera (Mean (± SD) = 4.9 (± 2.4) and 4.8 (± 2.2), respectively) compared to Brewers Bay and Hull Bay (Mean (± SD) = 4 (± 0.8) and 2.6 (± 1.6), respectively). Moreover, dawn was the observation period with the highest cleaning activity (see Supplementary Table S2).

The skin microbiome of the cleaner goby *Elacatinus evelynae*

Bacteria-associated 16S rRNA amplicons present in the skin of 44 *E. evelynae* cleaner gobies from four reefs in the US Virgin Islands (Brewers Bay, Hull Bay, Donkey Bight and West Lameshur) were sequenced, generating 1,245,579 raw reads, 1,099,501 filtered sequences, and 1222 ASVs. From those, 223 ASVs were present in Brewers Bay, 586 ASVs in Donkey Bight, 341 ASVs in West Lameshur, and 457 ASVs in Hull Bay (Fig. 2). The most abundant bacterial phyla (≥ 3%) across all samples were Proteobacteria (58%), Bacteroidota (16%), Firmicutes (7%), Actinobacteriota (4%), and Cyanobacteria (4%). *Pseudomonas* was the most abundant genus in Brewers Bay (17.7%), *Ekhidna* in Hull Bay (14.8%), and *Psychrobacter* in Donkey Bight and West Lameshur (15.7% and 15.5%, respectively). The most abundant genera (≥ 3%) found for each locality are detailed in Table 1. Differences in phyla abundance among localities were not found ($p > 0.05$), while significant differences were found for the genera *Alcanivorax*, *Cloacibacterium*, *Halomonas*, *Pseudomonas*, and an unclassified genus from Pseudomonadaceae family ($p < 0.04$) (Supplementary Table S3).

Although a total of 43 ASVs were common among reefs (Fig. 2), no single ASV was present in all sampled fish. Nevertheless, two ASVs identified as *Rubrobacter* sp. and *Cloacibacterium* sp. were found in all samples from Donkey Bight ($n = 12$), therefore constituting the core microbiota in that locality.

Variation of the skin microbiome of cleaner gobies with cleaning activity

Alpha-diversity of the bacterial communities associated with the cleaner goby skin was positively (i.e., increasingly) correlated to the number of clients and client genera visiting the cleaning stations and inspected for all reefs except for West

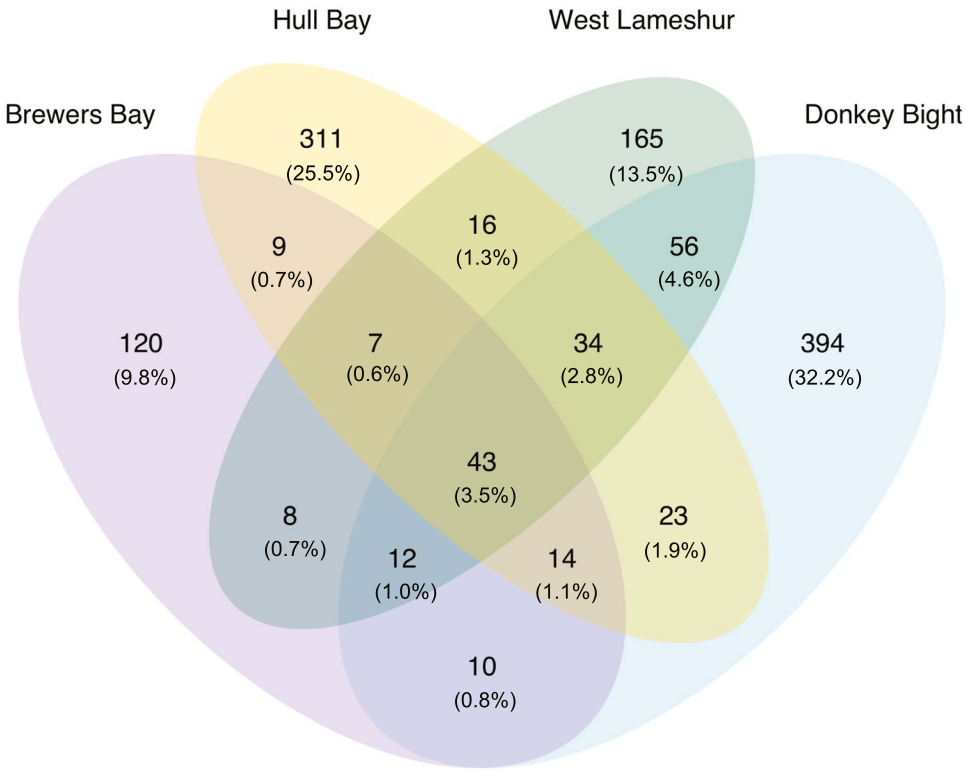

Fig. 2 Venn diagram with the numbers and percentages of shared and unique ASVs amongst localities

Lameshur, which showed an inverted pattern, i.e., higher number and diversity of clients corresponded to lower values of alpha-diversity (Fig. 3; Supplementary Figure S1). However, none of the trends were statistically significant ($p > 0.24$, Supplementary Table S3). Moreover, average inspection time did not correlate with alpha-diversity ($p > 0.15$, Supplementary Table S3).

Beta-diversity was not explained by the cleaning activity variables (i.e., the number of clients visiting the cleaning station, number of clients inspected, average inspection time, number of client genera visiting and inspected) with none of the beta-diversity indices ($p > 0.08$, Supplementary Table S3). However, there was a significant positive correlation between cleaner gobies' microbial beta-diversity using the unweighted UniFrac distance and the number of client genera inspected in Brewers Bay and Hull Bay (Mantel test, $p < 0.02$, Supplementary Table S4). In Donkey Bight, there was also a positive correlation between goby microbial beta-diversity using the BC index and number of client genera visiting and inspected (Mantel test, $p < 0.003$, Supplementary Table S4), and the same correlation was found in West Lameshur with the weighted UniFrac distance (Mantel test, $p < 0.02$, Supplementary Table S4).

Diversity of the skin microbiome of cleaner gobies across reef locations

Skin bacterial alpha-diversity was significantly different among reef localities ($p < 0.02$, Supplementary Table S3), with pairwise comparisons showing that cleaner gobies from Donkey Bight harbored significantly higher alpha-diversity compared to those from the other localities ($p < 0.04$; Fig. 4). No differences were observed in the alpha-diversities of individuals from the remaining locations.

Bacterial community structure (beta-diversity) was significantly different amongst reef localities with all beta-diversity indices ($p < 0.003$), with significant pairwise differences between Donkey Bight and each of the other reef sites ($p < 0.02$; Fig. 5a–c and Supplementary Table S5). Beta-dispersion was also significantly different among locations considering the Bray Curtis ($F = 5.94$, $p = 0.002$; Fig. 5d) and weighted UniFrac ($F = 6.97$, $p = 0.001$; Fig. 5e) indices. Pairwise comparisons of beta-dispersion for the Bray Curtis

Table 1 Percentage of the most abundant bacterial taxa collapsed by phyla and genera for each reef location. Values in bold represent an abundance ≥ 3%

Bacterial taxa	% of sequences			
	Brewers Bay	Hull Bay	Donkey Bight	West Lameshur
Phyla				
Actinobacteriota	2.2	**6.7**	**4.3**	**3.7**
Bacteroidota	**22.0**	**14.9**	**6.3**	**21.8**
Bdellovibrionota	**10.4**	0.1	0.2	0.8
Cyanobacteria	**6.8**	**3.4**	**3.1**	**5.1**
Firmicutes	**3.9**	**8.2**	**5.2**	**9.5**
Planctomycetota	**3.0**	0.1	0.4	0.02
Proteobacteria	**48.4**	**63.4**	**67.8**	**48.8**
Genera				
Acinetobacter	1.0	1.8	**3.1**	**4.0**
Alcanivorax	0.0	0.0	**4.8**	2.2
Cloacibacterium	**3.9**	0.2	**6.4**	0.8
Ekhidna	**12.7**	**14.8**	1.9	0.4
Endozoicomonas	0.0	0.1	0.8	**5.8**
Enterovibrio	0.0	**6.3**	0.2	**3.0**
Halomonas	0.0	0.1	**9.6**	**5.5**
Marinobacter	0.1	0.0	**2.6**	1.1
Mycoplasma	1.7	**4.8**	0.1	0.0
NS5 marine group (Flavobacteriaceae)	0.1	1.9	0.2	**2.9**
OM27 (Bdellovibrionaceae)	**10.3**	0.6	0.0	0.1
Pseudomonas	**17.7**	**10.3**	1.9	**8.9**
Psychrobacter	**9.1**	**3.7**	**15.7**	**15.5**
Stenotrophomonas	**4.3**	**2.6**	0.6	0.8
Synechococcus	**4.2**	**2.8**	0.7	**2.5**
Unclassified Neisseriaceae	0.0	**5.2**	0.1	0.0
Unclassified Pseudomonadaceae	0.0	0.0	**6.3**	**3.3**
Unclassified Rhodobacteraceae	**2.7**	0.5	0.5	0.1

index showed differences between Donkey Bight and West Lameshur, as well as between Donkey Bight and Hull Bay (TukeyHSD, $p < 0.003$; Fig. 5d). For the weighted UniFrac distance, differences were found between Donkey Bight and all remaining localities (Tukey HSD, $p < 0.03$; Fig. 5e).

Discussion

Cleaning stations have been shown to attract a wide diversity of fish species and thus, enhance local reef fish biodiversity and abundance (e.g., [40]). Because of the direct physical contact between cleaners and clients, there is the potential for cleaning stations to act as hubs for microbial exchange between fish. In this study, we used a 16S rRNA gene amplicon sequencing approach to test whether clientele diversity was associated with differences in the skin microbiome of the cleaner goby *E. evelynae* in the US Virgin Islands. Overall, the results showed increasing bacterial alpha-diversity with the number of clients and client genera inspected (except in West Lameshur), as well as a positive correlation between beta-diversity and clientele diversity. Moreover, our results showed differences in alpha- and beta-diversity amongst gobies from different sampled reefs with few shared ASVs among them and high inter-individual variation in microbiota diversity and structure.

Goby cleaning activity impacts skin microbial diversity

Recent studies have shown microbial changes in fish participating in symbiotic relationships. For example, microbial composition of clownfish mucus changes with contact with its anemone host [42]. Similarly, microbial interhost dispersal in zebrafish has also shown to influence diversity and composition of microbial communities that ultimately affected host immune system [14], and a "cleaner" ecotype of the Barbadian broadstripe cleaner goby *Elacatinus prochilos* harbored higher skin microbiota diversity than "non-cleaner" ecotypes [15]. However, the mechanisms involved in those changes are

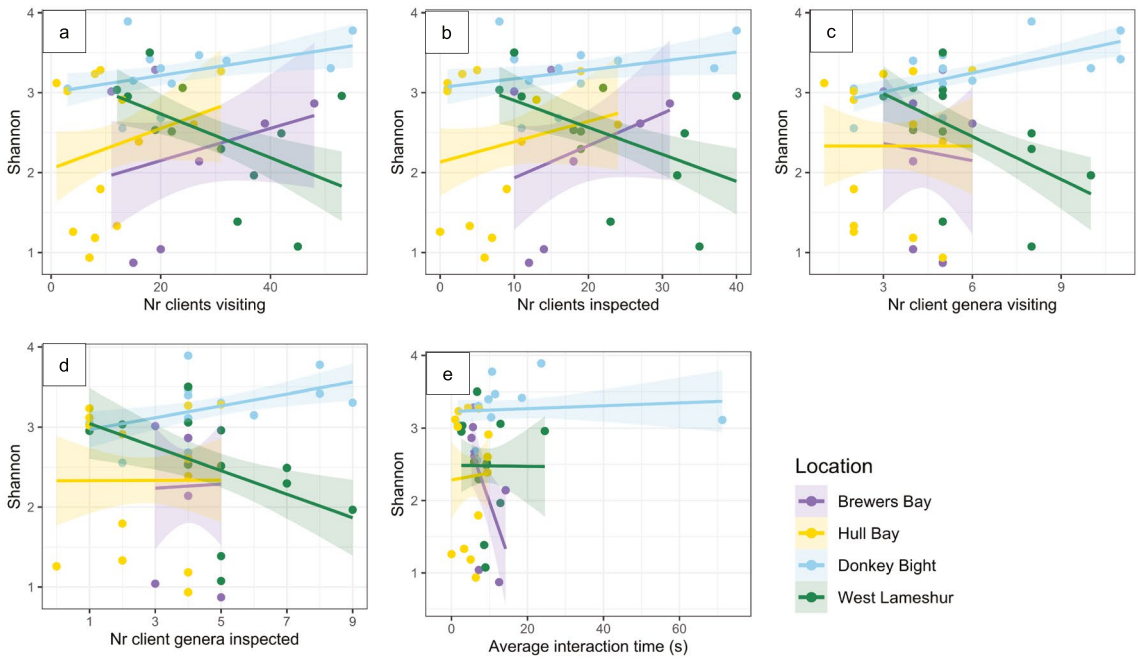

Fig. 3 Linear regression plots depicting the Shannon alpha-diversity measure versus each of the observed cleaning variables: **a** number of clients visiting the cleaning station, **b** number of clients inspected, **c** number of client genera visiting the cleaning station, **d** number of client genera inspected, and **e** average interaction time (seconds)

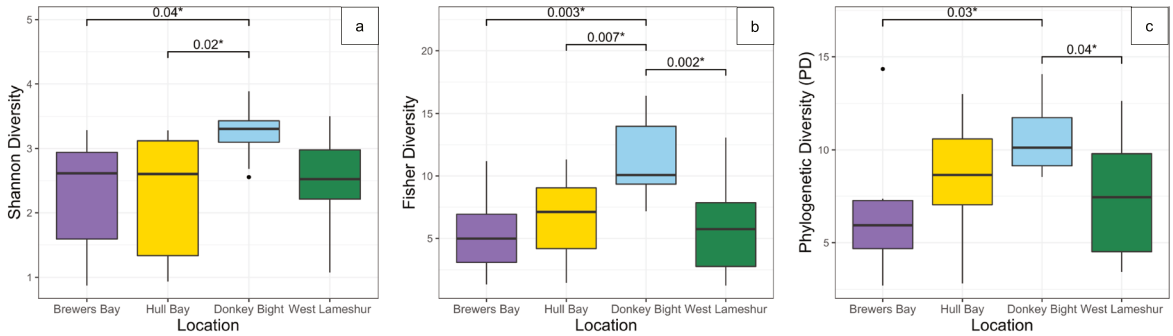

Fig. 4 Boxplots of the alpha-diversity measures for each locality with Tukey's HSD significance for pairwise differences: **a** Shannon diversity, **b** Fisher diversity, **c** Phylogenetic diversity (PD). *indicates significant differences

not yet well understood. Our results showed a positive correlation between goby skin bacterial beta-diversity and the diversity of clientele inspected (i.e., number of client genera) in all sampled reefs. Moreover, differences in clientele diversity visiting cleaning stations were also observed in our study. For example, although diversity of client species was high at West Lameshur, the most common clients on that reef were *Stegastes* damselfish, which are territorial fish only visiting cleaning stations within their territories, and usually less parasitized than other client species [24]. This could mean that despite the presence of a high diversity of fish species at West Lameshur, the territorial behavior of damselfish could have influenced which other potential clients gain access to cleaning stations located within damselfish territories. By contrast, the remaining sites showed higher visitation rates of larger fish species, such as striped parrotfish (*Scarus iseri*) in Brewers Bay, yellow goatfish (*Mulloidichthys martinicus*) in Hull Bay and ocean surgeonfish (*Acanthurus bahianus*) in Donkey Bight, all of which are "preferred" clients, likely due to larger body size and thus higher parasite burden [23, 41]. Larger

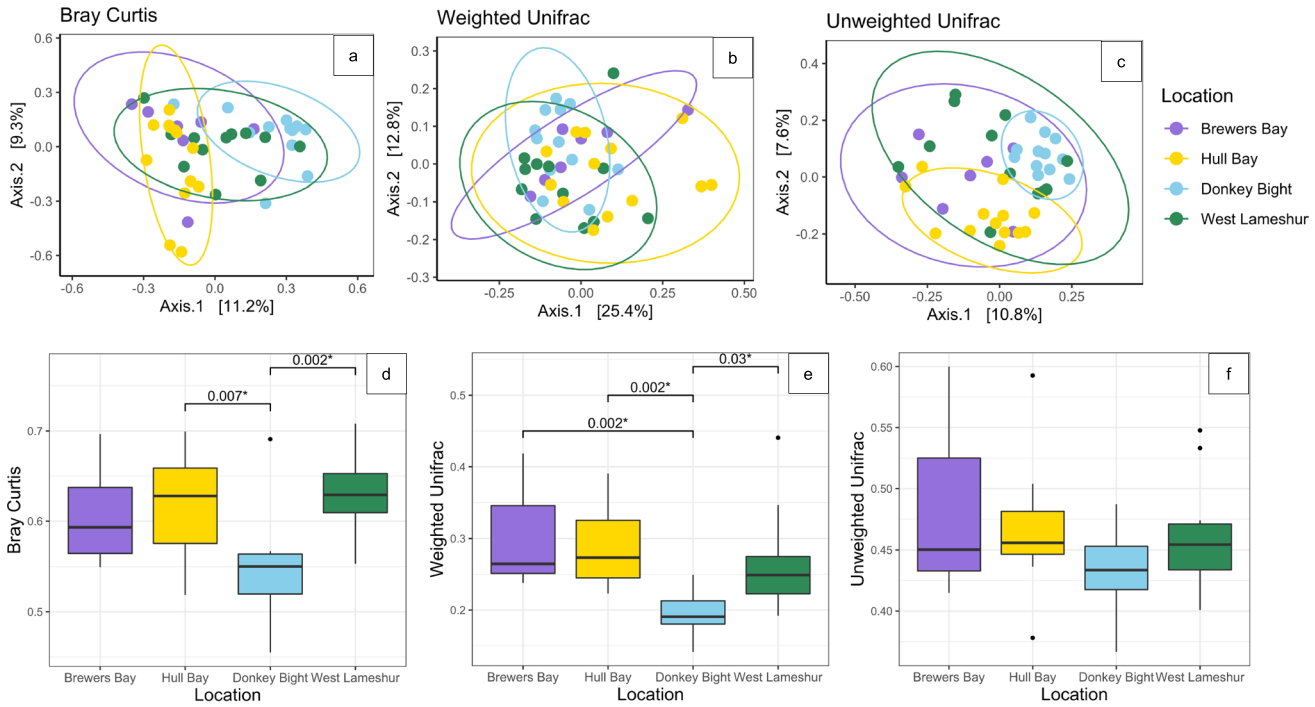

Fig. 5 **a**, **b**, **c** PCoA plots with beta-diversity distances grouped by locality with 95% confidence interval ellipse; **d**, **e**, **f** beta-dispersion represented by distance to centroid for each beta-diversity measure with pairwise differences indicated by an asterisk (*)

clients, therefore, engage in longer cleaning interactions [41], which could increase bacterial transfer. However, in our study, only client diversity was positively correlated with bacterial structure in the skin of cleaner gobies, indicating a stronger effect of client diversity compared to duration of inspection. Cleaner gobies inspect multiple client fish per day and engage in direct fish-to-fish contact [21, 44]. Although this creates the opportunity for exchange of microbes, given the interspecific nature of the interactions, actual exchange and persistence of microbes cannot be assumed. Our data, while correlative, support this hypothesis (i.e., microbial exchange increases with cleaning activity). The alternative explanation that cleaners with more diverse microbiota attract a more diverse array of clients seems less likely but cannot be ruled out without experimental manipulation.

Reef animal communities harbor some of the most diverse microbial communities of the marine environment [42] and it is estimated that the changes in host communities in a given location may impact microbial diversity in the entire reef, such as the loss of a host species due to environmental disturbances followed by a decrease in the microbial diversity of the reef [43]. In our study, we found a high diversity of clients visiting cleaning stations in West Lameshur (St. John). However, not only did cleaners from that location have similar bacterial alpha-diversity levels to the ones from Brewers Bay and Hull Bay (St. Thomas), which had lower visitation levels, but they also showed a contrasting relationship between microbial diversity and cleaning activity when compared to the gobies in all the other sampled reefs. A possible explanation for the differences found in West Lameshur might be related to the greater habitat degradation at this sampling locality [30], which could have altered local reef community dynamics.

Cleaner fish have been shown to remove significant numbers of crustacean ectoparasites from hosts [45], which could otherwise compromise client welfare by causing skin damage, feeding on blood, and acting as vectors for diseases (reviewed by [46]). Nonetheless, the gain of a seemingly easy meal for cleaners (client-gleaned ectoparasites and mucus) may come with a price: while obviously predatory clients may eat the cleaners [23], less obvious is the fact that clients may also be vectors of parasites, bacterial contamination, and consequently disease to cleaners [47]. Although frequent contact with other reef fish seems to potentiate chances of increased microbial diversity in cleaner fish, which may protect against infections [48], our study did not directly address the presence of potential pathogenic taxa, and further work should be performed to understand the potential risks of cleaning activity and their impacts on reef communities.

Goby skin microbiota varies across reef locations

Access by gobies to different reef fish species might be shaped by the level of reef degradation and therefore

influence cleaning interactions and consequently, goby microbiome. Spatial differences in skin microbiota have been reported for vertebrates, such as bats [49], amphibians [50], and marine species [51, 52], including reef fishes [5, 6]. Although our main goal was to examine the relationship between client diversity and microbial diversity, we also observed differences in goby skin microbial composition among reef sites. Interestingly, those differences include contrasting results from fish captured from different reef habitats within less than 500 m of each other. Even though they are located within the same bay, Donkey Bight and West Lameshur differ in coral cover [30]. Additionally, a mangrove swamp empties out near West Lameshur site, and therefore water quality parameters and reef communities are likely to vary among our sites [53], leading to differences in fish microbial consortia. In fact, several studies have shown that fish skin microbiota respond to changes in the physicochemical composition of the water (e.g., pH, dissolved oxygen [11] and temperature [13]).

Despite host taxonomy being considered one of the main factors influencing fish microbiomes [5], in our study, we identified high variability in the skin microbiome among *E. evelynae* individuals and no core microbiome. Our data shows that cleaner gobies share a considerably low proportion of bacterial taxa (3.5% common to all localities), even in a small geographic context (Fig. 2). Previous studies on reef fish species have found a skin core microbiome [5, 15], although fish were sampled in a small area and sampling size was also small. However, microbes are dynamic in time and space and the definition of a core microbiome may vary depending on the ecological question, and therefore different core definitions (i.e., thresholds) may include or exclude taxa [54]. Here, we have not found a core microbiome for cleaner fish at the 100% threshold (i.e., all samples share the same ASV). This suggests that the environment surrounding cleaning stations, which may also be responsible for differences in clientele diversity and abundance at each sampled site, plays an important role in cleaner fish skin microbiota.

Conclusions

This study suggests that fish-to-fish direct contact and specifically, access to a diverse clientele, influences the bacterial diversity and structures of cleaner gobies' skin. However, our study did not control for environmental factors and therefore, the extent to which microbial diversity of cleaner gobies can be influenced by the surrounding environment and social behavior needs to be further explored in controlled experimental conditions. Nonetheless, this study sets the stage for future research using cleaner gobies as models to understand microbial dynamics in coral reefs. Besides the cleaner gobies studied herein, the microbiome of other dedicated cleaners such as wrasses in Indo-Pacific reefs and the less studied but highly diverse group of cleaner shrimps [22] may also be influenced by cleaning behavior, and specifically by client diversity. Given current concerns over reef degradation worldwide and the importance of microbial commensals towards reef resilience, holistic studies examining microbial transfer to and from cleaner fish and other reef fish and the potential cascading effects deriving from such interactions are warranted. Additionally, microbial communities residing in areas surrounding cleaning stations, where fish largely congregate, should also be investigated due to their potential effects to the entire reef holobiont.

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Data availability Raw sequence reads are available in the NCBI's Short Read Archive under accession PRJNA756005.

Code availability Not applicable.

Declarations

Ethics approval Fish were collected under permit number DFW18072U from the US Virgin Islands Division of Fish and Wildlife and permit number VIIS-2018-SCI-0008 for sites within the Virgin Islands National Park, and under IACUC ethics protocol number 778227–1, PC Sikkel, PI.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest/Competing interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

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