



Assessing diets of marine fauna associated with emerging zoantharian habitats in the Canary Islands

S. Moreno-Borges¹ · V. Arranz² · S. Fernández-Martín¹ · S. Clemente¹ · R. Xavier^{3,4} · R. Vasconcelos^{3,4}

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Abstract

Population outbreaks of zoantharians in shallow water reefs have been observed globally, including intertidal and subtidal sites in the Canary Islands. This study investigated how zoantharians might be integrating into the local trophic network. For that purpose, we assessed the feeding patterns of common species in zoantharian-dominated habitats, focusing on finding consumers of *Zoanthus pulchellus* and *Palythoa caribaeorum*. Through DNA-barcoding and metabarcoding, the gut contents of 11 predatory species were analysed, aiming to characterize their diets and explore local species feeding on zoantharians. Analyses of diet revealed a diverse range of food items and trophic positions of some of the most common and frequent marine species in the archipelago. Furthermore, based on previous observations on the different impacts of *Z. pulchellus* and *P. caribaeorum* on shallow benthic ecosystems of the Canary Islands, a preliminary approach to identify their potential influence on feeding patterns of associated species was made. Even though DNA-metabarcoding did not detect zoantharians in the gut contents of any studied species, Sanger sequencing with zoantharian-specific primers indicated their consumption by subject species may be limited to only the crab *Platypodiella picta*. In addition, by focusing on some of the most common species, this study enhances our understanding of the local trophic network and provides an insight into trophic dynamics in zoantharian-dominated habitats.

Keywords Canary Islands · *Palythoa caribaeorum* · *Zoanthus pulchellus* · Gut content · Intertidal · Subtidal · DNA-metabarcoding

Introduction

Zoantharians are a group of colonial cnidarians frequently found in tropical and subtropical ecosystems. When the ecosystem is in equilibrium, they increase complexity and

diversity of the benthic communities (Soares et al. 2022). Depending on the species and environmental conditions, zoantharians can grow as a few isolated polyps or as large compacted colonies. Furthermore, many zoantharian species contain palytoxin (Moore and Scheuer 1971), a potent chemical whose ecological role remains uncertain (Guillen et al. 2020) but has been proposed to act repelling potential predators and reducing the number of organisms feeding on zoantharians (Sebens 1982; Francini-Filho and Moura 2010).

Recently, several regions around the globe have reported important population outbreaks of zoantharians in shallow water reefs (Cruz et al. 2015; López et al. 2020; Reimer et al. 2021). It has been observed that these colonial cnidarians can grow over other native benthic communities and colonize large areas, consequently homogenizing the substratum and causing important negative impacts on local ecosystems (Soares et al. 2022). Researchers proposed several factors as potential triggers for this phenomenon, such as rising sea surface temperatures (SST), seawater eutrophication,

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✉ S. Moreno-Borges
smorenob@ull.edu.es

¹ Department of Animal Biology, Edaphology and Geology, Faculty of Sciences, University of La Laguna, 38200 San Cristóbal de La Laguna, Spain

² School of Natural Sciences, Massey University, Auckland, New Zealand

³ Centro de Investigação Em Biodiversidade E Recursos Genéticos, CIBIO, InBIO Laboratório Associado Campus de Vairão, Universidade Do Porto, 4485-668 Vairão, Portugal

⁴ BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, Vairão, Portugal

and opportunistic settlement on free substratum left available by the decline of other native benthic communities (Reimer et al. 2021). Depending on the studied region, it is likely that a combination of these causes may also be acting simultaneously.

Marine environments off Macaronesian Islands are understudied and several researchers claim that more efforts should be made toward gathering basic information on species distribution and ecology (Florencio et al. 2021). In the Canary Islands, the presence of zoantharians was mentioned for the first time several decades ago (Hartog and Holthuis 1984), mainly in small and sporadic colonies on the intertidal rocky bench and shallow submerged platforms. In total, there are five species recorded in the islands belonging to the suborder Brachycnemina (López et al. 2019): *Palythoa caribaeorum*, *Palythoa* aff. *clavata*, *Palythoa grandiflora*, *Isaurus tuberculatus*, and *Z. pulchellus*.

As the eastern boundary of this subtropical archipelago is located 100 km away from the Saharan coast, the eastern islands are affected by an upwelling current of cold water, generating an SST gradient that can differ up to 2 °C from the western islands (Barton et al. 1998). Consequently, due to the environmental preferences of zoantharians, there is an observed tendency to build more extensive populations under warmer conditions on the western islands (López et al. 2020). In addition, several reports found signs of population outbreaks in intertidal and subtidal sites across the whole archipelago, causing a significant modification of associated benthic assemblages in comparison to algae-dominated systems typical of the region (González-Delgado et al. 2018; Clemente et al. 2022; Moreno-Borges et al. 2022). The main hypotheses for the spread of these cnidarians on these islands include the mixed effect of more favourable thermal conditions due to the rising SSTs during the last decades (Vélez et al. 2015), and the anthropogenic pressure that led marine ecosystems to unbalanced states, such as the occurrence of sea urchin barrens with few erect macroalgae (Hernández et al. 2008).

Ecological consequences of zoantharian-dominated habitats in the Canary Islands are still greatly unknown, but some initial studies correlated these areas with low algal coverages and changes in the biodiversity of the associated communities (González-Delgado et al. 2018; Moreno-Borges et al. 2022). From all zoantharians in the archipelago, *Z. pulchellus* and *P. caribaeorum* have shown the strongest capacity to spread over large areas (> 100 m²) and occupy large proportions of the substratum. However, *P. caribaeorum* colonies are usually more dominant in the benthos than *Z. pulchellus* (López et al. 2020). An important characteristic that differs between both zoantharians is their distinct growth and patterns of spread over the substratum (Durante et al. 2018; López et al. 2020). *P. caribaeorum* colonies present a thick coenenchyme that holds polyps together covering the

entire rocky substratum, reducing its structural complexity and leading to a loss of ecological niches for many organisms (Soares et al. 2022). Meanwhile, *Z. pulchellus* with a much thinner coenenchyme might not develop this type of cohesive layer, allowing other communities to settle and live beside or in between polyps (López et al. 2018). As a consequence, in the archipelago, *P. caribaeorum*-dominated habitats have been related to significant reductions of fish biodiversity, mainly affecting densities of herbivores and invertivores (Moreno-Borges et al. 2022). This was explained by the drastic reduction of macroalgal stands in the same area (four-fold reduction with respect to macroalgae in control habitat), as well as, to the difficulties that invertivores may find hunting small invertebrates in *P. caribaeorum* colonies (Moreno-Borges et al. 2022). Altogether, the aforementioned observations raised concerns about how zoantharians outbreaks might be leading to an unbalanced situation where none or few native species are adapted to forage in these new ecological niches, or even further, to prey on zoantharians.

In subtropical regions, where shallow ecosystems are mainly structured by macroalgal stands, zoantharians outbreaks are changing entire marine assemblages. These changes can promote a cascading effect by modifying the links of the trophic network through the replacement of macroalgal canopies. Considering the background information by Moreno-Borges et al. (2022) that characterized fish assemblages associated with mat-forming zoantharians in the Canary Islands, this study goes one step beyond characterizing the diets of common species of the Islands that are associated with zoantharian-dominated habitats (*Z. pulchellus* and *P. caribaeorum* zones). We especially aimed to detect whether any of the studied predators are feeding on zoantharians. For this purpose, DNA-barcoding and metabarcoding approaches were used to identify prey items in the gut contents of 11 common predatory species in the archipelago.

Material and methods

Study area, sampling sites, and studied species

Samples for this study were collected in Tenerife (Canary Islands, Spain). We chose four localities where zoantharian colonies occupied an area larger than 100 m² (Fig. 1). Two of these localities were intertidal benches where colonies of *Z. pulchellus* spread inside tidepools, and the other two were subtidal localities with large patches of *P. caribaeorum* colonies at 3–5 m depth.

A total of 11 predatory species, encompassing the most representative and frequent taxa at their respective habitats, were included in this study (Table 1). Approximately, 15

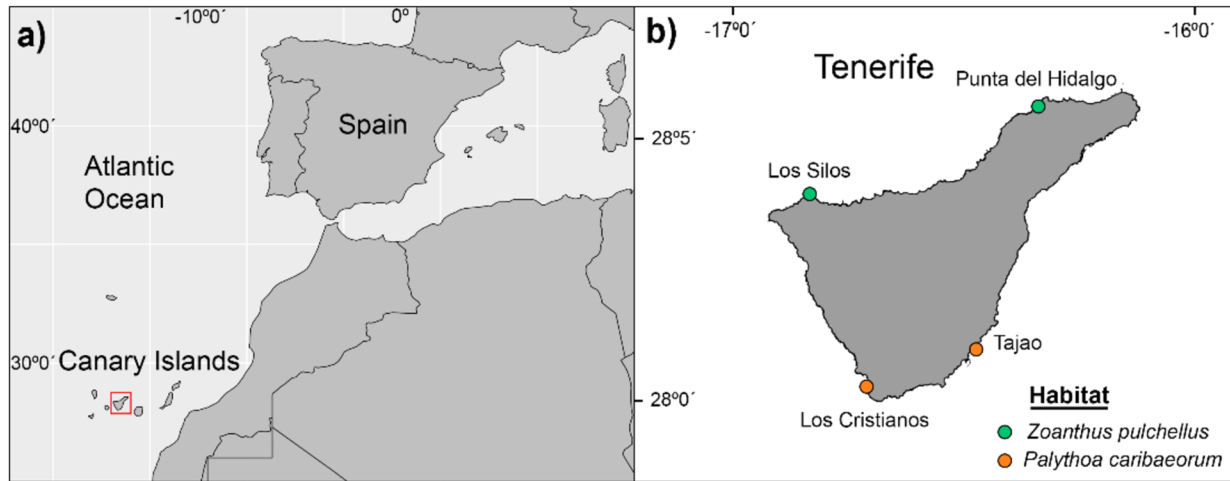


Fig. 1 Study area. **(a)** Location of the Canary Islands. **(b)** Location of the sampling sites at Tenerife, including intertidal benches with dominance of *Zoanthus pulchellus* (green) and subtidal localities within *Palythoa caribaeorum* zones (orange)

Table 1 Species collected for this study, indicating the taxonomic classification, scientific and common name, trophic role found in bibliography, distribution

Phylum	Class	Species				Habitat	
		Scientific name	Common name	Trophic role	Distribution	Z	P
Mollusca	Gastropoda	<i>Stramonita haemastoma</i>	Red-mouthed rock shell	Invertivore	A, EP, M	17	–
Annelida	Polychaeta	<i>Eurythoe complanata</i>	Iridescent fire-worm	Carnivore, opportunist	A, IP, M	17	–
Arthropoda	Malacostraca	<i>Pagurus anachoretus</i>	Hermit crab	Omnivore, opportunist	M, Mac	18	–
		<i>Palaemon elegans</i>	Rockpool shrimp	Omnivore, detritivore	EA, M	14	–
		<i>Cronius ruber</i>	Red swimcrab	Carnivore generalist	A, EP	–	7
		<i>Platypodiella picta</i>	Harlequin crab	Invertivore zoanthivore	SEA	–	13
Echinodermata	Holothuroidea	<i>Holothuria sanctori</i>	–	Detritivore	EA, M	14	15
Chordata	Actinopterygii	<i>Parablennius parvicornis</i>	Rockpool blenny	Herbivore	Mac	15	–
		<i>Thalassoma pavo</i>	Ornate wrasse	Invertivore	EA, M	14	16
		<i>Mauligobius maderensis</i>	Madeira goby	Invertivore	Mac	10	–
		<i>Similiparma lurida</i>	Canary damsel	Omnivore	Mac	14	12

A Atlantic, EA Eastern Atlantic, EP Eastern Pacific, IP Indo-Pacific, M Mediterranean Sea, Mac Macaronesia, SEA South-East Atlantic), and number of samples in each type of habitat (Z area dominated by *Z. pulchellus*, and P, by *P. caribaeorum*)

specimens of each species were collected in each type of habitat, although given the complexity of collecting such a varied diversity of species it was not possible to collect an even number of specimens per species across each two sites within the same habitat nor an equal number of total species studied in each habitat (Table 1). Sampling was carried out during August 2021 and, due to the wide variety of studied taxa, different methods of collection were used. Most invertebrates were collected by hand, sometimes with the help of snorkelling or SCUBA gear, while for the pantropical crab, *Cronius ruber*, pots were deployed before sunset and recovered the next morning (González et al. 2017). Fish were

caught using a line with a small fishhook, always avoiding any kind of bait that could interfere with the molecular identification of the true diet components.

DNA extraction, PCR amplification and sequencing

Each predatory specimen was dissected and the gut content was carefully separated, avoiding the host tissue as much as possible. Tools were sterilised between samples to avoid cross-contamination. Furthermore, dissections and DNA extractions were performed in a dedicated room to minimize the risk of contamination. DNA was extracted from the

full gut content using DNeasy Blood & Tissue kit (Qiagen, Netherlands) following the manufacturer's instructions.

The PCR (polymerase chain reaction) approach was used to target a 313 base pairs (bp) fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) region using the primer pairs m1COIintF and jgHCO2198 (Geller et al. 2013; Leray et al. 2013) due to their versatility to bind to the DNA of a wide range of metazoan prey items. The QIAGEN® (Hilden, Germany) Multiplex PCR kit was used following the manufacturer specifications. Sample amplifications were performed following the generic touchdown protocol (Leray et al. 2013) used for this combination of primers (an initial step at 95 °C for 5 min, followed by 16 cycles of 10 s at 95 °C, and a touchdown annealing of 30 s beginning at 62 °C until 46 °C; and elongation at 72 °C for 60 s; this was followed by 24 cycles of 10 s at 95 °C, 30 s at 46 °C and 60 s at 72 °C, followed by a 10 min final elongation at 72 °C). PCR was run in triplicate and amplification success was verified on 1.2% agarose gels. After gel inspection, a second PCR procedure was used to improve the amplifications of 123 out of 196 samples. Annealing time was increased to 45 s during the touchdown and to 29 the number of posterior cycles at 46 °C annealing temperature. For the PCR, 4 µl of DNA template was added to a mix consisting of 12.5 µl of QIAGEN® Multiplex PCR Master Mix, 0.5 µl of each primer (10 µM) and 7.5 µl of UltraPure DNase/RNase-Free Distilled Water in a 25 µl total reaction volume. For each PCR batch, a negative control was included using UltraPure DNase/RNase-Free Distilled Water instead of DNA template. Samples amplified with the first or second PCR protocol are hereafter referred to as P1 and P2, respectively. PCR reactions were purified by magnetic separation following the Agencourt AMPure XP Purification protocol (Beckman Coulter, Brea, USA). PCR products were quantified (Epoch, BioTek) and diluted to an equal concentration of 5 ng/µl and pooled together into a single product. Repeated samples through P2 PCR protocol were kept as additional samples for sequencing.

Amplicon libraries were prepared following the Illumina MiSeq protocol 'COI Sequencing Library Preparation' (<https://support.illumina.com>). Finally, P1 and P2 samples were sequenced in the Illumina MiSeq sequencer using the MiSeq Reagent Kit V2 150 bp×2 (Illumina, San Diego, CA, USA). Sequencing was performed by technicians from the Centre for Molecular Analysis (CTM), at CIBIO-InBIO research facilities (Vairão, Portugal).

To maximize the possibility of zoantharians' detection in the gut samples, samples were also screened with a zoantharians-specific PCR approach using the primers (COIZP) for the COI region (López et al. 2018). The PCR protocol was adapted from López et al. (2018), with an initial step at 94 °C for 2 min, followed by 30 cycles of 60 s at 94 °C, 60 s at 60 °C and 60 s at 72 °C, followed by a 10 min final

elongation at 72 °C. PCR products obtained were checked using 1.2% agarose gel electrophoresis and positive amplifications were purified and sequenced in both directions through Sanger sequencing. DNA sequences generated were aligned using MEGA7 (Kumar et al. 2016) and blasted against GenBank (Benson et al. 2018) and BOLD (Ratnasingham and Hebert 2007) reference sequences databases.

Bioinformatic analyses of metabarcoding samples

Forward and reverse DNA sequences could not be merged since they measured 125 bp or less, and were analysed and filtered separately using the bioinformatics toolkit included in QIIME2 (Bolyen et al. 2019). First, primers were removed allowing a mismatch of 0.05% with cutadapt (Martin 2011). We used DADA2 plug-in (Callahan et al. 2016) to denoise, dereplicate, and filter out chimeras (using the consensus method) and build an ASV (amplicon sequence variants) table for each direction. ASVs passing the initial quality control steps were taxonomically assigned using the MIDORI2 reference sequence database (Leray et al. 2022). For taxonomic assignment, we first performed a BLASTn (Altschul et al. 1990) with an e-value of 1–e10 for high-quality matches using the default max_target_seqn, which recovers the first 500 possible matches (Shah et al. 2019). Then, we used MEGAN 6.24.1 (Huson et al. 2016) and the Lowest Common Ancestor algorithm for taxonomic assignment within the NCBI taxonomy framework. The taxonomic assignment obtained for reverse fragments did not add new information to forward fragments; henceforth from this step on we only used the forward reads due to their higher sequencing quality.

The decontam R package v1.4 (Davis et al. 2018) was used to filter the ASV table from possible contaminants using the prevalence method, removing all ASVs that were more prevalent in negative controls than in positive samples. To account for the expected degree of tag switching in multiplexed metabarcoding analysis (Costello et al. 2018), the abundance renormalization approach described in Wangensteen and Turon (2015) was used, by removing the reads corresponding to a cumulative frequency lower than 3% for each particular ASV from each sample.

Lastly, the ASVs were clustered into Operational Taxonomic Units (OTUs), as a proxy for species level in downstream analysis, using a 97% similarity threshold in VSEARCH v2.13.6 (Rognes et al. 2016). The resulting OTU tables were further filtered to remove sequencing artefacts or erroneous OTUs. The LULU curation algorithm (Frøslev et al. 2017) was used to collapse OTUs into their parent OTUs depending on their similarity and co-occurrence patterns. OTUs based on DNA sequences shorter than 100 bp were discarded. OTUs assigned to Phylum level or higher and non-marine organisms were considered non-informative

and manually discarded. Rarefaction curves were employed to check whether sequencing depth was appropriate (Fig. S1). Rarefaction was performed using the Phyloseq R package v1.42 (McMurdie and Holmes 2013).

All sequences were deposited in GenBank (accession number for metabarcoding raw sequences: PRJNA998031; and Sanger sequencing: OR188781-OR188787). Furthermore, they can also be found at GitHub (<https://github.com/smorenob/MetabarcodingDIETS>), together with the bioinformatic pipeline followed in this study.

Data processing and statistical analyses

For the purpose of this study, the resulting OTU table was transformed into presence/absence (PA) and the analysis of species' diets was based on frequency of occurrence (FO) of each prey species among specimens of the same species. Prior to further analyses, differences in the results obtained by different amplification protocols (P1 and P2) were checked. A permutational analysis of variance (PERMANOVA) based on Jaccard distance obtained from the OTUs PA dataset was conducted, with 'PCR protocol' as a fixed factor with two levels. The test results indicated that there were no significant differences between protocols ($F=1.53$, $p=0.11$). We used the Betadisp test to test for heterogeneity of the variances between groups ($F=0.12$, $p=0.74$) (Oksanen et al. 2022). Consequently, the OTUs PA dataset from P2 samples were combined with their respective P1 in subsequent statistical analyses.

OTUs taxonomically assigned to the studied predatory species were considered 'self-hits' and discarded (Casey et al. 2019). Diet of each study species in each habitat was reconstructed, using the FO for data visualization and paying special attention to the detection of zoantharians in their diets.

In addition, under the premise that, in the Canary Islands, *Palythoa caribaeorum* may be causing certain impact to specific trophic guilds given its stronger dominance over the substratum and monopolization of the benthic space in comparison with *Z. pulchellus* (López et al. 2020; Moreno-Borges et al. 2022), we did a preliminary approach to identify potential differences in feeding patterns of certain common species between these two studied habitats. For this purpose, diet of species that were present in both zoantharians habitats (*Thalassoma pavo*, *Similiparma lurida* and *Holothuria sanctori*) were compared based on broad prey groups. Multivariate permutational analyses of variance (PERMANOVA) were run for each set of specimens of the three species, using 'Habitat' as fixed factor and based on the Bray–Curtis dissimilarity matrix of numbers of OTUs per prey group classified by Phylum. A second set of analyses following the same procedures were conducted with prey groups classified by Order. Similarity percentage analyses

(SIMPER) with 999 permutations were used to identify significant dissimilarities in prey composition between habitats (Pinho et al. 2022).

Statistical analyses were conducted in the statistical environment R.4.0.3 (R Core Team 2020) using the vegan package v2.6–4 (Oksanen et al. 2022).

Results

DNA sequencing

Illumina sequencing produced circa 7 million sequence reads from 323 samples (196 P1 samples + 123 P2 samples + 4 negative controls). After quality filtering (primer removal, denoise, dereplication, and chimera removal) circa 5 million reads were retained with a modal sequence length of 125 bp and a mean of 104 bp. From this dataset, blanks meant circa 10,000 reads that were used to identify 28 ASVs as contaminants. The final dataset after removing possible contaminants and accounting tag switching consisted of 2006 ASVs, with an average of 13,680 sequence reads per sample. ASVs were clustered into OTUs at 97% similarity, producing 1076 OTUs. After collapsing highly similar OTUs into their parent OTU, and manually discarding non-informative OTUs, a total number of 326 OTUs remained.

In the case of the predatory species *Cronius ruber* and *Platypodiella picta* almost all the reads (> 99%) were 'self-hits' and the remaining 1% corresponded to non-informative OTUs, therefore they were not considered in further analyses.

Diet in habitats dominated by *Z. pulchellus*

A total of 253 OTUs were detected in the guts of species collected in rockpools dominated by *Z. pulchellus*, including 14 phyla, 28 classes, and 79 orders. In general, largest FOs by phyla in the composition of diets in this habitat were: arthropods (26.5%), red algae (Rhodophyta) (19.5%), and brown algae (Ochrophyta) (18.3%), followed by annelids (11.1%), and molluscs (10%). Red algae was the phylum with the largest diversity (103 OTUs), followed by arthropods (48 OTUs), molluscs (34 OTUs), brown algae (22 OTUs), annelids (18 OTUs), while there were only two OTUs taxonomically assigned to bryozoans.

The lowest OTU diversity was observed in the gut of the rockpool shrimp *Palaemon elegans* (nine OTUs assigned to five phyla), while the canary damsel *Similiparma lurida* had the highest dietary diversity in this habitat (123 OTUs assigned to 10 different phyla) (Table 2). Most diet items of *Holothuria sanctori*, *Parablennius parvicornis* and *Pagurus anachoretus* were taxonomically assigned to algae (FO > 70%; Fig. 2a), mainly brown (Ochrophyta) and red

Table 2 Mean number of OTUs by Phylum of prey items detected in the guts of the studied predators in each type of zoantharian-dominated habitat (*Z. pulchellus* and *P. caribaeorum*)

PHYLUM	<i>Z. pulchellus</i>									<i>P. caribaeorum</i>		
	<i>H. s</i>	<i>P. p</i>	<i>P. a</i>	<i>S. h</i>	<i>S. l</i>	<i>E. c</i>	<i>P. e</i>	<i>T. p</i>	<i>M. m</i>	<i>H. s</i>	<i>S. l</i>	<i>T. p</i>
Chlorophyta	1 (0.8)	–	0.6 (0.9)	–	–	0.1 (0.3)	–	–	–	–	–	–
Rhodophyta	0.4 (0.7)	2.9 (4.5)	0.4 (0.8)	1 (1.3)	13.5 (8)	0.4 (0.7)	0.1 (0.4)	0.1 (0.4)	–	–	1.3 (2)	–
Ochrophyta	0.7 (0.7)	1 (1.6)	0.3 (0.5)	0.7 (1.6)	0.1 (0.4)	0.3 (0.5)	0.1 (0.4)	–	–	0.2 (0.4)	0.5 (1.3)	–
Nematoda	–	–	–	–	–	–	–	–	0.2 (0.4)	–	–	–
Nemertea	–	–	–	–	0.3 (0.5)	–	–	0.1 (0.4)	0.5 (0.5)	–	–	0.4 (0.6)
Platyhelminthes	–	–	–	–	0.1 (0.4)	–	–	0.3 (0.5)	0.1 (0.3)	–	–	0.4 (0.5)
Annelida	0.1 (0.3)	0.3 (0.5)	0.1 (0.4)	0.1 (0.3)	2.1 (1.3)	0.1 (0.3)	0.3 (0.5)	1.2 (1)	0.7 (0.8)	0.1 (0.3)	1.2 (0.9)	1 (1.3)
Porifera	–	–	–	–	0.5 (0.7)	–	–	–	–	–	–	–
Bryozoa	–	0.1 (0.3)	–	–	0.1 (0.3)	–	–	–	0.1 (0.3)	–	–	–
Arthropoda	0.3 (0.5)	0.3 (0.5)	0.1 (0.4)	1 (1.2)	1.1 (1.2)	0.8 (0.7)	0.7 (0.5)	4.4 (1.9)	4.7 (2.1)	0.4 (0.5)	1.9 (1.7)	7.7 (4.3)
Rotifera	–	–	0.1 (0.4)	–	–	0.1 (0.4)	–	–	–	–	–	0.2 (0.4)
Cnidaria	–	–	–	–	0.4 (0.7)	0.1 (0.3)	–	0.1 (0.4)	–	–	0.2 (0.4)	0.1 (0.3)
Echinodermata	–	–	–	–	–	–	–	0.5 (0.7)	0.1 (0.3)	–	–	0.8 (0.8)
Mollusca	0.2 (0.4)	0.2 (0.4)	0.1 (0.3)	–	0.9 (1.1)	0.2 (0.4)	0.3 (0.5)	1.6 (1.3)	0.8 (1)	0.6 (0.7)	0.5 (0.5)	1.6 (1.3)

Standard deviation is shown in brackets. (*H. s* *Holothuria sanctori*; *P. p* *Parablennius parvicornis*; *P. a* *Pagurus anachoretus*; *S. h* *Stramonita haemastoma*; *S. l* *Similiparma lurida*; *E. c* *Eurythoe complanata*; *P. e* *Palaemon elegans*; *T. p* *Thalassoma pavo*; *M. m* *Mauligobius maderensis*)

algae (Rhodophyta), and a lower percentage of green algae (Chlorophyta). *Stramonita haemastoma*, *S. lurida*, *Eurythoe complanata*, and *P. elegans* showed mixed diets including algae and invertebrates, such as arthropods and annelids. *Thalassoma pavo* and *Mauligobius maderensis* were the only predatory species that showed a diet mostly based on a wide diversity of invertebrates with large occurrences of arthropods, molluscs, and annelids.

Diet in habitats dominated by *P. caribaeorum*

A total of 118 OTUs, that were taxonomically assigned to 10 phyla, 23 classes, and 52 orders, were detected in the gut samples of species collected from the habitats dominated by *P. caribaeorum*. In this habitat, most OTUs were taxonomically assigned to arthropods (49 OTUs) followed by molluscs (19 OTUs), annelids (18 OTUs), red algae (13 OTUs), and brown algae (7 OTUs), while only one OTU was assigned to platyhelminths. In terms of FOs, the most relevant groups in diets were arthropods (31%) followed by molluscs (19.9%), brown algae (17.5%), and annelids (14.6%).

In the *P. caribaeorum* zone, the lowest diet diversity was observed in the holothurian *H. sanctori* (15 OTUs of 4 phyla), followed by the canary damsel *S. lurida* (48 OTUs of 6 phyla), and the ornate wrasse *T. pavo* with the highest diet diversity (83 OTUs assigned to 8 phyla) (Table 2). *H. sanctori* showed the highest proportion of algae (FO = 41.2%), specifically brown algae

(Fig. 2a), with arthropods and molluscs also as important diet components, when associated to this habitat. The canary damsel *S. lurida* had a mixed diet composed by red and brown algae, as well as molluscs, annelids, and arthropods. Some specimens also had cnidarians (4.5%), more specifically hydrozoans. Finally, the diet of *T. pavo* included a wide variety of invertebrates, with arthropods being the most frequent diet item found in their guts (42.2%). Cnidarians identified as anthozoans were also found (FO < 1%).

Identifying predators of zoantharians

Result from the metabarcoding approach indicated that the gut content of several specimens of *T. pavo*, *S. lurida*, and *E. complanata* contained cnidarian taxa (Table 2). Most of the cnidarian OTUs ($N=6$) were assigned to hydrozoan orders, but some OTUs were assigned to anthozoans identified as actinarians ($N=1$) and alcyonaceans ($N=1$). There were no OTUs taxonomically assigned to zoantharians using the DNA-metabarcoding approach and universal primers. However, results from DNA sequences obtained with Sanger sequencing using the zoantharian-specific COIZP primers showed that *P. picta* (exclusively collected in *P. caribaeorum* habitats) was the only studied species that had zoantharian DNA in its gut. Seven out of the 13 specimens of *P. picta* were positive for the presence of zoantharian, taxonomically assigned to *P. caribaeorum*.

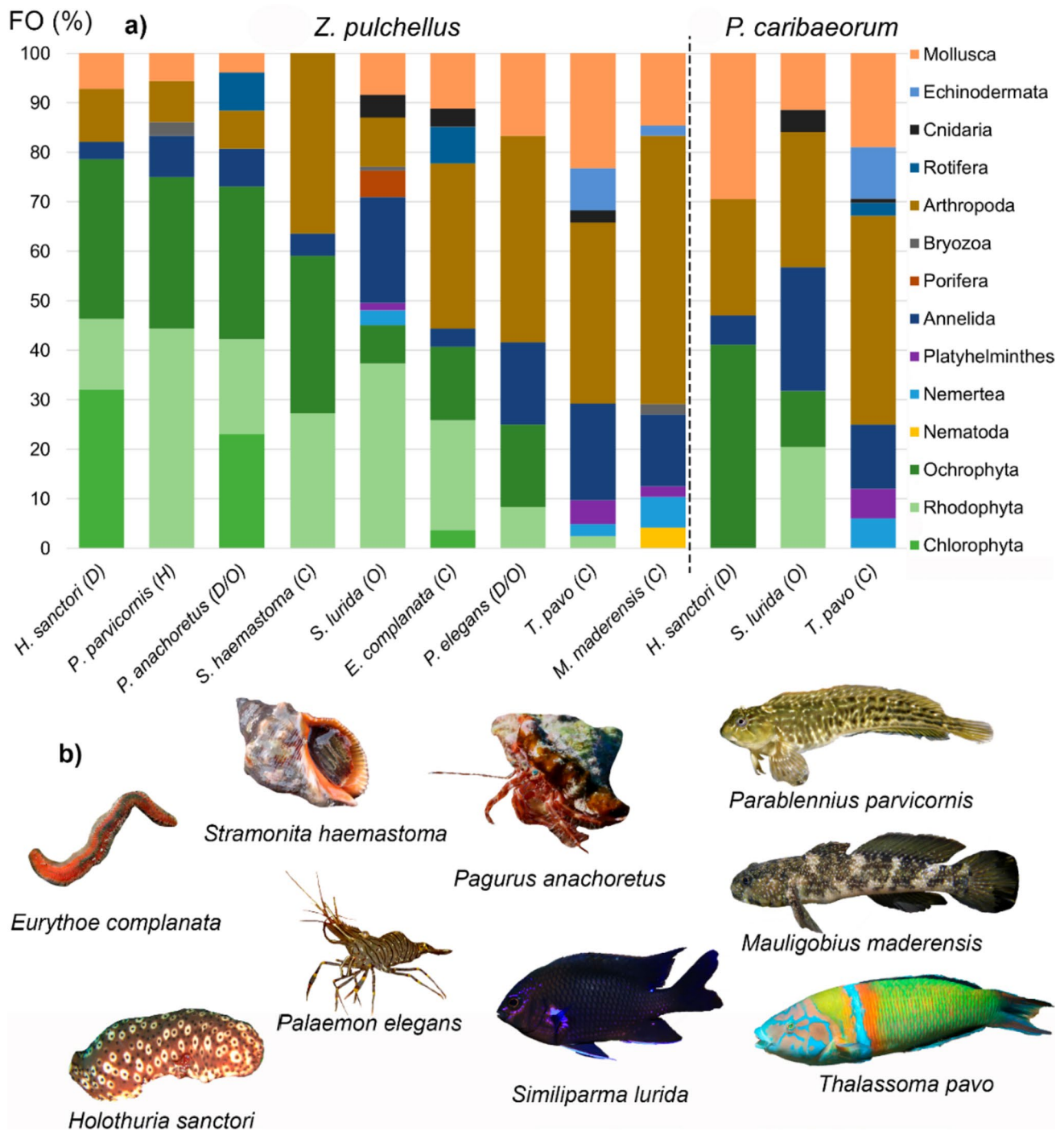


Fig. 2 DNA metabarcoding results at Phylum level. (a) Frequency of occurrence (FO) of diet items in the guts of studied predatory species found in habitats dominated by *Z. pulchellus* and *P. caribaeorum*. Letters next to predatory species names denote trophic role found in

bibliography (*D* detritivores, *H* herbivores; *O* omnivores, *C* carnivores/invertivores). (b) Images of each predator included in the bar plot

Diet diversity in habitats dominated by *Zoanthus* vs. *Palythoa*

Analyses of variance comparing the OTU diversity in the diets of *H. sanctori*, *S. lurida*, and *T. pavo* between

zoantharian habitats indicated significant or marginally significant differences for all the species regardless of grouping diet items by Phylum or Order (Table 3). Thus, results indicated different feeding patterns of the same species in each habitat.

Table 3 One-way permutational ANOVAs assessing differences in OTU diversity in the diet of *H. sanctori*, *S. lurida*, and *T. pavo* between habitats dominated by the zoantharian *P. caribaeorum*, and by *Z. pulchellus*

	Predator	df	MS	pseudo-F	P(perm)
Phylum	<i>H. sanctori</i>	1	1.48	5.67	0.003
	Residual	18	0.26		
	<i>S. lurida</i>	1	1.53	9.06	0.001
	Residual	22	0.17		
Order	<i>T. pavo</i>	1	0.32	3.23	0.025
	Residual	28	0.09		
	<i>H. sanctori</i>	1	1.37	4.43	0.003
	Residual	18	0.31		
	<i>S. lurida</i>	1	1.36	5.07	0.001
	Residual	22	0.27		
	<i>T. pavo</i>	1	0.37	1.74	0.073
	Residual	28	0.21		

Regardless the predatory species studied, diversity of consumed algae was larger at *Z. pulchellus* rockpools than at habitats dominated by *P. caribaeorum*, especially of red algae (Fig. 3). On the other hand, dietary diversity of invertebrates at each habitat varied between phyla (Fig. 3a), with amphipods and isopods particularly diverse in the diets of *T. pavo* from *P. caribaeorum* habitats (Fig. 3b).

Discussion

This study proved the lack of predation pressure upon mat-forming zoantharian species, to substantially control emerging zoantharian-dominated systems in shallow benthic habitats off the Canary Islands. Just one predatory species, the harlequin crab *Platypodiella picta*, was identified by applying molecular techniques. Nevertheless, the study also provided an overview of the diets of several common marine species in the Canary Islands, some of which have global distributions and others are restricted to the Macaronesia region. It is the first study to use DNA-metabarcoding techniques to examine the diets of a wide range of marine species in the Macaronesia, addressing significant knowledge gaps in this region (Florencio et al. 2021). Previous knowledge about the diets of most studied species was primarily based on occasional field observations or visual inspection of digestive tract contents (Hartog and Holthuis 1984; Navarro et al. 2013). However, these methods are limited by the inability to identify highly digested contents or accurately determine prey items in the case of predators consuming small or inconspicuous organisms (Francini-Filho and Moura 2010; Gil et al. 2020).

DNA-metabarcoding results showed that there were specimens containing cnidarians in their guts, but these were

taxonomically assigned to hydrozoans, actiniarians, and alcyonaceans and no zoantharians were detected. Nevertheless, targeted PCR using species-specific primers revealed that, among the studied species, the harlequin crab *P. picta* was actively feeding on *P. caribaeorum*. Highly versatile primers, such as m1COIntF and jgHCO2198 (Leray et al. 2013), can be of great utility to characterize diet diversity, however they clearly miss some groups like zoantharians (López et al. 2018). In this sense, as any other technique, DNA-metabarcoding also present some drawbacks (Sousa et al. 2019), and results must be interpreted accordingly. Some other issues to be consider are the inability of this technique to exclude secondary predation, especially when analysing broad diets, such as those of omnivores (Tercel et al. 2021), and the necessity to include internal controls and replication to minimize the occurrence of erroneous sequence due to protocol errors and/or cross-contamination between samples and other exogenous sources (De Barba et al. 2013).

Results highlighted main feeding habits of common species of the Canary Islands, useful for a general characterization of diets (herbivores, carnivores, omnivores, detritivores), but taking into account that specimens were collected in greatly transformed habitats by the proliferation of zoantharians on the substratum. These feeding patterns hinted by the observed diversity and FOs of prey items matched, in general terms, with main trophic roles attributed by previous bibliography to each of the studied species. However, some unprecedented findings will also contribute to expand the current knowledge on feeding habits of certain species. The rockpool blenny *P. parvicornis*, typically considered an herbivore (Zander 1986), contained remnants of annelids, bryozoans, arthropods, and molluscs in their gut contents, suggesting that they do not exclusively rely on herbivory. The canary damsel *S. lurida* had the most diverse diet, but until now little information was available regarding its feeding ecology, aside from being categorized as omnivorous (Quignard and Pras 1986a). Lastly, the wrasse *T. pavo* has been classified as a strict carnivore (Quignard and Pras 1986b), but a small presence of algae (<5%) in specimens from rockpools was detected, which could be a result of non-targeted intake while preying in the algae canopy. On the basis of the limited information on feeding patterns of these species, these observations could be interpreted as common broad diets but first-detected thanks to the use of molecular techniques or, alternatively, as changes in diets due to the new conditions found at zoantharian-dominated habitats. In any case, these findings demonstrate the inherent capability of each of these species to exploit wider resources and play more versatile ecological roles than previously thought.

Furthermore, although no comparisons with other habitats outside zoantharian-dominated habitats were performed here, diet comparisons of *H. sanctori*, *S. lurida*, and *T. pavo*

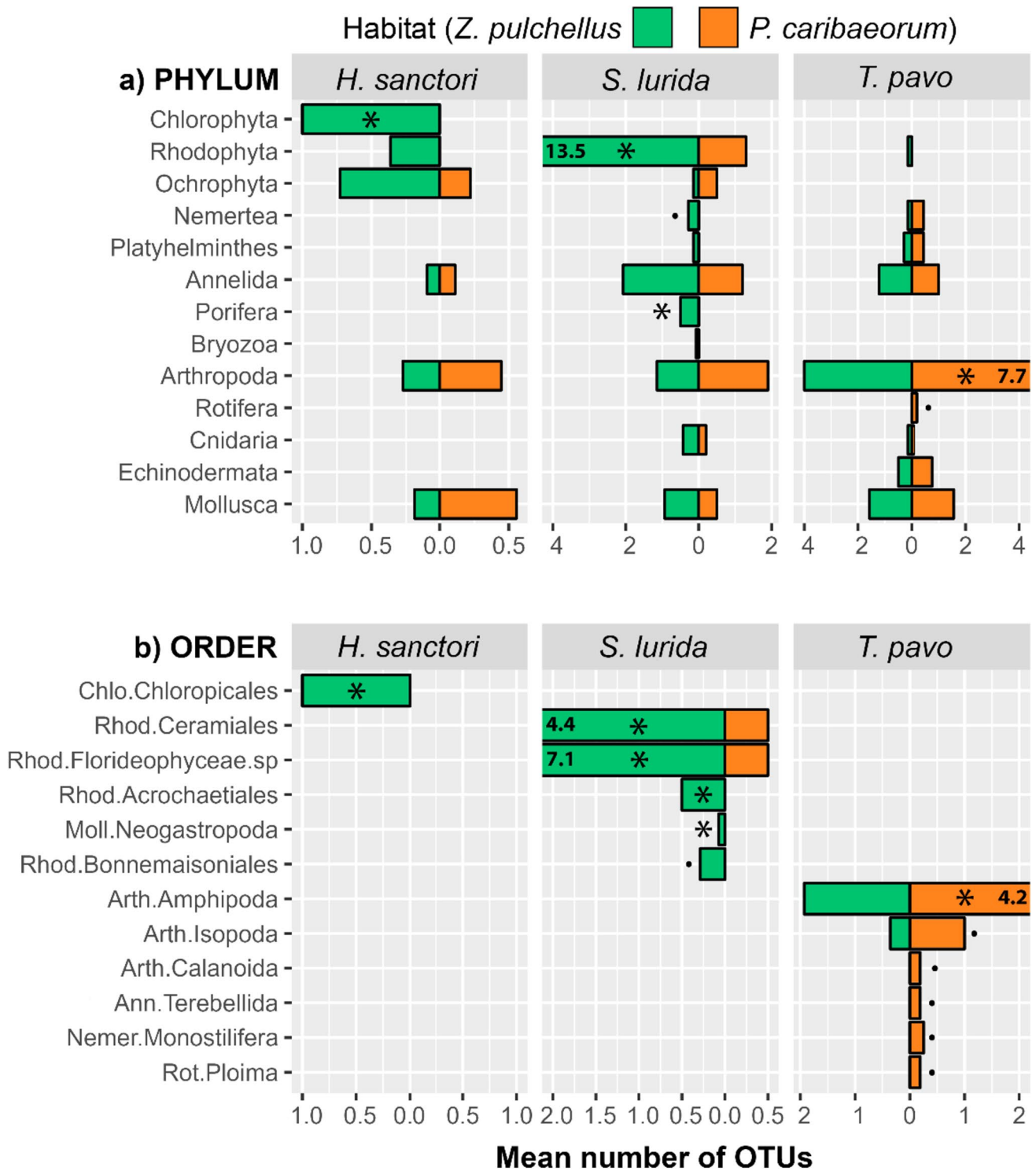


Fig. 3 Bar plots of average number of OTUs in the diet of *H. sanctori*, *S. lurida* and *T. pavo* between habitats dominated by *P. caribaeorum* and by *Z. pulchellus*. Data are shown by (a) Phylum and (b) Order in each habitat. Given the total number of different orders reg-

istered, only those that showed significant (*, $P < 0.05$) and almost significant (•, $P < 0.1$) dissimilarities in SIMPER analysis between habitats are shown. Large values were written if not fitted within the graph range

between *Z. pulchellus* and *P. caribaeorum*-dominated habitats showed three main differences: (1) lower red algae diversity and FO in *H. sanctori* and *S. lurida* when associated to *P. caribaeorum* extensions; (2) invertebrate prey were more diverse in the diet of *S. lurida* associated to *Z. pulchellus* dominated habitats with the exception of arthropods; (3) *T. pavo* showed a similar diet at both habitats, but arthropods were more diverse in *P. caribaeorum* habitats, mainly due to higher diversity of isopods and amphipods. Taking into account that these comparisons were based on wide general prey items groups (at Phylum and Order level), these results might be interpreted in terms of differences in the availability of prey between studied habitats, which may come either from the environmental differences that characterize each of the studied areas, or the potential effects of zoantharian proliferations on local benthic ecosystems and their different strategies building colonies over the substratum (Durante et al. 2018; López et al. 2020; Clemente et al. 2022), or a combination of both. In this sense, population outbreaks of *P. caribaeorum* have shown a stronger dominance over the substratum than *Z. pulchellus*, mainly by smoothening the rock-bed surface and leaving few free space for other competing benthic communities (González-Delgado et al. 2018; Moreno-Borges et al. 2022; Soares et al. 2022). In fact, certain concern about the integrity of coastal food webs was raised on previous studies that reported significant impacts of zoantharian dominance in local communities, showing alterations in species composition and trophic guilds of associated communities (Cruz et al. 2015; González-Delgado et al. 2018; Moreno-Borges et al. 2022). Predatory species here studied have shown feeding patterns that fit with their main trophic roles, although, further studies specifically assessing changes in the trophic network between zoantharian-dominated habitats and macroalgal stands will be needed to fully conclude about whether zoantharians dominance is determining availability of prey or even feeding habits of predators.

So far, the fact that the only confirmed species feeding on zoantharians in the Canary Islands is *P. picta*, may denote a lack of predation pressure on these cnidarians. In other regions where zoantharians have been an important part of the ecosystems for a long time, there are records of several species preying on them (Francini-Filho and Moura 2010). The sergeant damsel *Abudefduf saxatilis* is one of these species, and it is also among the new-arrived tropical species to the archipelago (Pajuelo et al. 2016). Therefore, zoantharian habitats may provide an adequate ecological niche for these new-arrived species to establish. In fact, given the estimations of rising SST for the next years (IPCC 2022) and the increasing anthropogenic impacts, more outbreaks of thermophilic zoantharians can be expected throughout the Canary Islands and other subtropical regions (López et al.

2020; Reimer et al. 2021). Consequently, this might provide opportunities for migrating species to establish in habitats where they can compete with local organisms. Future studies following the evolution of zoantharian-dominated habitats during the next years will be essential to understand the extent of their ecological impact on the local communities.

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Author’s contributions Sabrina Clemente, Raquel Xavier, Raquel Vasconcelos and Sergio Moreno-Borges contributed to the study conception and design. Material preparation and data collection was performed by Sonia Fernández-Martín and Sergio Moreno-Borges. Analyses were performed by Raquel Xavier, Raquel Vasconcelos, Vanessa Arranz and Sergio Moreno-Borges. The first draft of the manuscript was written by Sergio Moreno-Borges and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All sequences were deposited in GenBank (accession number for metabarcoding raw sequences: PRJNA998031; and Sanger sequencing: OR188781-OR188787), furthermore they can be

also found at GitHub (<https://github.com/smorenob/MetabarcodingDIETS>) together with the bioinformatic pipeline followed in this study.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study had the authorization from the Ministry for the Ecological Transition and Demographic challenge from the Spanish government (ref SGBTM/BDM/AUTSPP/50/2021). Ethic guidelines for the collection of studied species here were followed.

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