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Export of bathyal benthos to the Atlantic through the Mediterranean outflow: Sponges from the mud volcanoes of the Gulf of Cadiz as a case study

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ABSTRACT

The Mediterranean is a semi-enclosed sea, with a narrow natural connection —the Strait of Gibraltar— through its western basin to the North Atlantic. Many studies have investigated how the inflow of North Atlantic Surface water into the Mediterranean shapes the faunal composition and abundance of the shallow-water benthic communities of the Western Mediterranean. However, the reverse effect remains little explored, that is, at what level the relatively deep (>200 m deep) outflow of Mediterranean water (MOW) exports bathyal Mediterranean benthos into the North Atlantic and what is the fate of the exported fauna. In this study, we have investigated that process, using the bathyal sponge fauna known from a total of 9 biogeographical areas in the Northeastern Atlantic and 9 in the Western and Central Mediterranean, which accounted for a total of 456 spp. Prior to this general analysis, an exhaustive description of the bathyal sponge fauna (82 spp.) associated to 8 mud volcanoes located in the Gulf of Cadiz (Eastern North Atlantic) was conducted. This was necessary because the bathyal sponge fauna in the North Atlantic zone adjacent to the Strait of Gibraltar remained relatively poorly studied and that situation hindered relevant comparisons with the much better known bathyal fauna of the Western Mediterranean. The results of the clustering, ordination and regression analyses first revealed that the bathyal sponge fauna described from the mud volcanoes field in the Gulf of Cadiz was not essentially different from that previously described in pre-existing studies of other bathyal environments in the Gulf of Cadiz. The large scale subsequent assessment across the Atlantic-Mediterranean biogeographical gradient revealed that the sponge faunas of all Western Mediterranean areas form a relative cohesive group, except for the idiosyncratic nature of the Tyrrhenian Sea. More importantly, the deep-sea sponge fauna of the Gulf of Cadiz (in the easternmost Atlantic side of the Atlantic-Mediterranean gradient) showed more affinity with the fauna of the Western Mediterranean than with the fauna of the remaining Northeastern Atlantic areas considered in the study (i.e., Cape Verde, Canary Islands, Madeira, the Moroccan slope, Lusitanian Banks, Southern Azores Banks and Azores). The Mediterranean area with the highest faunal similarity to the Gulf of Cadiz was the Alboran Sea, followed by the Gulf of Lion, the Strait of Sicily and, the Gulf of Taranto, sharing collectively about 17% of their species. These patterns of faunal affinities clearly illustrate the importance of the MOW in transporting components of the Mediterranean deep-sea sponge communities towards the bathyal communities of the Gulf of Cadiz. The contrasting low faunal affinity between the deep-water sponge fauna of the Gulf of Cadiz and the remaining North Atlantic areas considered in the analyses also revealed that the Mediterranean faunal export is largely circumscribed to the Gulf of Cadiz. It is likely that the North-Atlantic trajectory of the MOW, turning north after the Strait of Gibraltar and staying attached to the slope of the Iberian margin, hinders subsequent colonization of the slopes of the Macaronesia region by the deep-water Mediterranean sponges exported to the Gulf of Cadiz. The results of this study, combined with previous literature on biogeographical sponge transport by marine currents, suggest that the sponge fauna provides a useful tool to reveal the future shifts in the biogeographic patterns predicted in our man-impacted and changing ocean.

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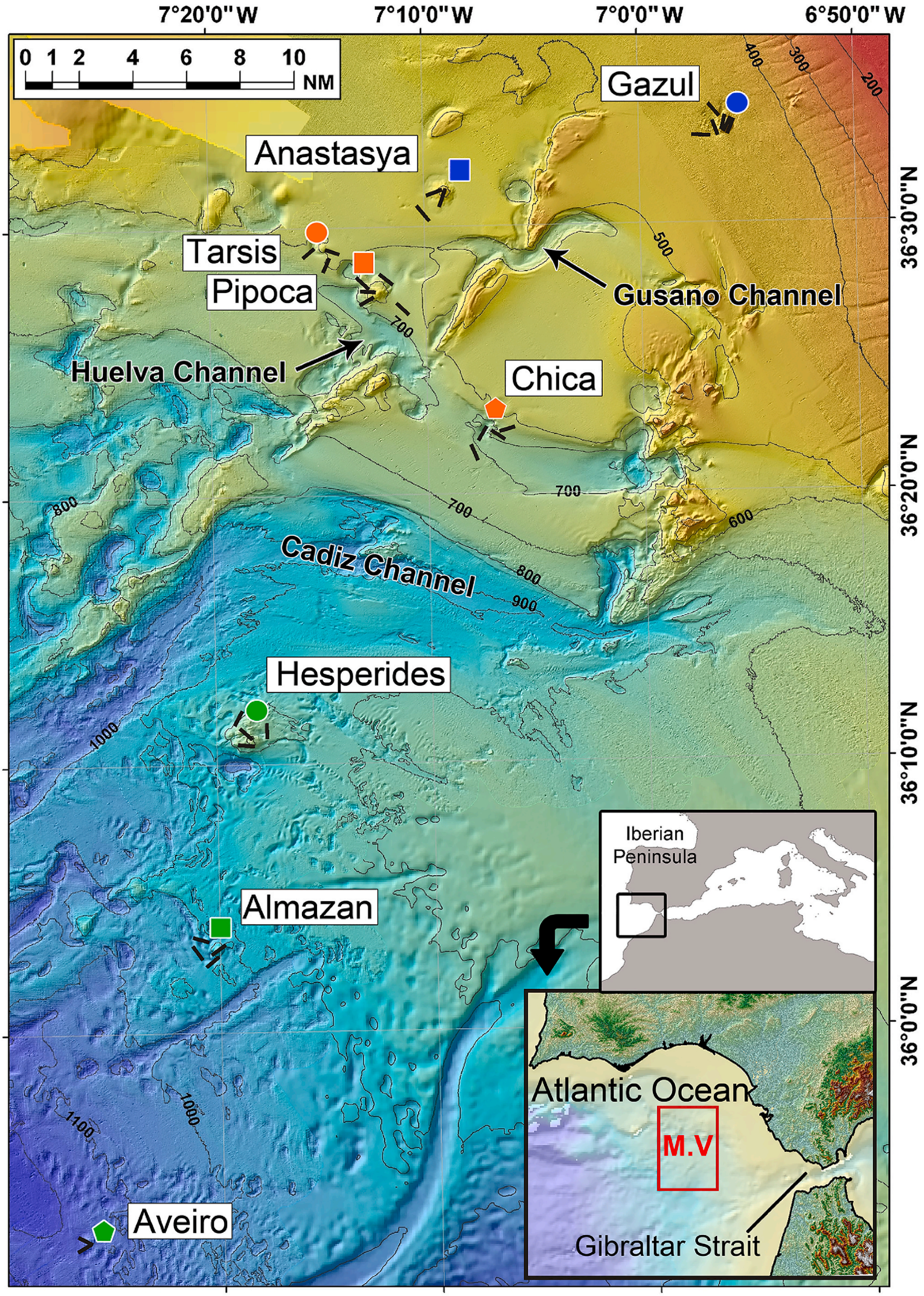

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Fig. 1. Map showing the location of the eight sampled mud volcanoes investigated in the mud volcano fields of the Gulf of Cadiz. The insets show the general location of the mud volcanoes into the Atlantic-Mediterranean at two different spatial scales. The mud volcanoes (indicated by color symbols in the largest map) were located in zones across three depth ranges: upper zone (blue symbols; 300–550 m), intermediate zone (orange symbols; 600–730 m) and deep zone (green symbols; 800–1200 m). The black lines indicate the location of beam trawl transects where the sponges were collected. The arrows indicate the location of some of the erosive channels produced by the Mediterranean Outflow Water (MOW) on the Gulf of Cadiz slope.

1. Introduction

The Northeastern Atlantic Ocean and the western Mediterranean Sea are connected through the Strait of Gibraltar. The North Atlantic Surface (NAS) water (0 to about 150 m depth) enters the westernmost area of the Mediterranean (i.e., Alboran Sea), while the Mediterranean Outflow Water (MOW) runs below (at 200–300 m) in the opposite direction and spreads along the North-Atlantic Gulf of Cadiz, divided in two main branches at depths of c.a. 400–800 m and 1200–1500 m (Madelain, 1967; Zenk and Armi, 1990; Baringer and Price, 1999; Gasser et al., 2017; Sánchez-Leal et al., 2017), flowing close to the sea bottom. Two Mediterranean water masses contribute to the MOW: the Mediterranean

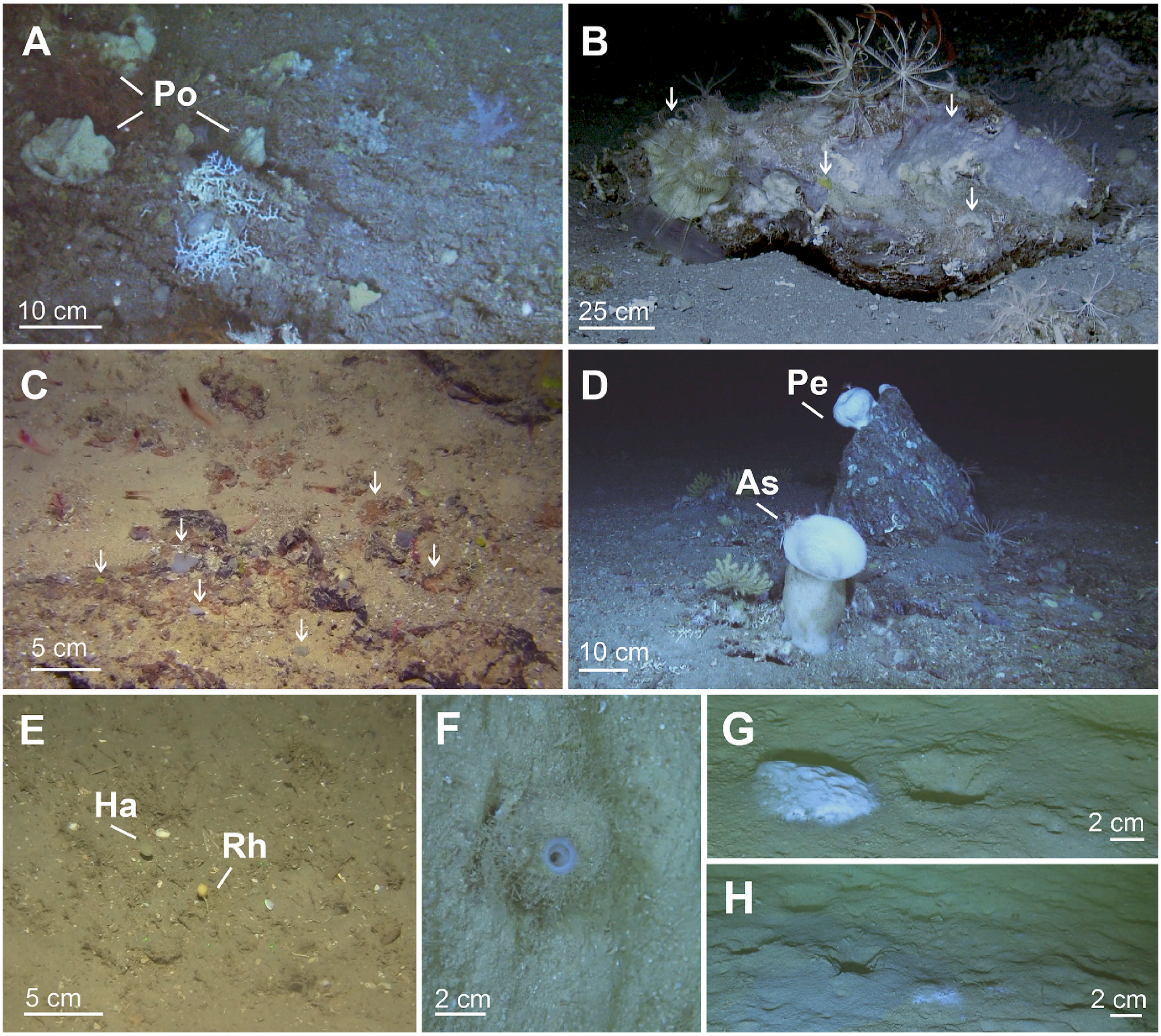

Fig. 2. Images of the sponge fauna of the mud volcanoes recorded by the Liropus ROV. **(A–B)** The hard substratum offered by the abundant carbonate slabs at Gazul mud volcano facilitated a rich benthic fauna of filter and suspension feeders. The sponge *Poecillastra compressa* (Po) is common in picture **A.** Likewise, abundant unidentifiable sponges occur on the carbonate slab in picture **B**, which are marked by arrows. **(C)** Carbonate slabs at Hesperides mud volcano showing a variety of small unidentified sponges (marked with arrows). **(D)** Bottom at Pipoca mud volcano characterized by predominant soft sediment with scarce carbonate slabs, which host the sponges *Asconema setubalense* (As) and *Petrosia crassa* (Pe). **(E)** Soft bottom at Chica mud volcano showing two different lollypop-shaped sponge species, *Haliclona pedunculata* (Ha) and *Rhyzaxinella pyrifera* (Rh). **(F)** Soft bottom at Almazan volcano showing the hexactinellid *Pheronema carpenteri,* an abundant species at this volcano. **(G–H)** Soft bottoms at Anastasya mud volcano deprived from sponges and showing white mats of sulphur-reducing bacteria, adjacent to presumptive methane-venting pockmarks.

Table 1

Summary of major features of the studied mud volcanoes, including total number of beam trawl transects (N bt), number of beam trawl transects retrieving sponges (N sbt), average (±SD) depth (=D, in m) for the set of beam trawl transects conducted at each volcano, average (±SD) abundance of authigenic carbonates (Carbonate = C) as scored from a semi-quantitative approach (0 to 3), average (±SD) venting activity of methane-rich fluid (Methane = M) as scored from a semi-quantitative approach (0 to 3), and intensity of bottom fishing activity (Fishing = F) as scored from a semi-quantitative approach (0 to 3). Averages (±SD) were calculated considering only trawls that retrieved sponges. Sponge species richness (N sp), total number of sponge individuals (N ind), and density of individuals (N ind x 10^{-3} m^{-2}) at each mud volcano are also shown.

Mud volcano	N bt	N sbt	Depth (m)	Carbonate	Methane	Fishing	N sp	N ind	N ind (x10^{-3} m^{-2})
Gazul	7	6	453 ± 32	2.00 ± 0.89	0.50 ± 0.55	0.50 ± 0.84	15	370	30.72
Anastasya	6	3	524 ± 32	0.00 ± 0.00	2.00 ± 1.73	2.67 ± 0.58	8	42	6.07
Tarsis	6	3	602 ± 23	0.33 ± 0.58	0.67 ± 1.15	2.33 ± 1.15	9	99	16.63
Pipoca	5	5	616 ± 60	0.60 ± 0.89	0.80 ± 1.10	0.40 ± 0.89	38	249	25.92
Chica	4	4	673 ± 36	0.75 ± 0.50	0.00 ± 0.00	1.00 ± 1.41	27	299	37.01
Hesperides	4	4	765 ± 52	1.75 ± 1.50	1.50 ± 1.73	0.00 ± 0.00	19	105	14.30
Almazan	4	4	904 ± 25	1.00 ± 0.82	1.00 ± 1.15	0.00 ± 0.00	27	270	35.74
Aveiro	2	2	1124 ± 21	0.00 ± 0.00	1.50 ± 2.12	0.00 ± 0.00	9	225	61.12

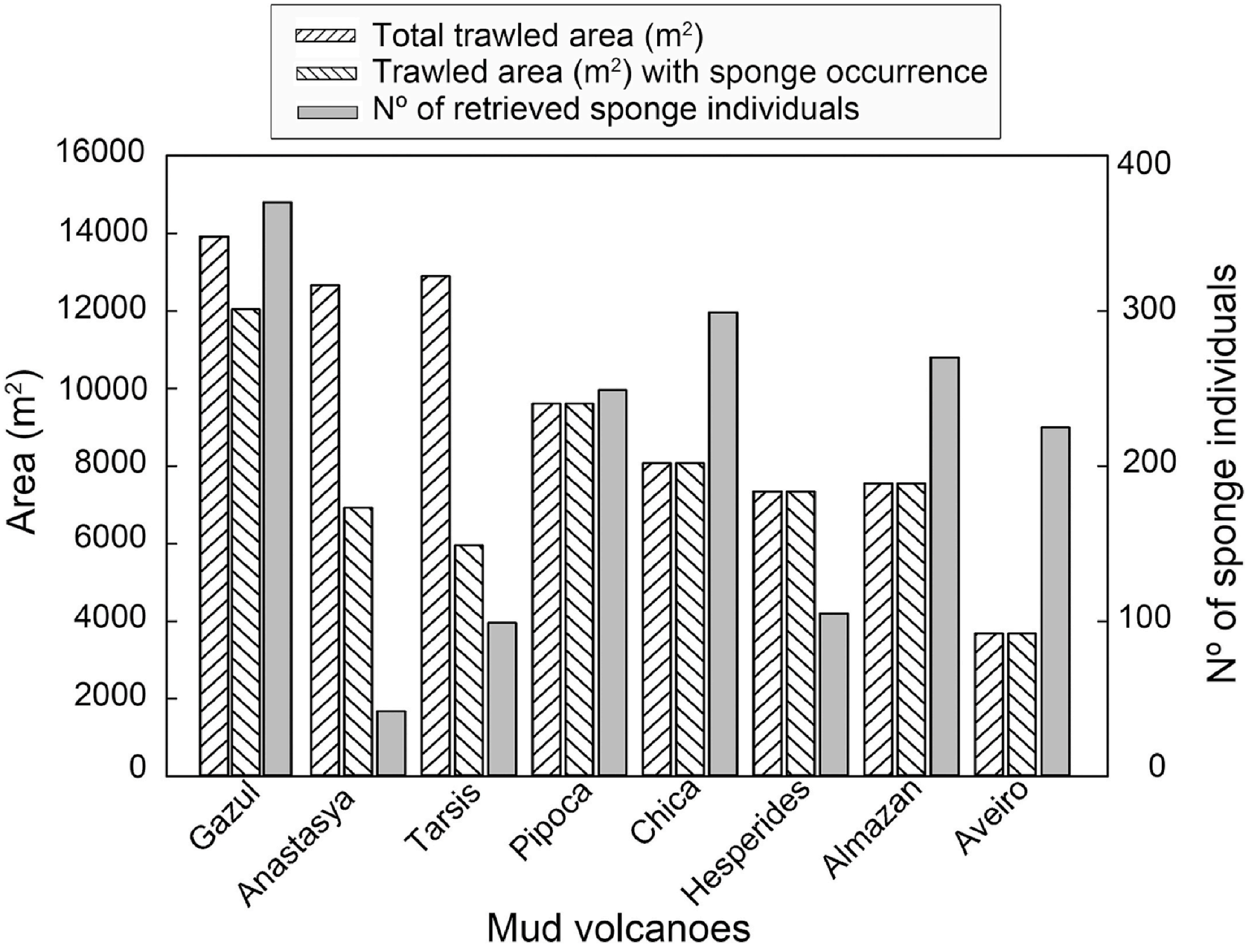

Fig. 3. Plot summarizing the total trawled area (m^2), the trawled area where sponges were found (m^2) and the number of collected sponge individuals (grey-shaded columns) for the sampled volcanoes.

Levantine Intermediate Water (LIW), originated in the Eastern Mediterranean, and the Western Mediterranean Deep Water (WMDW), originated in the Gulf of Lion. The former runs along the western basin at depths between 200 – 800 m and acts as the major contributor to the MOW, being responsible for a 90% of the MOW's composition. The WMDW, responsible for a 10%, flows under the LIW (Stommel et al., 1973; Bryden and Stommel, 1984; Lascaratos et al., 1993; Malanotte-Rizzoli, 2001).

It has already been documented that the NAS brings the presence of Atlantic sessile species into the Alboran Sea, the westernmost basin of the Mediterranean (Pérès and Picard, 1964; Templado et al., 2006). Biogeographical studies specifically focused on the sponge fauna also reflect such a process (Templado et al., 1986; Pansini, 1987; Maldonado, 1992, 1993; Maldonado and Uriz, 1995; Maldonado et al., 2011; Xavier

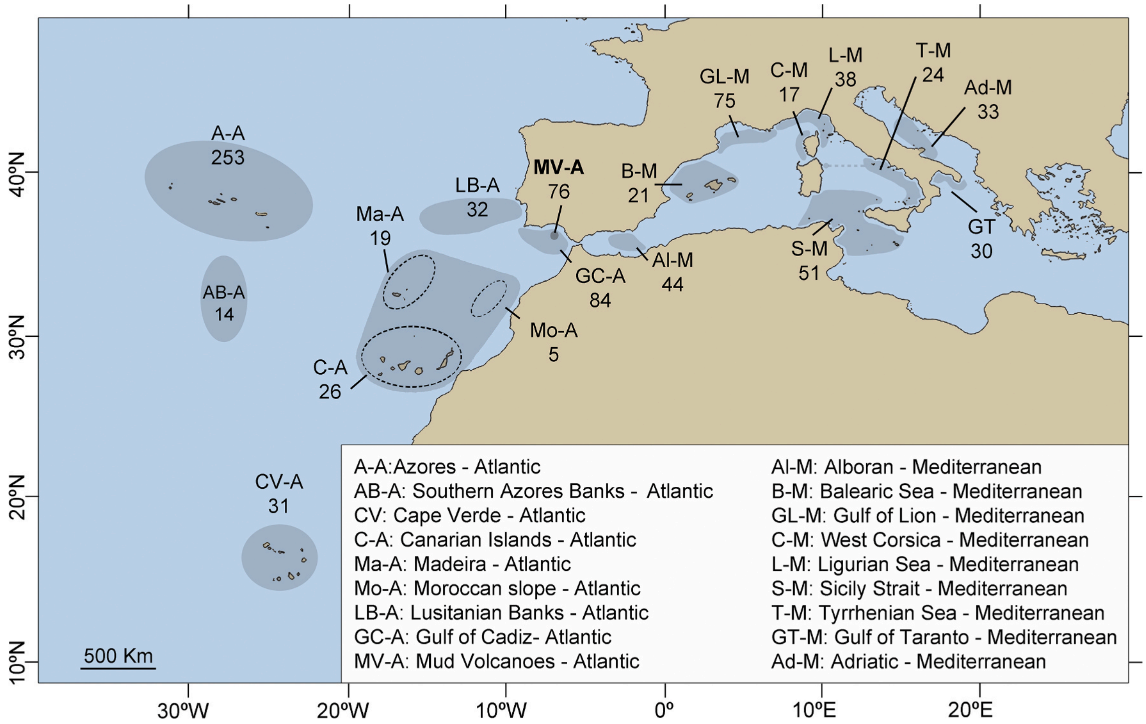

Fig. 4. Map of the Atlantic-Mediterranean region showing the biogeographical areas (grey shades) and their sub-areas (see codes in legend) considered for the comparison of affinities for the sponge fauna. Geographically close sub-areas which showed not significant faunal differences after exploratory Jaccard-based cluster analyses were unified into a larger area, but still indicated in the map by dashed lines. Each area and subarea is labelled with a code (see graphic legend). The number of sponge species recorded to date within the 200–1500 m depth in each area and subareas is also indicated.

and Van Soest, 2012). However, little is known about the effects of a putative faunal transport in the opposite direction, that is, from the deep Mediterranean into the North Atlantic, which would be due to the MOW. Very few studies have assessed whether the MOW exports Mediterranean benthic invertebrates to the Atlantic deep-sea communities and which could be the fate of the exported fauna. Most available information comes from the results of the French oceanographic cruises "Balgim", which sampled the benthos at the continental slope of the Ibero-Moroccan Gulf and the western zone of the Alboran Sea from May 25 to June 22 in 1984. Two Balgim studies based on 24 species of ascidians (Monniot and Monniot, 1988) and 146 species of bryozoans (Harmelin and d'Hondt, 1993) suggested minimal presence of Mediterranean species exported to the Atlantic communities, but the one based on sponges (Boury-Esnault et al., 1994) suggested a different pattern: from a total of 96 sponge species collected, 50 were present exclusively at the Atlantic side, 28 at the Mediterranean side and 17 occurred in both. Since the Balgim times, the study of the North Atlantic and Mediterranean depths has been tremendously facilitated by progressive improvements of the technologies for deep-sea exploration during the past 30 years. As a result, numerous subsequent records on deep-sea benthos have been obtained, which need to be considered in updated faunal analyses. Herein we are re-examining the "biogeographical message" of the deep sponge fauna after expanding considerably the database used for these analyses (up to 456 spp.) relative to that in previous studies. Our main objective was to conduct a comprehensive re-examination of the MOW role in the interrelationships between the bathyal faunas of the Northeastern Atlantic and the Mediterranean, using sponges as a case study.

The faunal comparison in the concerned area of this study incorporates all taxonomic studies on sponges subsequent to Boury-Esnault et al. (1994), including diverse locations within both the eastern North Atlantic and the Western Mediterranean. Yet, the sponge fauna of the bathyal communities in the Gulf of Cadiz, despite being in a Northeastern Atlantic zone strongly affected by the MOW trajectory, was less known than its bathymetric equivalent in the Western Mediterranean, a situation that prevented reliable comparisons. For this reason, prior to the global analysis at a large-scale Atlantic-Mediterranean gradient, we conducted a detailed analysis of the rich sponge fauna (82 spp.) recently described from mud volcano habitats in the Gulf of Cadiz (Fig. 1). Since the late 90's, a variety of hydrocarbon fluid venting structures, including mud volcanoes, has progressively been described at the Gulf of Cadiz, with more than 70 mud volcanoes discovered to date, at depths from 200 to 4000 m (Gardner, 2001; Pinheiro et al., 2003; Somoza et al., 2003). The presence of hydrocarbons (mainly consisting of methane, together with propane, butane and ethane in smaller amounts) facilitates the proliferation of consortia of archaea and bacteria that live out of anaerobic methane oxidation coupled with sulphate reduction (Boetius et al., 2000; Boetius and Suess, 2004; Díaz del Río et al., 2014). The methane venting is also relevant to sessile invertebrates, since it results in precipitation of methane-derived authigenic carbonate structures (hereafter referred to as carbonates), such as slabs, crusts and chimneys (Hovland et al., 1987; Suess, 2014). Once venting ceases, the carbonate structures provide suitable hard substrates for subsequent colonization by deep-sea sessile fauna (sponges, gorgonians, cold-water corals, etc.). This pioneering fauna may in turn facilitate the arrival of additional organisms, triggering a global increase of the benthic biodiversity on the carbonates (Levin, 2005; Rueda et al., 2012; González-García et al., 2020). In European waters, mud volcanoes are classified as sensitive habitats: habitat 1180 "Submarine structures made by leaking gases" EUR 27, 2007, and their sponge fauna has only recently been explored (Fig. 2). After the recent taxonomic description of the main sponge fauna associated to a field of mud volcanoes located at bathyal depths in the northern Gulf of Cadiz (Sitjà et al., 2019), this study further investigates between-volcano faunal patterns. More importantly, the sponge fauna of those mud volcanoes is herein collectively incorporated as a crucial element to undertake a more representative Atlantic-Mediterranean assessment of the transport of bathyal fauna by the MOW.

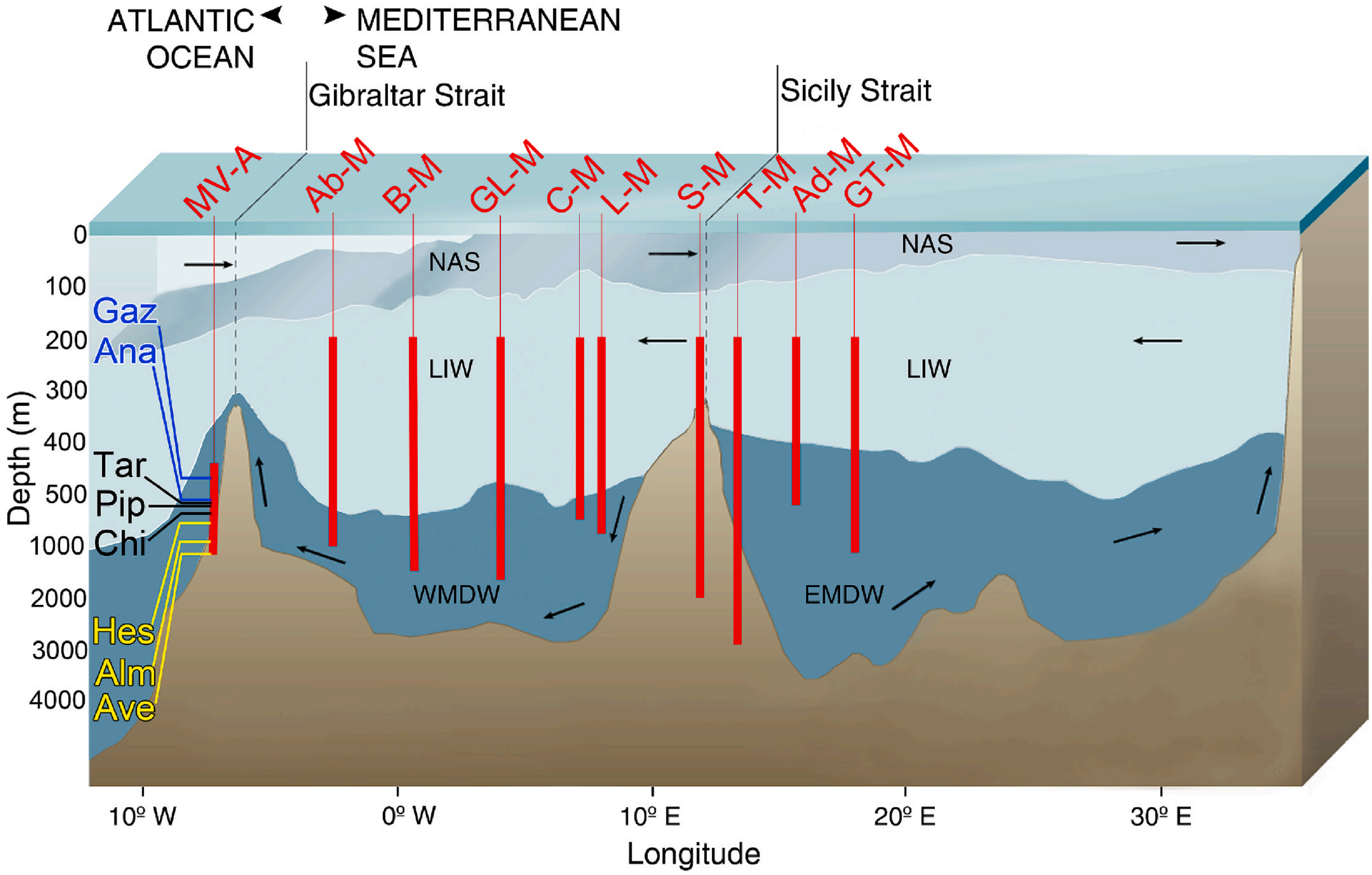

Fig. 5. Schematic profile of the Mediterranean and its connection to the North Atlantic (modified from GRID-Arendal, 2013), indicating the bathymetric range (thick red lines) and longitude of the Mediterranean areas and the mud volcanoes at the Gulf of Cadiz considered in this study. The major water masses (in black upper case) occurring in the depicted region and their prevailing direction (black arrows) are also indicated (NAS= North Atlantic Surface water, LIW = Levantine Intermediate Water, WMDW & EMDW = Western & Eastern Mediterranean Deep Waters, respectively). Biogeographical areas (red uppercase labels) follow the codes given in Fig. 3 (Ab-M = Alboran – Mediterranean; B-M= Balearic Sea – Mediterranean; GL-M = Gulf of Lion – Mediterranean; L-M = Ligurian Sea – Mediterranean; C-M= West Corsica – Mediterranean; S-M= Sicily Strait – Mediterranean; T-M = Tyrrhenian Sea – Mediterranean; Ad-M = Adriatic Sea – Mediterranean; GT-M = Gulf of Taranto – Mediterranean). To the left, the blue, black and yellow, lower case labels for the mud volcanoes are Gaz = Gazul, Ana = Anastasya, Tar = Tarsis, Pip = Pipoca, Chi = Chica, Hes = Hesperides, Alm = Almazan and Ave = Aveiro.

2. Materials and methods

2.1. Study area at the Gulf of Cadiz

The Gulf of Cadiz is situated at the Northeast Atlantic, between the SW Iberian margin and the NW Moroccan margin (Fig. 1). The sponge fauna collected from eight mud volcanoes located on the continental slope from 300 to 1200 m depths in the northern Gulf of Cadiz was recently described by our team (Sitjà et al., 2019). Strictly speaking, the eight studied mud volcanoes are indeed six mud volcanoes and two diapir-mud volcanoes, but for the sake of simplicity they will be referred to hereafter as mud volcanoes. The mud volcanoes are distributed in three zones (Fig. 1). The shallowest, consisting of Gazul and Anastasya, is located in the upper continental slope, from 300 to 550 m. Tarsis, Pipoca and Chica are located at intermediate depths of 600–730 m. Hesperides, Almazan and Aveiro make the deepest zone, from 800 to 1200m depths. This deepest zone is physically separated from the two others by the Cadiz Contourite Channel (Fig. 1), an orographic incision of the substrate caused by the erosive effect of the MOW. There are two others MOW-eroded contourite channels, one at the intermediate zone, called the Gusano Channel, relatively close to Anastasya volcano, and the other, the Huelva Channel, close to Chica and Pipoca volcanoes (Fig. 1). Therefore, there is strong evidence that the MOW trajectory directly impacts on the studied mud volcanoes (Sánchez-Leal et al., 2017).

2.2. Sampling procedures at the Gulf of Cadiz

A total of 38 collecting transects (hauls) were performed by beam trawl, sampling collectively about 75,000 m^2. Sampling was conducted in the frame of the EU LIFE + INDEMARES project (subproject “Chimeneas de Cádiz"). Information on number of beam trawl transects and mean (±SD) sampling depth in each volcano is given in Table 1. Information on the area sampled at each volcano is given in section 2.4 “*Between-volcano faunal affinities*”. Details on the expeditions, sampling and taxonomic identification procedures of the sponge fauna are given elsewhere (Sitjà et al., 2019). Further “in vivo” details of the sponge communities and their environment were documented during the cruises using both an underwater towed observation vehicle VOR ‘Aphia 2012’ and the ROV ‘Liropus 2000’. Four environmental variables were recorded for each sampling transect: depth (D), semi-quantitative abundance of methane-derived authigenic carbonates (C), methane-rich fluid emission (M), and a semi-quantitative assessment of the intensity of bottom fishing (F) activities by the trawling fleet. The semi-quantitative assessment of the C, M, and F parameters consisted on scoring them from 0 to 3 for each sampling station, based on observations, as it follows: 1) Carbonate formations (C), such as chimneys, crusts and slabs, were scored by their abundance in the samples retrieved by the trawls and, when possible, these data were confronted with underwater images obtained during VOR and ROV transects. The criteria for the semi-quantitative categorization was 0 = no carbonate piece

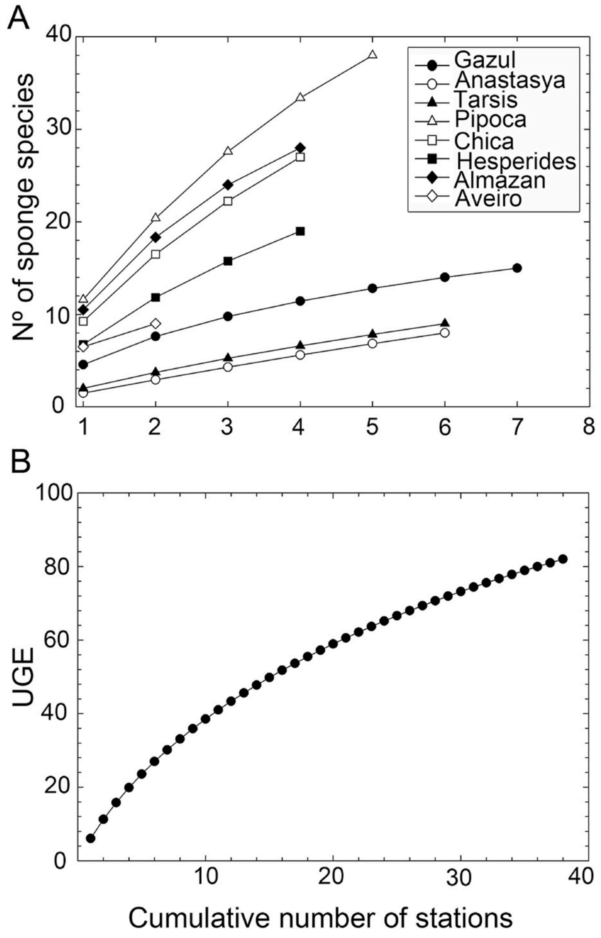

Fig. 6. **(A)** Species accumulation curves for each of the eight mud volcanoes obtained from randomizing samples through the Ugland, Grey and Ellingsen method (UGE). They show the increment in the number of different species with increasing numbers of trawl transects. **(B)** Species accumulation curves for data on number of species retrieved from trawl transects of all volcanoes are pooled together for analysis.

retrieved per trawl, 1 = one C piece retrieved per trawl, 2 = two to five C pieces retrieved, and 3 = more than five C pieces retrieved, often larger than 50 cm in length. 2) The scoring of the methane-rich fluid emission was ranked according to the presence of chemosynthetic bacterial mats and the abundance of chemosymbiotic invertebrates recovered by the trawls and box corers from the various mud volcanoes. It was categorized as 0 = no evidence of chemosynthetic-chemosymbiotic communities; 1 = <50 individuals of chemosymbiotic invertebrates m^{-2}; 2 = 51–500 individuals of chemosymbiotic invertebrates m^{-2}; 3 = >500 individuals of chemosymbiotic invertebrates m^{-2}. 3) The trawl fishing activity was assessed by tracking the activity of each vessel of the trawling fleet for the period January to December 2011, using the Vessel Monitoring System (VMS) datasets supplied by the Spanish General Secretary of Fisheries (Spanish Ministry of Agriculture and Fisheries). Categories were 0 = no trawling vessel operating in that area during 2011; 1 = 1 trawling vessel; 2 = 2–5 trawling vessels; 3= >5 trawling vessels.

The final score given to a mud volcano for each of the four environmental variables was the mean (±SD) value obtained from the set of beam trawl transects conducted in such a mud volcano (Table 1). Prior to any analysis, each of the 4 variables was standardized by its own maximum to render their ranges comparable.

2.3. *Sampling effort at the Gulf of Cadiz*

From the list of sponge species identified, a 'species x sample' matrix was created, discriminating the number of individuals of each species (abundance) retrieved from each beam trawl transect. Previous to any analysis or standardization, the sampling effort performed on a mud volcano was tested through a species accumulation curve to examine at what level the collected fauna was representative of the global sponge fauna occurring at that volcano. The species accumulation curves were built following the Ugland, Grey and Ellingsen (UGE) method (Ugland et al., 2003) in the Plymouth Routines Multivariate Ecological Research (PRIMER 6) software (Clarke and Gorley, 2006). A curve was obtained for each volcano throughout 999 randomizations. Then, a global curve was built for the combination of all the samples of all volcanoes.

2.4. *Between-volcano faunal affinities*

To analyze the faunal affinities between volcanoes, each 'species x volcano' matrix was collapsed across beam trawls, showing just the total number of individuals of each species for each volcano. The matrix included only the beam trawl transects that retrieved sponges (n = 31). When plotting the total trawled area versus either the area with sponge occurrence for each volcano or the total number of collected individuals (Fig. 3), it was made evident that some trawl transects at Gazul, Anastasya and Tarsis collected no sponge. This was probably due to the fact that some hauls had initially been designed in the project frame for purposes other than the collecting sponges and targeted specific habitats in which sponges are typically absent.

Before any analysis, the number of individuals of each species in each volcano was standardized to total trawled area and such abundance per m^2 was then square root transformed in order to minimize the effect of highly abundant species in the subsequent multivariate analyses. By using the PRIMER 6 software, the affinities between pairs of volcanoes based on the Bray-Curtis similarity index were calculated and represented in a dendrogram accompanied by a similarity profile (SIMPROF) permutation analysis to test for evidence of a genuine (statistically significant) clustering within our set of mud volcanoes. Following, a similarity percentages (SIMPER) test was run to obtain the percentage contribution of the species to the clustering pattern among volcanoes.

To assess the influence of the recorded environmental variables on the faunal patterns among the mud volcanoes, correspondence analyses were performed using the software for Canonical Community Ordination, CANOCO 4.5. To avoid distortion of ordination scores by rare species the 'down-weight option' available in the software was used. The explainable faunal variation in our 'species x volcano' matrix was assessed by using a correspondence analysis (CA) to reveal how the species were distributed along the volcanoes with no constraint. Then, the portion of variation related to the environmental variables was estimated using canonical correspondence analysis (CCA). The statistical significance of the first canonical axis and all canonical axes of the resulting model was tested by the Monte-Carlo test using 500 permutations. Four environmental variables (C, D, F, and M) were initially considered for the CCA analysis. Subsequently, we discriminated between "natural" environmental variables (i.e., D, C and M) and "man-driven" environmental variables (i.e., F) and calculated the faunal variation uniquely explained by each of the sets, by the two sets together and that remaining unexplained, following an analysis of variation partitioning (Borcard et al., 1992). The 'forward selection' option was then used to unmask co-variation and select the most relevant environmental variables in terms of explaining faunal variance, as indicated by Monte-Carlo permutation tests with 500 permutations and the variance inflation factors (VIF). Finally, a second CCA was performed constrained by only the most relevant environmental variables. Additionally, the relationship of the resulting environmental vectors and the main axes of the ordination space was examined by Spearman rank correlation for both models of four and two environmental variables.

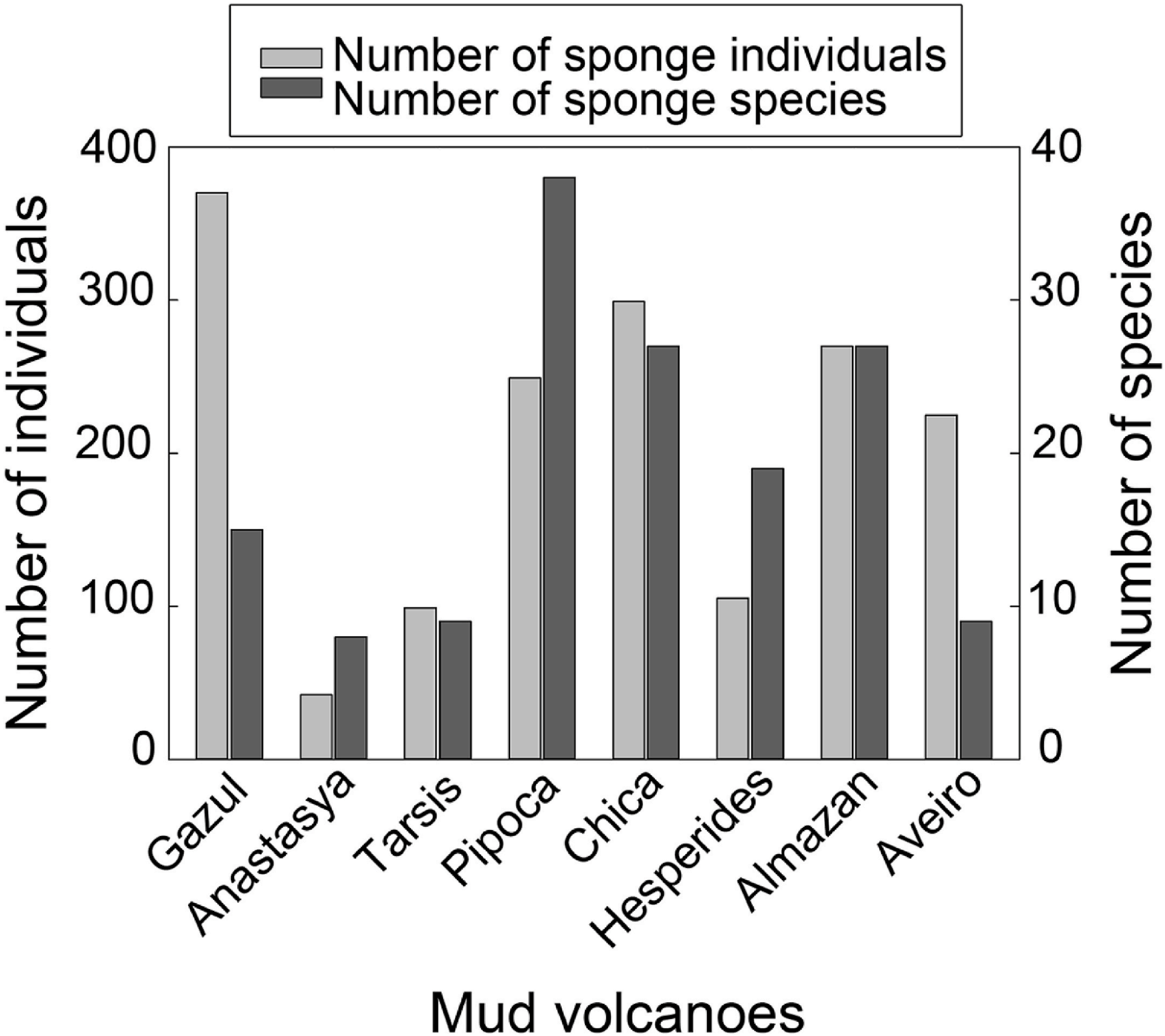

Fig. 7. Comparative summary of the number of individuals and species in the sponge fauna collected from each mud volcano.

Table 2
List of the most abundant species (i.e., those accounting each for more than 3% of the total abundance) in the total of the studied mud volcanoes. The taxonomic class to which they belong is indicated (Dem = Demospongiae; Hex = Hexactinellida), together with the abundance of each species along the total sampling stations (Ab), their abundance proportional to the total of sponge individuals (% Ab) and the number of trawls (N sbt) in which each of the species were present.

Class	Species	Ab	% Ab	N sbt
Dem	*Thenea muricata*	366	22.0	12
Hex	*Pheronema carpenteri*	181	11.0	8
Dem	*Petrosia (Petrosia) crassa*	169	10.2	10
Hex	*Asconema setubalense*	117	7.1	18
Dem	*Desmacella inornata*	110	6.6	16
Dem	*Haliclona (Gellius) angulata*	74	4.5	6
Dem	*Lycopodina hypogea*	71	4.3	1
Dem	*Poecillastra compressa*	64	3.9	8
Dem	*Stylocordyla borealis*	58	3.5	3
Dem	*Haliclona (Rhizoniera) bouryesnaultae*	49	3.0	6

2.5. *Faunal affinities across the Atlantic-Mediterranean gradient*

To examine the faunal affinities along the Atlantic-Mediterranean gradient, a presence/absence matrix was built containing all deep-sea sponge species (456 spp.) recorded for a variety of bathyal biogeographic areas distributed along the considered Atlantic–Mediterranean gradient (Supplementary Data File S1). Areas recognized as biogeographic entities were based in pre-existing literature (Pansini and Longo, 2003; Spalding et al., 2007). The approach included records from a total of 18 initial areas, including areas from the Central and Western regions of the Mediterranean and, in the Atlantic, the Gulf of Cadiz, southern Lusitania and Macaronesia (Fig. 4, Table S1). The nine areas from the Atlantic were Azores (A-A), Southern Azores Banks (AB-A), Cape Verde (CV-A), Canary Islands (Ca-A), Madeira (Ma-A), Morocco (Mo-A), Lusitanian Banks (LB-A), the Gulf of Cadiz mud volcanoes (MV-A), and the remaining slope communities of the Gulf of Cadiz (GC-A). In the Mediterranean, nine areas were considered: Alboran Sea (Al-M), Balearic Sea (B-M), Gulf of Lion (GL-M), Ligurian Sea (L-M), West Corsica (C-M), Tyrrhenian Sea (T-M), Strait of Sicily (S-M), Gulf of Taranto

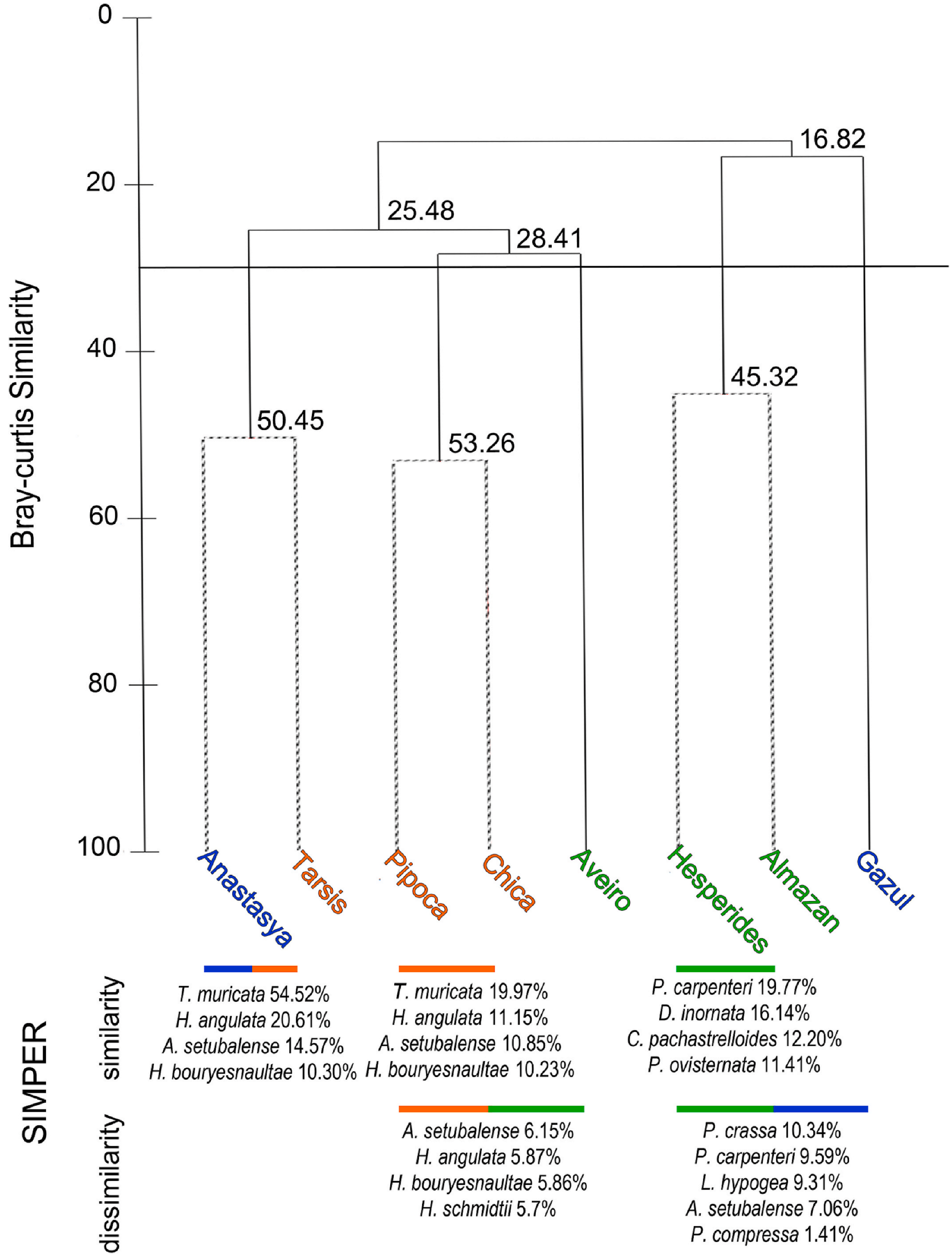

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Fig. 8. Dendrogram based on Bray-Curtis similarity between the eight mud volcanoes, also summarizing the results of the SIMPROF and SIMPER tests. Mud volcanoes are labelled by colors according to the depth range of volcano zones they belong to (upper zone = blue, intermediate zone = orange, lower zone = green). The numerical values at the nodes correspond to the percentage of similarity of the sub-groups. The horizontal solid line represents the 30% similarity threshold. Dashed lines represent genuine groups with statistical significance according to SIMPROF test. SIMPER results are shown at the bottom, indicating the species most contributing to the similarities or dissimilarities between the volcano clusters (see color lines for the cluster entities involved in the comparison). Species contribution is expressed in percentages. Length and color of the horizontal lines match the labels, indicating the subgroup to which the SIMPER results are referring to.

(GT-M), and Adriatic Sea (A-M).

An exploratory Jaccard-based dendrogram and subsequent SIMPROF tests were performed (data not shown) to detect: 1) areas with strong affinities representing statistically significant genuine groups, which can be combined with each other into a single biogeographic area; and 2) cluster members with no statistically significant structure, which should stay independent for the analyses. These exploratory tests suggested that the fauna of the Canary Islands (Ca-A), Madeira (Ma-A) and Morocco (Mo-A) could be grouped into a unit, hereafter referred to as C+Ma+Mo-A. Following this readjustment, a total of 16 areas were finally submitted to the definitive analyses of the bathyal sponge fauna along an Atlantic-Mediterranean gradient impacted by the MOW. The geographical location of the initial and collapsed areas and their respective numbers of species are summarized in Fig. 4.

Because the available taxonomic studies for the sponge fauna of most locations often lacked robust and/or consistent information about the abundance of the species, the use of quantitative or semi-quantitative analyses was impracticable. The compiled database included only those records deeper than 200 m, this threshold being defined by the upper depth limit of the LIW and the MOW. Since both the LIW and the WDMW contribute (in a 90% and 10%) to the composition of the MOW, no lower depth limit was settled for the Mediterranean records. Because the MOW spreads in the Atlantic at about 1500 m, this depth was established as the lower limit for the records in the Atlantic Ocean (Fig. 5). Therefore, the considered depth ranges facilitate the possibility that species living in the Mediterranean below 1500m can be detected into the Atlantic inventories if brought to shallower depths by the MOW trajectory in the North Atlantic region. All sponge records were double checked by contrasting them against the World Porifera Database (Van Soest et al., 2019), so that those currently considered as inaccurate or incorrect taxonomic records in such database were excluded. Likewise, records identified as cf. (n = 6) and those with an unknown collection depth were also discarded. The two controversial species *Leiodermatium lynceus* and *Leiodermatium pfeifferae* were included in the database as a single taxonomic entity called *Leiodermatium* spp. complex. This responds to their questionable validity as different species, since they have been reported to lack robust differential characters (Carter, 1873; Kelly, 2007; Maldonado et al., 2015). The readjustments resulted in a total of 456 species. Supplemental Data File S1 details all the species, sampling depths, geographic locations and bibliographic records.

To assess the sponge faunal affinities across the Atlantic-Mediterranean deep-sea gradient, the “species x area” matrix of presence/absence data was analyzed by PRIMER Vo. 6 software to conduct cluster analyses based on Jaccard similarity, along with SIMPROF tests. Jaccard similarity matrices were further processed by Multidimensional Scaling (MDS) to visualize affinity patterns in 2D ordination plots.

Subsequently, the putative effect of the MOW on the faunal affinities was also tested by linear regression analysis, under the assumption that, if the Mediterranean outflow has no major effect on shaping the faunal affinities, the geographical distances among the studied deep-sea areas should largely predict the faunal similarities: the greater the geographical distance between two areas, the lower their faunal affinity. Geographical distances were obtained by using Google Earth software. We considered the shortest possible distance between the centroids of two studied areas through the marine medium, meaning that all distances between Atlantic and Mediterranean areas were calculated from trajectories through the Strait of Gibraltar. The relationship between the pattern of faunal similarity and actual geographical distances among the areas was examined by linear regression using SigmaPlot Vo.13 software.

3. Results

3.1. Sampling effort and sponge abundance at the Gulf of Cadiz

The effectiveness of sampling, as analyzed by the UGE method, showed for each of the volcanoes a rising accumulation curve not reaching the asymptotic stage. This pattern suggests that probably additional sponge species would have been retrieved by increasing the sampling effort in all volcanoes (Fig. 6A). Uneven sampling effort across volcanoes was also detected, with shallower volcanoes typically being more sampled than the deepest ones (Fig. 3). The enormous logistic and economic effort required to increase sampling effort was simply not affordable for the deepest mud volcanoes. Therefore, these weaknesses in the approach have been taken into consideration when conducting and interpreting the faunal analyses. Interestingly, the global sampling effort across the mud volcanoes field appears to be more representative. When pooling all volcanoes, the species accumulation curve starts describing a trajectory that approaches to an asymptotic stabilization (Fig. 6B).

From the 38 trawl transects performed in the eight mud volcanoes, seven of them retrieved no sponge and 31 provided a total of 1659 sponge specimens belonging to 82 different species (Table 1). The volcanoes with high total numbers of sponge individuals were —in decreasing order from 370 to 225 individuals— Gazul, Chica, Almazan, Pipoca and Aveiro (Table 1, Fig. 7). A comparatively high number of species (i.e., richness) was found at Pipoca, Chica, Almazan, Hesperides and Gazul, ranging from 38 to 15 species (Table 1, Fig. 7).

The pattern suggests a relatively even representativeness of the sponge species across the mud volcanoes, Gazul (the shallowest) and Aveiro (the deepest) being exceptions. The latter two showed a relatively low number of species in relation to the number of individuals because of disproportionate abundances of *Petrosia (Petrosia) crassa* in Gazul and *Thenea muricata* in Aveiro. The former species represented 40% of the collected individuals in Gazul and the latter about 60% of those collected in Aveiro. These results, together with ROV images, suggest that Gazul hosts aggregations of *P. crassa,* probably favored by an abundance of hard substrate in the form of carbonates, and that aggregations of *T. muricata,* a soft-bottom specialist, occur at Aveiro. Other mud volcanoes with lower but still fairly relevant abundances and high species richness were Pipoca, Chica, and Almazan. Interestingly, the first two are located close to the Huelva Channel, eroded by the MOW.

Taxonomically, the Class Hexactinellida represented 18% of the total of sponges identified and class Demospongiae 82%, both classes being present in all the studied volcanoes. The most abundant species were *T. muricata*, *Pheronema carpenteri* (Fig. 2F), *P. crassa* (Fig. 2D), *Asconema setubalense* (Fig. 2D), and *Desmacella inornata* (Table 2).

3.2. Between-volcano faunal affinities

The Bray-Curtis faunal similarity analysis distributed the mud volcanoes in two main groups (Fig. 8). At the global level, the within-group similarity was low (i.e., 16.8 and 25.4%, respectively) and none of the groups had its main node with statistical support. The main dichotomy in two volcano groups did not reflect a bathymetric pattern, since the smallest of the clusters (i.e., 16.8% similarity) included the shallowest

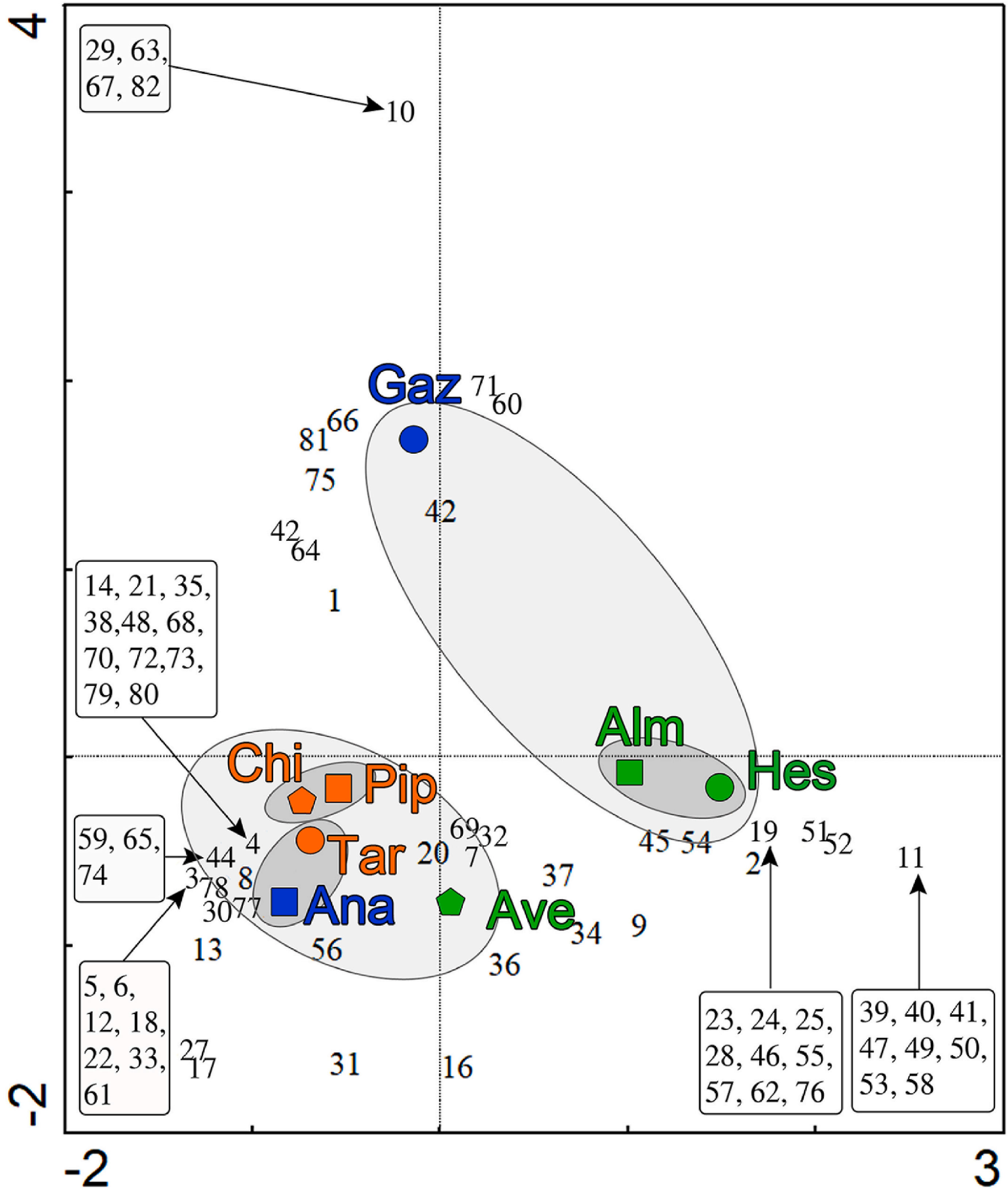

Fig. 9. Biplot showing the bidimensional space formed by the first and the second axes of an unconstrained CA depicting the relative position of the mud volcanoes —labelled in color (upper zone = blue, intermediate zone = orange, lower zone = green)— and their associated sponge species —labelled as numbers. Numbers that overlapped at exactly the same spot in the biplot space are depicted inside boxes and their original position is indicated by the arrow head. Shaded areas correspond to the affinities observed from the dendrogram. Correspondence of each number to its respective species name is given in Table 3. Note that some species are clearly related to some volcanoes, such as *Poecillastra compressa* (#60) and *Petrosia crassa* (n#71), which abound in Gazul volcano.

Table 3
Numerical coding used as labels for the scientific names of the sponge species in the ordination analyses in Figs. 9 to 11.

Code	Species	Code	Species
1	*Asconema setubalense*	42	*Jaspis incrustans*
2	*Pheronema carpenteri*	43	*Jaspis johnstonii*
3	*Lanuginella* cf *pupa*	44	*Jaspis sinuoxea*
4	*Rhizaxinella elongata*	45	*Neoschrammeniella bowerbankii*
5	*Rhizaxinella pyrifera*	46	*Geodia anceps*
6	*Suberites carnosus*	47	*Penares euastrum*
7	*Topsentia glabra*	48	*Geodia* cf *spherastrella*
8	*Stylocordyla borealis*	49	*Geodia megastrella*
9	*Cladorhiza abyssicola*	50	*Geodia pachydermata*
10	*Lycopodina hypogea*	51	*Characella pachastrelloides*
11	*Anisocrella hymedesmina*	52	*Characella tripodaria*
12	*Crella fusifera*	53	*Pachastrella monilifera*
13	*Crella pyrula*	54	*Pachastrella ovisternata*
14	*Coelosphaera tubifex*	55	*Nethea amygdaloides*
15	*Coelosphaera cryosi*	56	*Thenea muricata*
16	*Forcepia forcipis*	57	*Thrombus abyssi*
17	*Forcepia luciensis*	58	*Vulcanella gracilis*
18	*Hymedesmia koehleri*	59	*Discodermia ramifera*
19	*Hymedesmia mutabilis*	60	*Poecillastra compressa*
20	*Hymedesmia peachii*	61	*Rhabderemia profunda*
21	*Hymedesmia pennata*	62	*Bubaris vermiculata*
22	*Iotroata polydentata*	63	*Myrmekioderma indemaresi*
23	*Sceptrella insignis*	64	*Phakellia robusta*
24	*Clathria campecheae*	65	*Phakellia ventilabrum*
25	*Antho signata*	66	*Axinella vellerea*
26	*Antho erecta*	67	*Axinella* cf *rugosa*
27	*Mycale lingua*	68	*Janulum spinispiculum*
28	*Myxilla incrustans*	69	*Eurypon clavatum*
29	*Myxilla rosacea*	70	*Acantheurypon pilosella*
30	*Podospongia lovenii*	71	*Petrosia crassa*
31	*Desmacella annexa*	72	*Petrosia raphida*
32	*Desmacella inornata*	73	*Petrosia vansoesti*
33	*Dragmatella aberrans*	74	*Cladocroce fibrosa*
34	*Hamacantha johnsoni*	75	*Cladocroce spathiformis*
35	*Hamacantha lundbecki*	76	*Haliclona* cf *fulva*
36	*Hamacantha schmidtii*	77	*Haliclona angulata*
37	*Hamacantha papillata*	78	*Haliclona bouryesnaultae*
38	*Craniella cranium*	79	*Haliclona utriculus*
39	*Weberella verrucosa*	80	*Pleraplysilla spinifera*
40	*Leiodermatium pfeifferae*	81	*Dysidea fragilis*
41	*Jaspis* cf *eudermis*	82	*Spongia officinalis*

volcano (Gazul) along with two others of the deepest zone (Hesperides, Almazan). Likewise, the largest of the two clusters (25.48% similarity) contained volcanoes from the three bathymetric zones (Fig. 8). However, a more detailed analysis of the structure of this larger group in 3 subclusters revealed a clear effect of depth in the faunal affinities. There was a subcluster formed by Anastasya (from the shallow zone) and Tarsis (from the intermediate zone); they made a genuine group (50.45% similarity) with statistical support, probably because, although Tarsis was considered *a priori* a volcano from the shallow zone, it actually occurs in a transitional zone between the shallow and the intermediate zones. A second subcluster formed by the two volcanoes of the intermediate zone, Pipoca and Chica, showed even higher faunal similarity (53.26%) with statistical SIMPROF significance. Finally, Aveiro, the deepest volcano, became associated to the two intermediate volcanoes, although with only 28.41% and without statistical support. Therefore, under the structure of the large clusters there was some "depth pattern". The situation was similar for the smallest of the two main clusters (Fig. 8): Gazul, being at the shallower zone, shared only 16.82% ($p = 0.001$) of faunal similarity with Hesperides and Almazan, which are supported pair with 45.32% of similarity. It is also worth noticing that these three mud volcanoes clustered consistently with their features regarding authigenic carbonates features, being the mud volcanoes with highest abundance of such hard substrate (Table 1).

According to the SIMPER test, the species contributing to the formation of the larger group are *T. muricata, Haliclona angulata, A. setubalense* and *Haliclona bouryesnaultae* (Fig. 8). The smallest cluster of volcanoes was mostly supported by the shared presence of *P. crassa*, *D. inornata* and *Poecillastra compressa* (Fig. 2A). The reasons for Gazul's distinctiveness were its singular abundance of *P. crassa* (10.34%) and the carnivorous sponge *Lycopodina hypogea* (9.31%) —of which 71 individuals were all found together in a single small boulder. The abundance of *A. setubalense* also contributed a 7.06%. Additionally, Gazul lacked *P. carpenteri* (9.59%), which was abundant in the two deep volcanoes of the group.

The bidimensional ordination space of an unconstrained CA (Fig. 9; Table 3) grouped the volcanoes in congruence with the results previously described from the dendrogram of Fig. 8. The first four axes explained a 75.3% of the faunal variation in the 'species x volcano' matrix (Table 4; Fig. 9). The first axis appeared related to depth and the second to the global number of sponge individuals at each volcano. Species like *Poecillastra compressa* (labelled as #60), *Axinella vellerea* (#66) and *Petrosia crassa* (#71) were associated to the position of Gazul volcano in the ordination space (Fig. 9). A wide variety of species was associated to the group formed by Pipoca, Chica, Tarsis and Anastasya, some of them being encrusting species (typically in Pipoca and Chica) along with soft-bottom specialists occurring in all the volcanoes of the group. The encrusting species with relevant contribution to this group of volcanoes were *Hymedesmia (Hymedesmia) pennata* (#21), *Jaspis sinuoxea* (#44) and *Janulum spinispiculum* (#68). The most relevant soft-bottom specialists were *Rhizaxinella elongata* and *Haliclona bouryesnaultae.* Other soft-bottom specialists, such as *Thenea muricata* (#56) and *Haliclona angulata* (#77) were more typically associated to the Anastasya position. Although Aveiro showed abundant *T. muricata* too, it had a distinctive abundance of *Hamacantha* spp. (#34, 37, 36). The group formed by Almazan and Hesperides shared importantly species like *Neoschrammeniella bowerbankii* (#45) and *Pachastrella ovisternata* (#54).

The four main axes of a CCA analysis constrained by four environmental variables (Fig. 10) explained 63.7% of the faunal variation in the 'species x volcano' matrix (that is, less than the unconstrained CA) and 100% of variability in the 'species-environment' matrix (Table 4). The Monte-Carlo Permutation test indicated that the first axis of the ordination space was almost significant ($p = 0.052$), while all the canonical axes tested together resulted clearly significant ($p = 0.034$), meaning that some of the environmental variables are responsible for at least

Table 4
Summary of statistics for the correspondence analysis (CA) and canonical correspondence analyses (CCA) constrained by four and two environmental variables. Eigenvalues are shown for the first four axes, together with the sum of all unconstrained (T. inertia) and canonical (C. inertia) eigenvalues. The percentage of variance in both faunal data (%V_{sp}) and fauna-environment relation (%$_{Vsp\text{-}env}$) explained by each axis is also given.

	CA		4-var CCA			2-var CCA		
	Eigen.	% V_{sp}	Eigen.	% V_{sp}	% $V_{sp\text{-}env}$	Eigen.	% V_{sp}	% $V_{sp\text{-}env}$
Axis 1	0.638	26.9	0.608	25.6	40.3	0.569	24	56.1
Axis 2	0.516	21.8	0.464	19.6	30.8	0.446	18.8	100
Axis 3	0.339	14.3	0.236	10.0	15.6	0.399	16.9	0
Axis 4	0.291	12.3	0.201	8.5	13.3	0.305	12.8	0
T. inertia	2.370	–	2.370	–	–	2.370	–	–
C. inertia	–	–	1.509	–	–	1.015	–	–

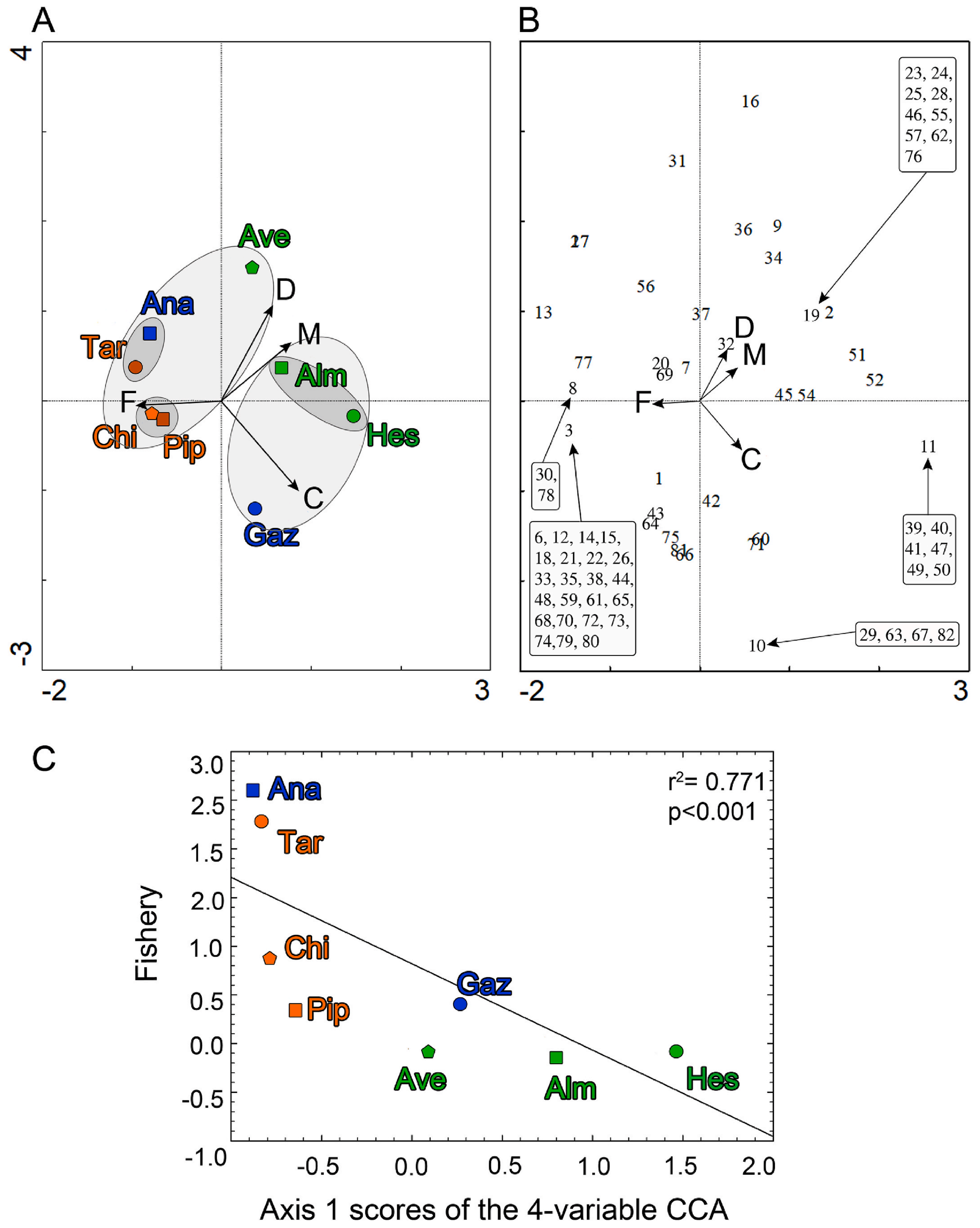

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Fig. 10. **(A)** Bidimensional ordination space formed by the first and the second axes of a CCA constrained by four environmental variables. It depicts the relative position of the mud volcanoes with relation to the environmental variables represented as vectors (C = Average abundance carbonated hard substrata; D = average depth; M = average intensity of methane venting; F = average intensity of bottom fishing). Shaded areas correspond to the affinities observed from the dendrogram. **(B)** Same CCA that in Fig. A, but showing the relative position of the sponge species in relation to the environmental variables. Species are labelled as numbers whose correspondence to scientific names is given in Table 3. Numbers that overlapped at exactly the same spot in the biplot space are depicted inside boxes and their original position is indicated by the arrow head. Some species, like *Podospongia lovenii* (#30) and *Haliclona bouryesnaultae* (#78) are closely related to the F vector. **(C)** Spearman correlation, showing the statistically significant association between the scores of the volcanoes along the first axis of the four-variable CCA and the average semi-quantitative scoring of the mud volcanoes relative to the intensity of bottom fishing in their benthic communities. The correlation coefficient and its "p" value are given at the top right corner.

Table 5
Summary of the variance partitioning analysis and VIF based on the four-variable CCA. Abbreviations are as follows: D = average depth; C = average abundance of authigenic carbonates; M = average methane venting activity; F = average fishing activity.

Variation component	Explained variation (%)	Variance inflation factor (VIF)
Benthic variation	47.64	D = 3.05; C = 2.27; M = 1.31
Fishery variation	9.20	F = 2.405
Shared variation	6.84	
Unexplained variation	36.33	

some of the variation within the 'species x volcano' matrix.

A subsequent variance partitioning analysis (Table 5) indicated that the variation explained by the set of natural variables (C = carbonates, D = depth, M = methane venting) was 47.64%, that is about 5 times more than that explained (9.20%) by the man-driven variable (F = bottom fishing activity). The shared variation explained was low (6.84%), therefore, given the also low percentage of variation from the F variable, it can be concluded that most of the variation explained by F is also explained by the set of natural variables. The unexplained variation was 36.33%. Indeed, a subsequent step-forward process of the variables to the elaboration of the model indicated that the C and D environmental variables significantly explained part of the variability (Inflation Factors = 2.3 and 3.0, respectively; $p = 0.034$ and $p = 0.036$, respectively) while M and F variables resulted not statistically significant, despite having relatively low inflation factors that did not denote co-variation (Inflation Factors = 1.3 and 2.4, respectively; $p = 0.414$ and 0.642). However, although the step-forward addition process to examine the effect of each variable on the model in terms of explaining the faunal variation at the CCA ordination space indicated that variable F was not statistically significant, the CCA biplot revealed a strong relation between the F vector and the first ordination axis (Fig. 10A–B; Table 4), which was also supported by a subsequent Spearman rank correlation test ($r^2 = 0.771$, $p < 0.001$; Fig. 10C).

The volcanoes Anastasya, Tarsis, Pipoca and Chica are the ones showing the strongest effects of bottom fishing activity (F) and are also characterized by soft-bottom sponge specialists, such as *Stylocordyla borealis* (#8), *Podospongia lovenii* (#30) and *Haliclona pedunculata* (#78), since soft bottoms are preferred activity grounds of the fishery fleet.

Following the indications of the step-forward analysis of the environmental variables, a subsequent CCA constrained by only the two significant variables (C and D) was conducted (Fig. 11). Its 4 main axis explained collectively 72.5% of the faunal variation in the 'species x volcano' matrix, that is, more than in the two previous ordination analyses. It also explained 100% of variability in the 'species-environment' matrix (Table 4). The Monte-Carlo Permutation test indicated that the first axis of the ordination space was significant ($p = 0.042$), and also all the canonical axes when they were tested together ($p = 0.002$). These results suggested that the environmental variables D and C are responsible for at least some of the variation within the 'species x volcano' matrix.

The two-variable CCA biplot depicted a global pattern of relative faunal affinities similar to that in the CA analyses, but rotated and having Gazul, Almazan and Aveiro volcanoes moderately changed their positions in the biplot (Fig. 11A). Significant spearman rank correlations (Fig. 11C–D) revealed that the C variable was associated to axis 1 of the ordination space and D variable to axis 2 (C: $r^2 = 0.552$, $p = 0.029$; D: $r^2 = 0.476$, $p = 0.047$). The volcanoes with higher C values (Gazul, Hesperides, Almazan) were located at the negative side of axis 1 and the deepest volcanoes (Aveiro, Almazan) at the positive side of axis 2. Compared to the unconstrained biplot (Fig. 9), Gazul clearly moved its position in the 2-variable CCA towards the C vector, indicating that the presence of carbonates has a strong influence on the abundance distribution of the sponge fauna of this volcano. Hesperides volcano, which conserved its position in the biplot despite the constraint, appeared relatively near the C vector, which can also be interpreted as a moderate influence of the carbonates on its sponge fauna. Almazan moderately moved towards the C vector, remaining in a central position between the C and the D, which can be interpreted as a moderate influence of these two variables on its sponge fauna. Aveiro volcano subtly moved towards the center, and remained near the D variable, implying that depth was more markedly influencing its sponge composition. The remaining volcanoes, from the shallow and intermediate zones, and with moderate presence to lack of carbonates, seemed to be unaffected by these two environmental variables. The distribution of some species was closely related to these two environmental variables (Fig. 11B; Table 3). Species such as *D. inornata* (#32), *Hamacantha (Hamacantha) johnsoni* (#34), *Hamacantha (Hamacantha) schmidtii* (#36) and *Hamacantha (Vomerula) papillata* (#37) were close to D vector, with growing abundances with increasing depth. Other species, such as *Jaspis incrustans* (#42), *Poecillastra compressa* (#60) and *Petrosia crassa* (#71), were close to the C vector, indicating their preference or need for hard substrates.

3.3. *Faunal affinities along a MOW-related Atlantic-Mediterranean gradient*

Fig. 12 summarizes the results of a cluster analysis based on the Jaccard similarity for a matrix of 456 sponge species from 9 deep-sea biogeographical areas of the Western Mediterranean and 7 from the Northeastern Atlantic — after pooling together the Canary Islands (C-A), Madeira islands (Ma-A), and the adjacent Moroccan slope (Mo-A). All Mediterranean areas were grouped together, except for the idiosyncratic Tyrrhenian Sea (T-M). The sponge fauna of the mud volcanoes (MV-A) and that previously known from other deep-sea environments in the Gulf of Cadiz (GC-A) were closely grouped together (28% similarity) with statistical significance, indicating that the potentially singular environment at the volcanoes is not selecting for a singular composition of sponge species, that is, the occurrence of a sponge fauna specialized in mud volcanoes is not supported from our study. More importantly, the two faunas from the Gulf of Cadiz (GC-A + MV-A) did not become associated to other Atlantic areas. Rather they clustered with the westernmost Mediterranean Area, the Central Alboran Sea (Al-M), with 18.79% similarity. These three regions became the sister group (16.98% similarity) of three other Mediterranean regions, the Gulf of Lion, the slope of Sicily Island and its surroundings, and the slope of the Gulf of Taranto, forming collectively a genuine subgroup (19.50%) with statistical support (Fig. 12).

The statistical support for the Gulf of Cadiz-Western Mediterranean node clearly illustrates the effect that the MOW has in transporting components of the Mediterranean deep-sea sponge communities towards the bathyal communities of the Gulf of Cadiz. The dendrogram

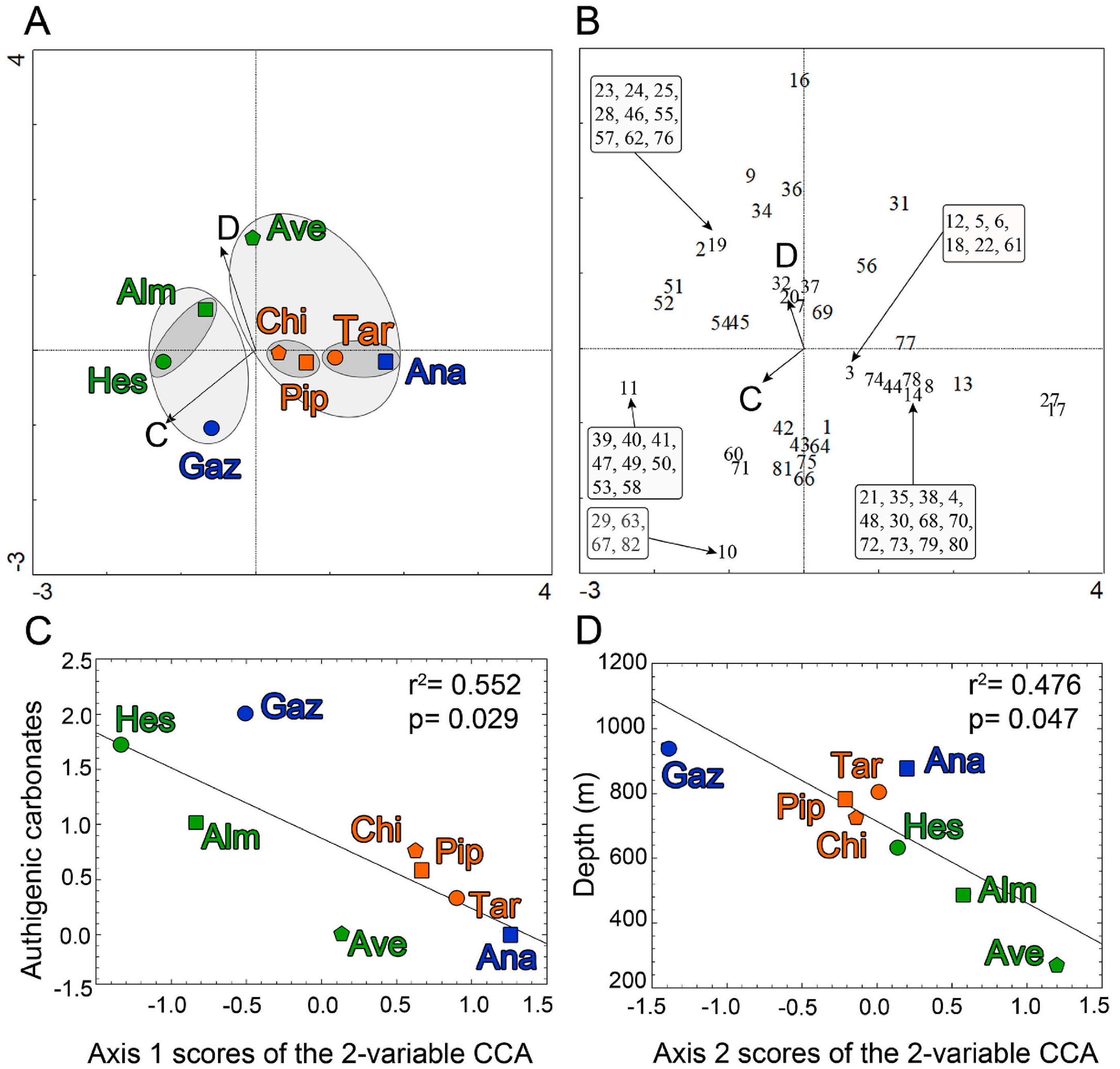

Fig. 11. **(A)** Bidimensional ordination space formed by the first and the second axes of a readjusted CCA constrained by only two of the four initial environmental variables (C = Average abundance of carbonated hard substrata; D = average depth), where volcanoes and environmental variables are represented. Shaded areas correspond to the affinities observed from the dendrogram. **(B)** Plot of the same CCA analysis, this time displaying the position of the sponge species and the environmental variables. The species are labelled as numbers which are referenced in Table 3. Numbers that overlapped at exactly the same spot in the biplot space are depicted inside boxes and their original position is indicated by the arrow head. Some species are evenly located near the vectors, indicating a major presence in determined environmental conditions. Note how some *Hamacantha* spp. (#34, 36, 37) occur near D vector, suggesting preference for deep habitats, while other species, such as *Petrosia crassa* and *Jaspis incrustans* occur near the C vector, suggesting dependence on hard carbonate substratum. **(C)** Spearman rank correlation indicating statistically significant, negative association between the scores of the volcanoes along the first axis of the two-variable CCA and their average value of abundance in authigenic carbonates. **(D)** Spearman rank correlation indicating statistically significant, negative association between the scores of the volcanoes along the second axis of the two-variable CCA versus their average depth. Spearman correlation coefficient and its "p" value are given at the top right corner for both 'B' and 'C' plots.

also showed that the Atlantic areas considered in the analysis and not directly affected by the MOW trajectory (Azores, Southern Azores Banks, Cape Verde, Lusitanian Banks, and Canary-Madeira-Moroccan slopes) had surprising low levels of faunal similarity (from 6.15 to 8.19%) among them. Only the Lusitanian Banks shared a modest affinity with the Canary-Madeira-Moroccan slopes (11.11%), and the Southern Azores Banks (8.93% affinity) (Fig. 12). The analysis also showed that the Tyrrhenian Sea was not part of the main Mediterranean cluster, implying some dissimilarity between their deep sponge faunas.

The MDS (Fig. 13) showed a global pattern similar to that of the dendrogram, identifying a cohesive group of Mediterranean areas to which the sponge fauna of the Gulf of Cadiz and the mud volcanoes were

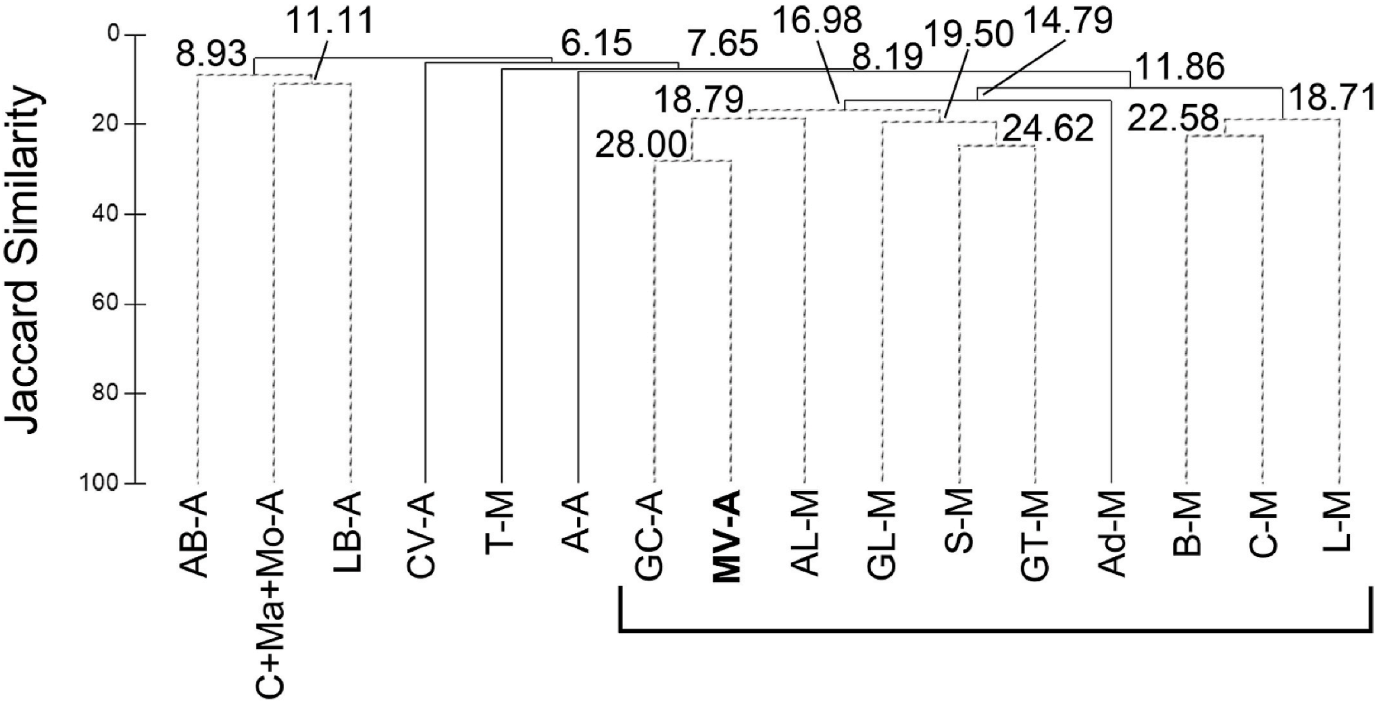

Fig. 12. Dendrogram based on Jaccard similarity displaying the affinities between the deep sponge fauna from different Atlantic and Mediterranean areas. Codes for biogeographical areas are as explained in Fig. 4. Values at the nodes correspond to the percentage of similarity between sub-groups. Dotted vertical lines represent genuine clusters with statistical support. Horizontal line at the bottom shows how the Mud volcanoes and Gulf of Cadiz group with all but one of the Mediterranean faunas considered in the study.

also associated. This again reflected the importance of the MOW trajectory on the composition of the deep-water sponge fauna of the easternmost Atlantic areas. Within the Mediterranean cluster, the deep-water sponge faunas showing the highest similarity were, on the one side, those of Gulf of Lion, Gulf of Taranto and Sicily, and, on the other side, those of the Balearic Sea and the western slope of Corsica. The distinct nature of the deep-water sponges at the Tyrrhenian Sea relative to the rest of Mediterranean deep-sea areas considered in the study was again highlighted by the analysis. The Atlantic areas not affected by the trajectory of the MOW did not form a cohesive group, but rather each area showed a relatively distinct fauna. While the distribution of the sponge faunas along the first ordination axis could not be related to a single predominant factor but to a mixed variety of them, the second axis appeared to be related to the latitude and the direction of the MOW. The Mediterranean areas (except for the idiosyncratic Tyrrhenian Sea) and those Atlantic areas impacted by the MOW, that is, the Gulf of Cadiz, the mud volcanoes, and, to a lesser extent, the Lusitanian Banks and Azores were located towards the top of the diagram. The Atlantic areas of Cape Verde, the Canary-Madeira-Moroccan slopes, and Southern Azores Banks were depicted towards the bottom of the ordination space.

The effect of the MOW in the faunal affinities was also tested by linear regression analysis, under the assumption that, if the MOW trajectory has no major effect on shaping the faunal affinities, the geographical distances among the studied deep-sea areas should largely predict the faunal similarities: the greater the geographical distance between two areas, the lower their faunal affinity. A regression analysis showed a very weak support to this hypothesis, since faunal similarity could be predicted with confidence from the geographical distance in only 34% of cases ($r^2 = 0.337$, $p < 0.001$; Fig. 14). Therefore, it can be concluded that the low association between geographical and faunal distances among the investigated deep-sea areas is largely the result of the MOW effect.

Out of the 456 species recorded for the considered depth range (≥200 m in the Mediterranean and 200–1500 m in the Atlantic) in the Atlantic-Mediterranean areas, a total of 280 (61%) species were only present in the Atlantic and 92 (20%) where present only in the Mediterranean. The number of species co-occurring in at least one Atlantic and one Mediterranean deep-sea area was 84 (18%). The deep-water sponge fauna of the Gulf of Cadiz, understood in this study as the combination of that in the mud volcanoes (MV-A) and that cited in the pre-existing literature of the Gulf of Cadiz (GC-A), consisted of 125 species, of which 63 were also present in the Mediterranean and 27 in their geographically nearest Mediterranean area, the Alboran Sea. Thirty five species co-occurred at the MV and GC areas, from which 12 were also present at the Alboran Sea: *P. carpenteri*, *A. setubalense*, *Rhizaxinella pyrifera*, *Cladorhiza abyssicola*, *Crella (Yvesia) pyrula*, *D. inornata*, *Hamacantha (Hamacantha) johnsoni, Pachastrella monilifera*, *T. muricata*, *Bubaris vermiculata*, *Phakellia robusta* and *Janulum spinispiculum*. This group of deep-water species makes the core of the faunal affinities between the Gulf of Cadiz and the westernmost Mediterranean. From those species, only three were also present in other four Mediterranean areas, which also showed faunal affinity with the Gulf of Cadiz (i.e., Al-M, Si-M, GT-M, NB + GL-M): *D. inornata*, *H. johnsoni* and *T. muricata*. Collectively, these results provide evidence that the composition of the deep-sea sponge communities of the mud volcanoes and, in general, of the Gulf of Cadiz is clearly affected by the outflow of Mediterranean water (MOW).

4. Discussion

4.1. The sponge fauna of the mud volcanoes

Previous studies on different groups of benthic organisms have been conducted across various mud volcanoes of the Gulf of Cadiz (Rodrigues et al., 2011; Rueda et al., 2012; Cunha et al., 2013; Delgado et al., 2013; González-García et al., 2020), but the sponge fauna remained hardly investigated until recently (Sitjà et al., 2019). Our study shows that the sponges of the mud volcanoes at the northern Gulf of Cadiz make a relatively cohesive fauna, which in turn shows significant affinities with the sponge fauna previously described from other bathyal communities

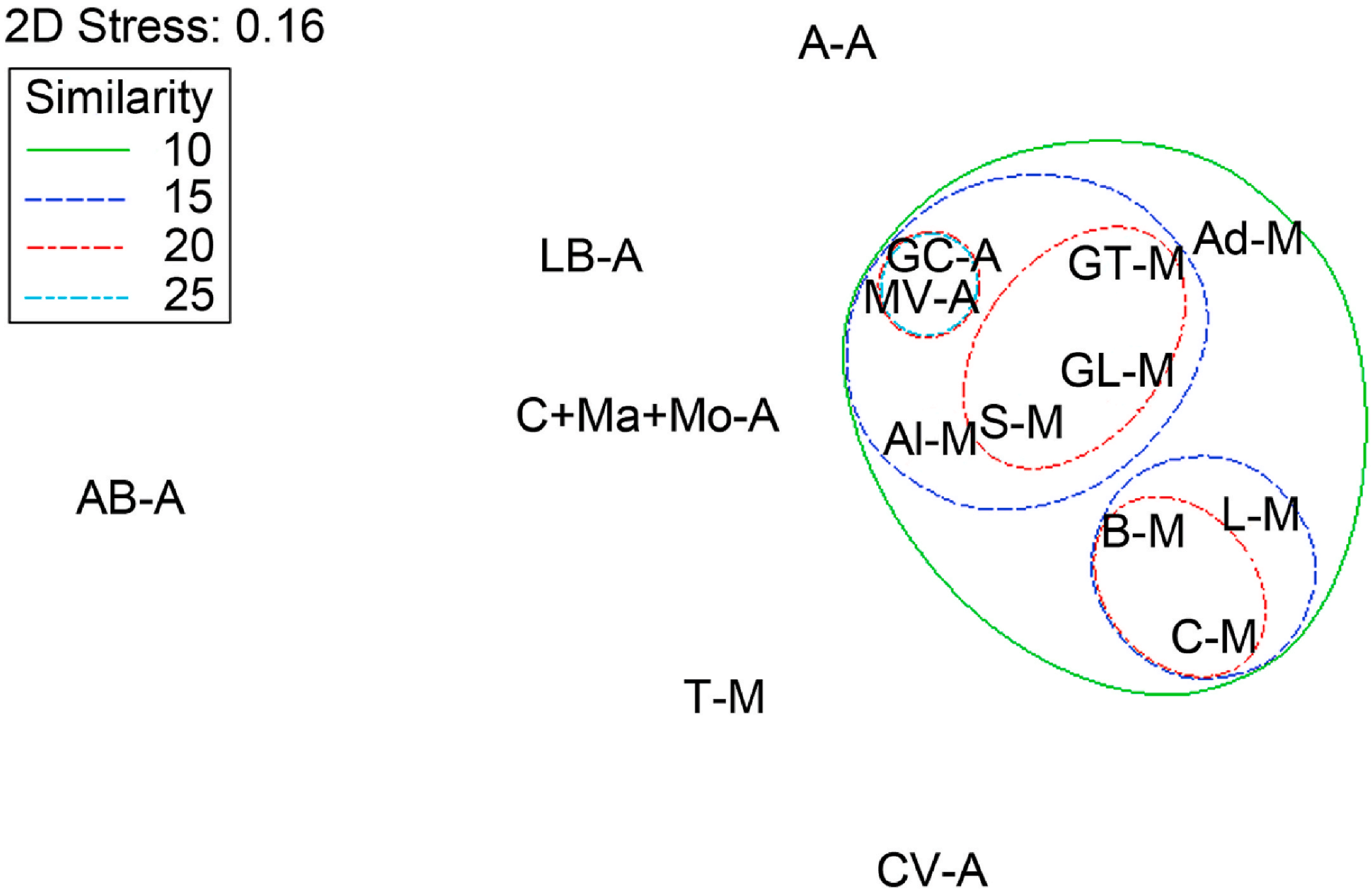

Fig. 13. MDS analysis based on the Jaccard similarity among the deep-sea biogeographical areas considered along the Atlantic-Mediterranean gradient. Codes for biogeographical areas are as explained in Fig. 4. Color lines define 10,15, 20 or 25% clines of faunal affinities. The position of the biogeographical areas clearly indicates, in congruence with previous cluster analyses, that the easternmost areas of the North Atlantic (MV-A, GC-A) are more related to the Mediterranean areas than to the remaining North Atlantic ones.

within the Gulf of Cadiz. Between-volcano comparisons also revealed that differences in faunal composition existed, being depth and presence of carbonate substrate the most relevant factors affecting both the composition and the abundance distribution of the sponges. Volcanoes such as Gazul and Hesperides (Fig. 2A–C), which had abundant authigenic carbonates exhumed by erosive processes, hosted relatively large abundance of sponges in terms of number of individuals and species richness respectively. The presence of carbonates has been reported to have positive effects in the abundance of other sessile organisms as well (Díaz del Río et al., 2014; González-García et al., 2020). Interestingly, while Gazul had the highest abundance of sponges among all the herein studied volcanoes, it showed a relatively low species richness, because its fauna was dominated by aggregations of *P. crassa*. The modest sponge species richness in Gazul contrasts with previous reports for other sessile groups, such as cnidarians and bryozoans, found to be comparatively very diverse in this volcano (Oporto et al., 2012; Díaz del Río et al., 2014; González-García et al., 2020). Yet, the abundance of hard substrates was not the rule in most volcanoes, which were characterized by prevailing soft bottoms with interspersed carbonate structures at varying density levels (Fig. 2D–E), including extremely rare presence of hard substrata (Fig. 2F–H). As a general rule, wherever soft bottoms abounded, species richness decreased comparatively, as typically in Anastasya, Tarsis and Aveiro, where the sponge fauna was dominated by a few soft-bottom specialists, which, sometimes formed aggregations. As typically happening in many other marine environments, in those volcanoes with relatively balanced proportions of hard and soft bottom (Pipoca, Chica, Almazan), species richness showed the highest values. This pattern has also been observed for other benthic organisms from Pipoca and Chica (González-García et al., 2020), in fact, their benthic communities have shown species accumulation curves similar to those herein obtained for sponges.

From a qualitative point of view, Gazul (the shallowest volcano) and Aveiro (the deepest volcano) hosted the most distinct sponge fauna relative to the other volcanoes. The main species responsible for the differentiation of Gazul from those mud volcanoes also characterized by hard substrates were *P. crassa* and *L. hypogea* —both occurring in small aggregations, along with *A. setubalense* and *P. compressa* (Fig. 2A). On the other side, the distinct nature of Aveiro derived from its aggregations of the soft-bottom sponge *T. muricata*. Another explanation might be the fact that the sampling methodologies favored the collection of larger species at this volcano, missing the small encrusting ones that might abound only on hard substrates.

The other environmental factor revealed by the CCA analyses as relevant to the sponge fauna of the volcanoes was depth, which has also been observed for the benthic communities of mud volcanoes in the depth ranges from Gazul to Tarsis (González-García et al., 2020). The general pattern for sponges, also pointed out in a previous study (Sitjà et al., 2019), was an increase in species richness with increasing depth. In the studied volcanoes, the analysis of the sponge fauna did not reveal significant detrimental effects by the bottom fishing activity. The CCA analyses indicated that fishing was not a significant variable for the constrained model addressing the explanation of the faunal variation in the "species x volcano" matrix. However, this could be a consequence of the fishing activity occurring with most intensity at the shallower volcanoes, which in turn are characterized by predominant soft bottoms (except Gazul). It is possible that the variation to be explained by the

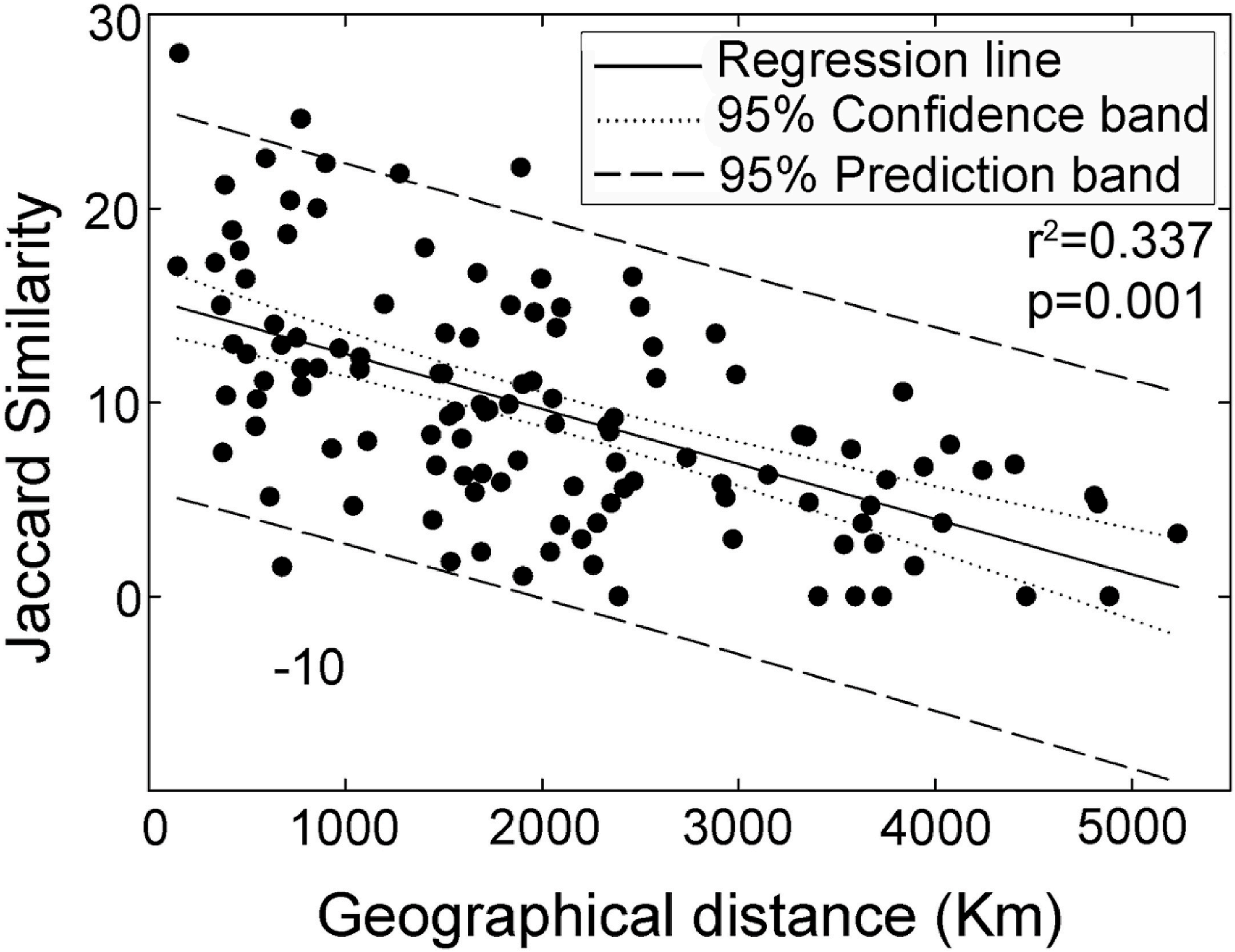

Fig. 14. Linear regression analysis showing that faunal distances between two areas (expressed as Jaccard faunal similarity) can only be predicted with confidence from the marine geographical distance in less than 34% of times. This poor predictive capability of the between-area geographical distances suggests that marine current (i.e., the MOW) is substantially affecting the pattern of faunal affinities.

bottom fishing activity was confounded with that explained by the two other variables, depth (D) and soft bottom (measured in our analyses as 1-C, that is, lack of carbonate abundance). It is worth noting that the 4-variable CCA model revealed a strong alignment between the F vector and the first ordination axis (Fig. 10A–B), a relationship also supported by a statistically significant Spearman rank correlation ($r^2 = 0.771$, $p < 0.001$; Fig. 10C). Therefore, a subsequent, more detailed study addressing the bottom fishing effects would be needed for this field of volcanoes, since in the current study fishing was processed as a semi-quantitative variable and the sampling effort at the volcanos was suboptimal for the sponge fauna. Likewise, methane venting was found to have no effect on the sponge fauna, but this, again, should be taken as tentative conclusion that might be affected by a limited sponge sampling and an incomplete acquisition of proper quantitative environmental data for the analyses. In other cold seep systems, highly specific nutritional symbioses between some sponge species and microbial symbionts have been described (Vacelet et al., 1995; Maldonado and Young, 1998; Rubin-Blum et al., 2019), suggesting that methane venting favors occurrence of a little diverse but highly specialized sponge fauna. In the mud volcanoes from the Gulf of Cadiz, no extensive dominance by such sponge fauna was detected. Therefore, the real importance of methane venting will only be elucidated by additional, more detailed studies using oceanographic manned submersibles or remotely operated vehicles rather than just trawling to both quantify methane concentrations and sampling sponge fauna from venting zones specifically.

In addition to the four environmental variables considered in the CCA analyses, it is obvious that many other factors can affect both the abundance and diversity of the sponge communities at the mud volcanoes. Local patterns of current and regional geological processes are among them (Van Rooij et al., 2010; Lozano et al., 2019). For instance, Palomino et al. (2016) found that zones of low-velocity bottom currents favored development of benthic communities related to seepage, as it could also be the case in Anastasya. Conversely, high-velocity currents could promote erosive processes that prevent sediment deposition and lead to exposure of the authigenic carbonates and any other hard substratum, favoring settlement of sessile organisms. At the same time, strong hydrodynamics associated to internal waves could favor resuspension and supply of food particles. This could be the case of Gazul, located near the shelf-break and characterized by abundant filter feeders and suspension feeders, being also known to shelter cold water corals (Rueda et al., 2016; González-García et al., 2020). Other volcanoes, such as Chica and Pipoca, which are exposed to one of the main branches of the MOW running through the adjacent Huelva Channel, also show abundant filter feeders (Rueda et al., 2016; González-García et al., 2020), in agreement with rich and abundant sponge fauna herein described from them.

4.2. Faunal affinities along the Mediterranean-Atlantic gradient

The significant similarity between the sponge fauna of the mud volcanoes (MV-A) and that previously known from other bathyal communities of the Gulf of Cadiz (GC-A) indicated that there does not appear to exist a sponge fauna specialized in the environment of the mud volcanoes. The detected similarity in the deep-sea sponge fauna between the Gulf of Cadiz (understood herein as MV-A + GC-A areas) and the Alboran Sea is in agreement with the only previous study about this issue (Boury-Esnault et al., 1994), but our results even expand the intensity of such an affinity, making clear that the MOW has a crucial role in exporting bathyal sponge fauna from the Western Mediterranean into the easternmost North Atlantic. The low faunal affinity between the deep-water sponge fauna of the Gulf of Cadiz and the remaining North Atlantic areas considered in the analysis also reveals that the effect of the Mediterranean faunal export is largely circumscribed to the Gulf of Cadiz. The reasons of this spatial constraint are unclear from the scope of our study. Nevertheless, it is likely that the exported Mediterranean sponges may somehow need the presence of the MOW water to survive, since it appears that once exported to the Gulf of Cadiz, they cannot disperse easily towards additional northeastern Atlantic areas (e.g., Azores, etc.). It could also be that the North-Atlantic trajectory of the MOW, turning North after passing the Strait of Gibraltar to stay attached to the Lusitanian slope, is hindering the colonization of the slopes of the Macaronesia (i.e., Azores, Morocco, Canary Islands, Madeira Islands, Cape Verde). Another potential reason —but less likely— for such a distribution could be that the pre-existing Atlantic deep-sea sponge fauna outcompetes rapidly the Mediterranean immigrants and avoid their proliferation.

The markedly low affinity of the sponge fauna from the Tyrrhenian Sea region with the rest of the Mediterranean reflects the long known hydrographic and hydrobiological singularity of this Mediterranean basin (Cognetti et al., 2000). There are some structural features of the Tyrrhenian Sea that contribute to its singularity relative to adjacent basins. For instance, the continental shelf of the Tyrrhenian Sea is very narrow and depth exceeds 2000 m throughout virtually the whole of the basin, reaching maximum values of 3840 m. Because the input from the continental waters is minimal, salinity remains constant at roughly 38 PSU. Temperature also remains constant all year in the deep sea, set at a relatively comfortable value of 13 °C, which allows survival in very deep water of species that in other Mediterranean areas can live only in shallower (i.e., warmer) ranges. There are many areas with active volcanism. The oxygen concentration (4.4 ppm) in deep waters is also comparatively low as well as primary productivity is in surface waters. In addition to those features, the known hydrodynamics also supports a distinctive character. At depths of 200–800 m, the Tyrrhenian Sea gets an inflow of the LIW. After passing the Strait of Sicily, a major branch of the LIW flows towards the Algerian basin and a secondary one deviates towards the Gulf of Lion running across the Tyrrhenian Sea. In the Tyrrhenian Sea, it turns cyclonically forming the South Western Tyrrhenian Gyre (SWTG) and further breaking down into smaller LIW branches that run across Corsica and the Algerian basin (Pinardi et al., 2015). Most of the deep sponge species cited from the Tyrrhenian Sea were located where the SWTG occurs, meaning that they are located out of the main LIW pathway. Contrastingly, the sponge faunas from the rest of the Mediterranean regions considered in this study are directly bathed by the main LIW flow, which contributes to explain the low affinity of the Tyrrhenian deep sponges to those of adjacent Western Mediterranean areas.

Why the exporting role of the MOW appears to be more effective for the sponge fauna than for other sessile groups, such as bryozoans and ascidians (Monniot and Monniot, 1988; Harmelin and d'Hondt, 1993), remains unclear. Previous studies showed that the sponge fauna was very suitable to illustrate how the North Atlantic Superficial water entering into the Mediterranean was transporting shallow-water northeastern-Atlantic fauna (Maldonado and Uriz, 1995; Xavier and Van Soest, 2012). Likewise, our study shows again that the sponge fauna is suitable to retrace the export of bathyal Mediterranean fauna towards the Atlantic by the MOW. The export of deep-sea sponges by the MOW explains why about 18% of the 456 species considered in our analysis of the global Atlantic-Mediterranean gradient co-occurred at both the Atlantic and the Mediterranean sides of the Strait of Gibraltar.

The global message is that the sponge faunas, either from shallow-water or deep-water, may be particularly suitable to detect early stages of between-sea faunal shifts (also ecological invasions at the deep-sea level) that might result from future alteration of major marine currents as foreseen for our future changing ocean.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2020.103326.

Authors contribution

MM designed the study. JLR and CF collected sponges and data on environmental variables. CS built the taxonomic database. CS and MM conducted the statistical analyses and assembled the first manuscript, with subsequent contributions by the remaining co-authors. All authors have approved the content of the submitted manuscript.

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