



# A relict oasis of living deep-sea mussels *Bathymodiolus* and microbial-mediated seep carbonates at newly-discovered active cold seeps in the Gulf of Cádiz, NE Atlantic Ocean

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## Abstract

Extensive beds of the deep-sea mussel *Bathymodiolus mauritanicus* (currently also known as *Gigantidas mauritanicus*) linked to active cold seeps related to fissure-like activity on Al Gacel mud volcano, Gulf of Cádiz, were filmed and sampled for the first time during the oceanographic expedition SUBVENT-2 aboard R/V Sarmiento de Gamboa. Al Gacel mud volcano is one of up to 80 fluid venting submarine structures (mud volcanoes and mud volcano/diapir complexes) identified in the Gulf of Cádiz as result of explosive venting of hydrocarbon-enriched fluids sourced from deep seated reservoirs. This mud volcano is a cone-shaped edifice, 107 m high, 944 m in diameter constituted by mud breccias and, partially covered by pavements of seep carbonates. Extensive beds of this deep-sea mussel were detected at the northern flank at 810–815 m water depth associated with bacterial mats around intermittent buoyant vertical bubble methane plumes. High methane concentrations were measured in the water column above living mussel beds. Other chemosymbiotic species (*Siboglinum* sp., *Solemya elarraichensis*, *Isorropodon* sp., *Thyasira vulcolutre* and *Lucinoma asapheus*) were also found in different parts of Al Gacel mud volcano. Al Gacel mud volcano may currently represent one of the most active mud volcanoes in the Gulf of Cádiz, delivering significant amounts of thermogenic hydrocarbon fluids which contribute to foster the extensive chemosynthesis-based communities detected. This finding is of paramount importance for linking extremophile bivalve populations along the North Atlantic, including cold seeps of the Gulf of México, hydrothermal vents of the Mid-Atlantic Ridge and now, detailed documented at the Gulf of Cádiz.

**Keywords** Deep-sea mussels · Chemosynthesis-based habitats · *Bathymodiolus mauritanicus* · Cold seeps · Seep carbonates · Mud volcanoes · Gulf of Cádiz

## Introduction

Bathymodioline deep-sea mussels (Mytilidae, Bathymodiolinae) are one of the dominant macroorganisms of chemosynthesis-based communities in hydrothermal vents on spreading ridges and back-arc basins and in cold-water seeps along subduction zones across the globe (Miyazaki et al. 2010; Laming et al. 2018). Although initially viewed

as oases within a barren deep ocean, hydrothermal vents and methane seep chemosynthesis-based communities are now recognized to interact with surrounding ecosystems on the seafloor and with the water column, and to affect global biogeochemical cycles (Levin et al. 2016).

Deep-sea mussels within the subfamily Bathymodiolinae are among the most iconic fauna to colonize deep-sea reducing habitats (cold seeps and hydrothermal vents) globally (e.g. Cosel 1982; Laming et al. 2018). Within this subfamily, the different *Bathymodiolus* (over 20 species) and *Gigantidas* species (ca. 10 species) can occur in both cold seeps and hydrothermal vents, with some species displaying different morphology but showing little genetic differentiation, which may indicate the high adaptability

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of some bathymodioline species to deep-sea reducing environments (Miyazaki et al. 2010). According to the “Evolutionary stepping stone hypothesis”, the ancestors of bathymodioline deep-sea mussels exploited resources from organic-fall habitats (e.g. sunken wood, whale carcasses) in their progressive adaptation to deep-sea reducing environments (Distel et al. 2000; Jones et al. 2006). These changes of habitat preferences resulted in adaptive trends in shell lengths (related to the availability of space and energy, and physiological trade-offs) and in the successive colonization of greater water depths (Lorion et al. 2013). During this process, these singular bivalves established symbiosis with chemosynthetic bacteria like sulfide and methane oxidizers, as an effective feeding strategy and tolerance to toxic hydrogen sulfide (Miyazaki et al. 2010; Rodrigues et al. 2010). Fueled by energy derived from chemosynthetic symbioses, their contribution to ecosystem productivity is conspicuous, with many bathymodioline species forming dense and extensive aggregates and beds (Petersen and Dutilleul 2009).

The first report of methanotrophic deep-sea mussels, living in the vicinity of cold seeps and consuming methane as the principal component of natural gas, was made by Childress et al. (1986) at the Gulf of México. Populations of other deep-sea mussels have been reported from some of the most important deep-sea cold-seep areas (around the world (Fig. 1a), including the Gulf of México (e.g. MacDonald et al. 1990), Western Africa (Olu-Le Roy et al. 2007), the Caribbean Arc-Barbados accretionary Prism (Cosel and Olu 1998), the Japanese Arc (Miyazaki et al. 2010), Papua New Guinea (Cosel and Janssen 2008) and recently, along the Costa Rica margin (McCowin et al. 2020), among others. In some of these areas, deep-sea mussels may also play an important role as ecosystem engineers, both through the formation of spatially heterogeneous biogenic reefs and in redistributing reduced-fluid emissions (Laming et al. 2018).

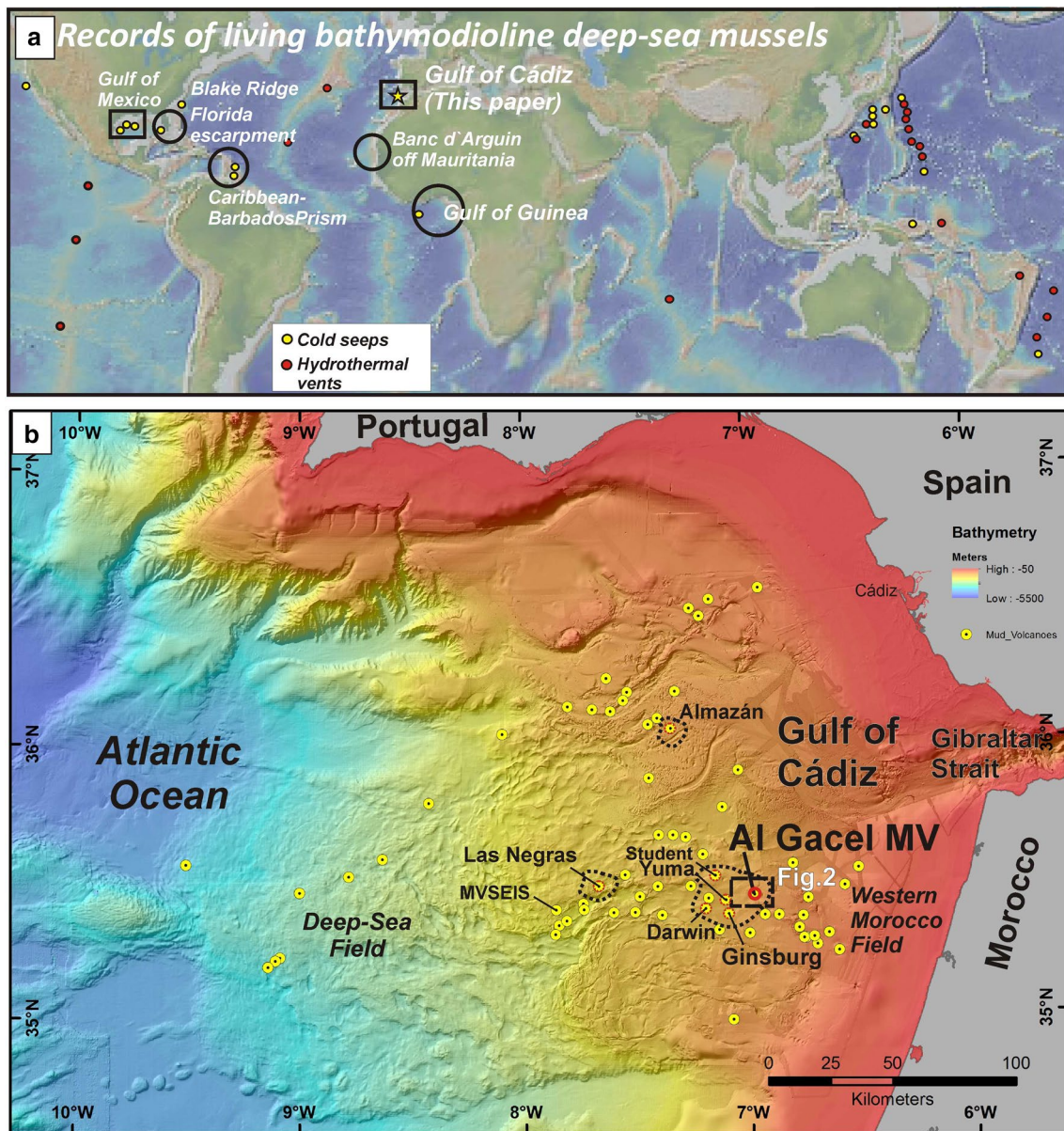
*Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus* according to Xu et al. 2019) was firstly caught as by-catch by commercial bottom trawlers along the African margin off Banc d'Arguin, Mauritania, and they were presumably associated with potential cold seeps occurring there (Cosel 2002). This species displays morphological similarities with the *Bathymodiolus childressi* clade (Childress et al. 1986) and were included in the same morphological “group” by Cosel (2002). Later on, phylogenetic studies made by Olu-Le Roy et al. (2007) pointed out some connectivity along the equatorial belt between the species originally described as *Bathymodiolus mauritanicus* living in the West Africa margin seep sites along the eastern Atlantic (Cosel 2002; Génio et al. 2008) and *Bathymodiolus childressi* occurring in the western Atlantic, especially in seep sites of the Barbados Prism (Cosel and Olu 1998) and the Gulf of México (Childress

et al. 1986). Miyazaki et al. (2010) include both deep-mussel species in the same hierarchical phylogenetic group 1–1, supporting the hypothesis that these bathymodioline species living on deep-sea cold seeps at both sides of the Atlantic Ocean could have been differentiated from amphi-Atlantic species and treated like that in further studies (Coykendall et al. 2019). Recent phylogenetic reconstruction based on three mitochondrial genes and two nuclear genes revealed that *B. mauritanicus* belongs to the *Gigantidas* genus (Xu et al. 2019) as it was already previously suggested by Jones et al. (2006). Some studies have revealed *Bathymodiolus* and *Gigantidas* species differentiation has not a single pattern and that habitat specific biotic and oceanographic characteristics play an important role, leading to deep-sea mussel population divergence at seeps and vents (Laming et al. 2018).

This study provides, for the first time, images and data of dense beds of living deep-mussels *B. mauritanicus* (currently assigned to *Gigantidas mauritanicus*) associated with cold seeps sourced from one of the most active mud volcano, Al Gacel MV, of the Gulf of Cádiz (GoC), NE Atlantic. Furthermore, this study suggests that the GoC was an area of formerly widespread populations of *Bathymodiolus mauritanicus* associated with a past higher activity of cold seeps, which has now waned, persisting in some isolated areas, such as Al Gacel MV that currently represent a relict oasis for this deep-mussel species (Fig. 1). The importance of this fact reconsider questions on the past fast spreading of the populations of *B. mauritanicus* colonizing active cold seeps at both sides of the Atlantic Ocean.

### Mud volcanoes and mud volcano/diapir complexes: the largest manifestations of cold seeps

Cold seeps are seafloor manifestations of fluid migration including methane and other hydrocarbons, brine, hydrogen sulfide, and sometimes carbon dioxide from the subsurface to the seabed and into the water column, occurring worldwide on both passive and tectonically-active continental margins (Ceramicola et al. 2018). Mud volcanoes (MVs) and mud volcano/diapir complexes (MVDs) are the largest seafloor expressions of cold seeps generated by the vertical and jointly migration of overpressured fluids (gas and water) and extrusion of mud-breccia sediments (Kopf 2002; Ceramicola et al. 2018). These fluid venting submarine structures occur globally in a wide variety of geological environments such as convergent margins as the GoC (Somoza et al. 2002, 2003; Wulff-Barreiro 2009; Palomino et al. 2016), the Calabrian accretionary prism (Ceramicola et al. 2014), the Barbados accretionary prism (Deville and Guerlais 2009) and the Caspian Basin (Blouin et al. 2020) but also in relation to sedimentary-related overpressures as



**Fig. 1** **a** Distribution of some living records of bathymodioline deep-sea mussels around the world in chemosynthesis-based communities of hydrothermal vents on spreading ridges and back-arc basins and in cold seeps along subduction zones (modified from Olu-Le Roy et al. 2007; Miyazaki et al. 2010). Black empty circles and squares represent the main seep areas with living bathymodioline deep-sea mussels at both sides of the Atlantic. **b** Distribution of fluid venting submarine structures, including mud volcanoes and mud volcano/diapir com-

plexes in the Gulf of Cádiz. Red circles show mud volcanoes where shells of *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus* according to Xu et al., 2019) have been previously reported: Darwin, Ginsburg, Student and Yuma (Génio et al. 2008) (surrounding Al Gacel) and Almazán in the Iberian field (Rueda et al. 2012). Al Gacel mud volcano is the only MV where living *B. mauritanicus* has been filmed associated with active seeps including fluid bubbling

in the Nile Deep Sea Fan (Dupré et al. 2014) or Black Sea (Bohrmann et al. 2003).

Due to the high volume of emissions and the deep roots of MVs, these structures are considered one of the most important global mechanisms for degassing of deeply buried, methane-oversaturated sediments (Dimitrov 2002). The main sink for methane in seafloor MVs is the anaerobic

oxidation of methane (AOM) mediated by archaea, operating most likely in cooperation with sulphate-reducing bacteria (SRB) (Boetius et al. 2000; Reitner et al. 2005a, b).

At cold seeps, a recent review of existing data estimated that 0.02 Gt of methane-C is consumed annually in the sediment metabolized by microbial action, with an additional 0.02 Gt methane-C escaping annually into the hydrosphere

(Boetius and Wenzhöfer 2013). The main biomineralization product resulting of the AOM are seep carbonates forming slabs or chimneys (Díaz-del-Río et al. 2003; Reitner et al. 2005a, b; Magalhães et al. 2012) transformed to oxides as a result of later exhumation forming ferromanganese nodules or pavements (González et al. 2007, 2012).

## Methods

In March 2014, the R/V *Sarmiento Gamboa* with the ROV *Luso* explored more than eleven MVs in the Moroccan margin of the GoC (Somoza and UTM-CSIC, 2018b). The ROV *Luso* is instrumented with high definition camera (1024 × 1024 pixel digital still camera), 2 robotic manipulators for biological/geological samples, CTD (conductivity, temperature and depth measurements) with fluorescence, turbidity and CO<sub>2</sub>, CH<sub>4</sub> and O<sub>2</sub> sensors and 4 Niskin bottles for water samples. Subsamples of 20 and 500 mL were taken immediately on board for CH<sub>4</sub> and CO<sub>2</sub> analyses, respectively, preserved with saturated mercuric chloride and stored in darkness. More than 107 h of video imagery were recorded from the mud volcanoes. Targets et al. Gacel mud volcano were selected using previous information from multibeam bathymetry and gravity cores during the MVS-EIS cruise (Somoza and UTM-CSIC, 2018a) on board the R/V *Hespérides*. Different *B. mauritanicus* specimens were sampled for further biosignature analysis of their shells. Furthermore, their gills were inspected to characterize their chemosynthetic symbionts, by 16S rRNA gene metabarcoding sequencing, and light and electron microscopy (Supplementary Material). Detailed information about the geophysical, sampling, mineralogical, and biogeochemical techniques can be found in the Supplementary Material.

### Cold seeps fueled by deep hydrocarbon reservoirs

Al Gacel MV is located on the north-eastern side of the middle Moroccan MV field (MMF) (León et al. 2012). This MV is smaller in size, when compared to the large MVs located at the MMF, such as Yuma and Ginsburg MVs that are up to 250 m high. Al Gacel MV is a 107 m high cone-shaped structure with a diameter at its base of 944 m, whose summit is located at 775 m depth (Fig. 2). Flanks generally have smooth slopes ranging from 2 to 20°, rarely up to 30°, and display several terrace levels on the southern flank interpreted as mudflow lobes. The summit forms a high reflective internal dome interpreted as active fluid venting.

High-resolution multichannel seismic data of Al Gacel MV revealed that the formation of this MV is related to the eruption of hydrocarbon-enriched sediments sourced from a subsurface reservoir. This MV stands over vertical fractures that act as a feeder complex connecting the MV to the

gas reservoir by fracturing the hosting Pliocene–Quaternary sedimentary units. The feeder complex is rooted in a seismic unit interpreted as the hydrocarbon reservoir characterized by high amplitude acoustic anomalies linked to the surface expression of MV by hydro-fractures that source with hydrocarbon-enriched fluids on the surface of the mud volcano (Fig. 2c). The top of the reservoir is located at 1.4 s two-way travel time (TWT).

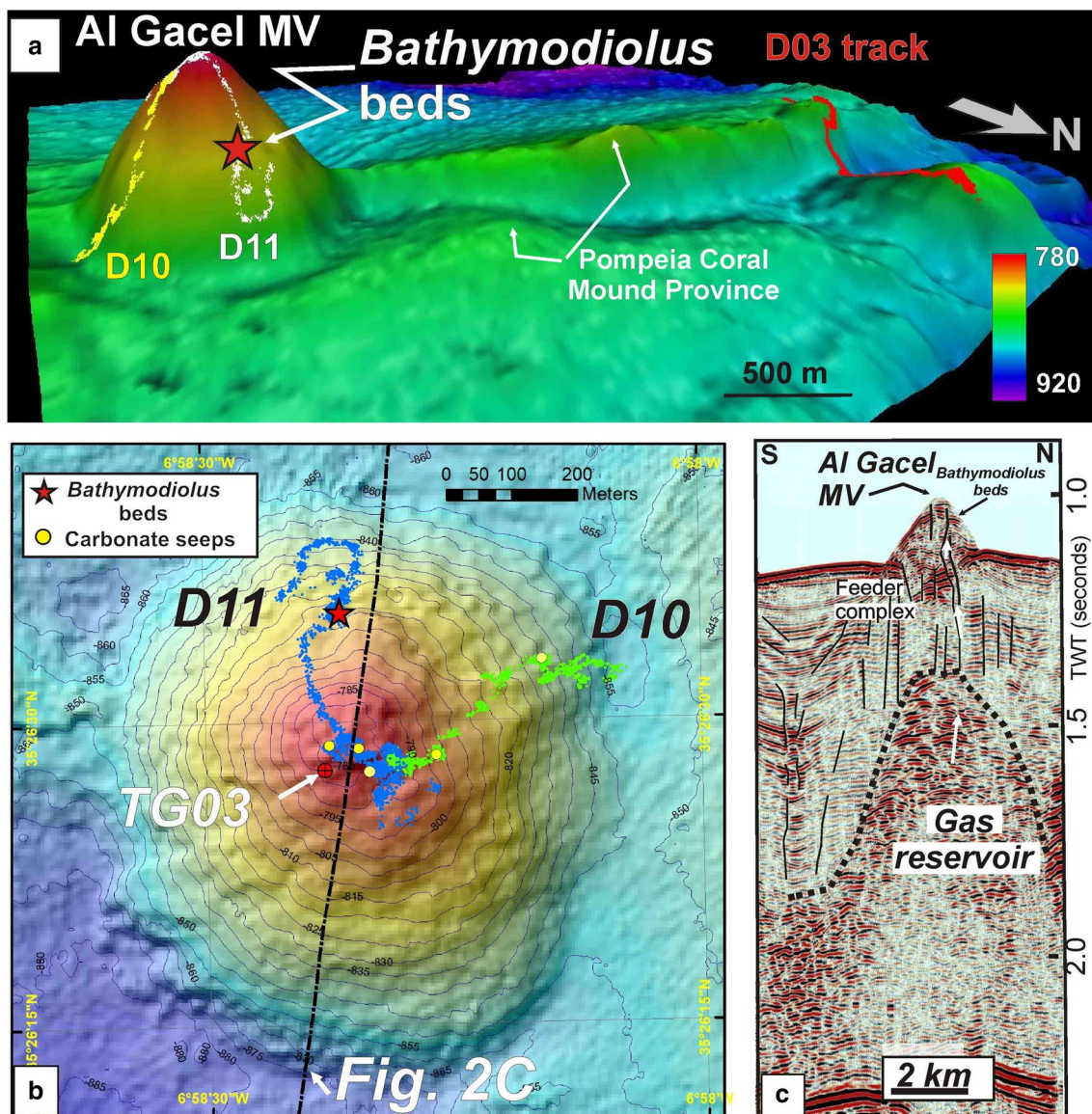
### Seafloor observations of living beds of *Bathymodiolus mauritanicus* and carbonate seeps

Visual observations made during two ROV transects across the summit and flanks of Al Gacel MV allowed to map the distribution of chemosynthesis and non-chemosynthesis-based habitats on the seafloor (Fig. 3a). Chemosynthesis-based habitats were mainly formed by: (i) beds of living *Bathymodiolus mauritanicus*, sometimes covered by microbial mats, in the N flank; (ii) Pockmark-like circular depressions, 10–50 m in diameter, completely covered by dead shells of *B. mauritanicus* and other typical cold seep species along the SE flank; and (iii) Dense populations of *Siboglinum* sp. worms at the bottom of pockmarks (1–2 m in diameter) located on the summit of Al Gacel MV (Rincón-Tomás et al. 2019, 2020) (Fig. 3a).

Extensive beds of living *B. mauritanicus* (*Bathymodiolus* beds) generally form linear aggregations (up to 10 m in length) and scattered sub-circular clumps surrounded by intermittent gas bubbling areas (diffuse to focused venting areas). These were identified at the northern flank of Al Gacel MV at 810–820 m (Figs. 3a and 4). These *Bathymodiolus* beds displayed average seafloor coverages up to 20%, with densities reaching 280 individuals m<sup>-2</sup>. Similar abundances of large (up to 8 cm shell length) and small size individuals (< 2 cm) have been detected in collected mussel clumps using the ROV manipulators, with the small size individuals being more common in the interior part of the clumps. The living *Bathymodiolus* beds showed peaks of methane concentration (Fig. 2b).

Otherwise, pockmark-like circular depressions with diameters from meters to tens of meters harbouring shell graveyards with abundant shells of typical cold seep bivalves (mainly of *B. mauritanicus* and *Lucinoma asapheus*) were detected at the southeastern flank of Al Gacel MV at 790 m depth (Fig. 3a). Live individuals of other typical cold seep bivalves as *Solemya elarraichensis* (large and small-size individuals) and *Isorropodon* sp., as well as recent remains of *B. mauritanicus*, were collected from the muddy bottoms of the south-eastern flank of Al Gacel MV at 773 m depth.

Large seep carbonate build-ups (up to 2–3 m wide) forming pavements and blocks were frequently detected and scattered around the summits of the MV. These seep carbonates were colonized by deep-sea gorgonians

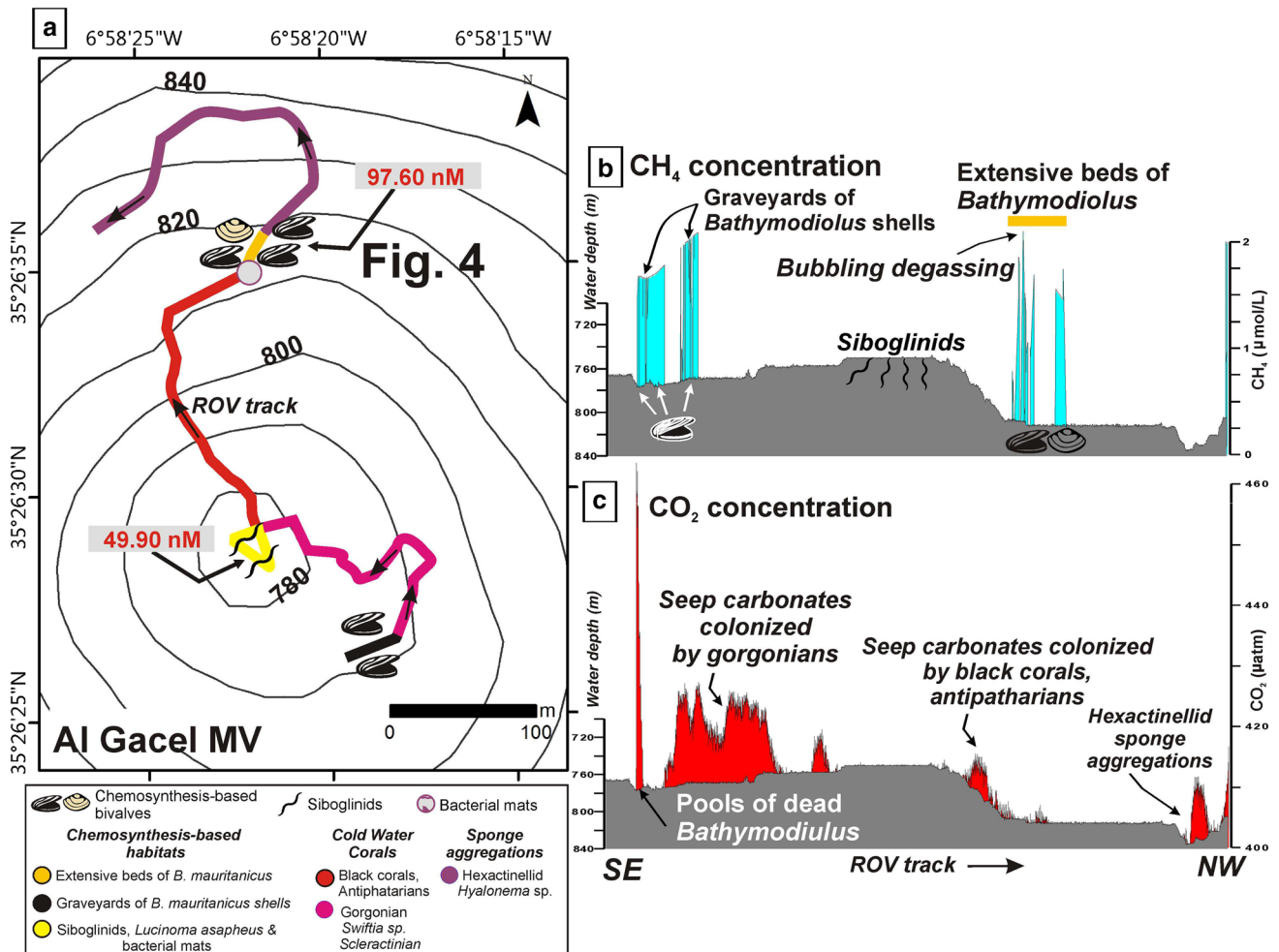


**Fig. 2** **a** 3D view of Al Gacel MV displaying the areas with extensive beds of *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) marked by a red star (*Bathymodiolus* beds in figure legend). **b** Multibeam bathymetric map of Al Gacel MV showing the

location of the ROV transects (D10 and D11), the gravity core TG03 and the seismic line shown in **c**. Grid resolution is 15 m and contour lines (isobath lines) are every 5 m. **c** Multichannel seismic line showing the gas reservoir beneath Al Gacel MV

(*Swiftia*), antipatharians (*Bathypathes*, *Leiopathes*, *Stichopathes*), bamboo corals (*Chelidonisis*, *Acanella*) and demosponges (Fig. 3a). These habitat-forming species conformed complex habitats that were located in areas with high concentrations of carbon dioxide (Fig. 3c). Otherwise muddy bottoms of the flanks contained abundant graveyards of scleractinian corals (*Desmophyllum pertusum*, *Dendrophyllia*) and stony octocorals (*Corallium*). Cementation by seep carbonates of sediments (sizes of 20–30 cm) underlying by dense populations of siboglinids appear at the center of unit pockmarks, of ca. 1–2 m in diameter. Recent data from the same expedition suggested that these

dense siboglinid populations may favour the formation of hotspots of seep carbonates on the seafloor by the action of symbionts sulphide-oxidizing bacteria by isolating these highly hydrogen sulfide toxic acidic environments not compatible with carbonate formation (Rincón-Tomás et al. 2019). At the basal part of Al Gacel MV, muddy bottoms with some patches containing graveyards of scleractinian corals and small seep carbonates were also colonized by hexactinellid sponges (*Phoronema*, *Hyalonema*) and gorgonians (*Radicipes* on soft bottoms, *Swiftia* on dispersed seep carbonates (Fig. 3a).



**Fig. 3** a Track of the ROV Dive 11 across Al Gacel MV displaying the distribution of the main chemosynthesis and non-chemosynthesis-based habitats and the location of stations where water samples for methane gas analyses were collected by the ROV; B and C Estimative concentration of gases measured by the ROV sensors during Dive 11;

**b** High methane concentration linked to the occurrence of *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) (*Bathymodiolus* in figure); **c** High carbon dioxide concentrations are linked to the occurrence of seep carbonates and non-chemosynthesis-based habitats

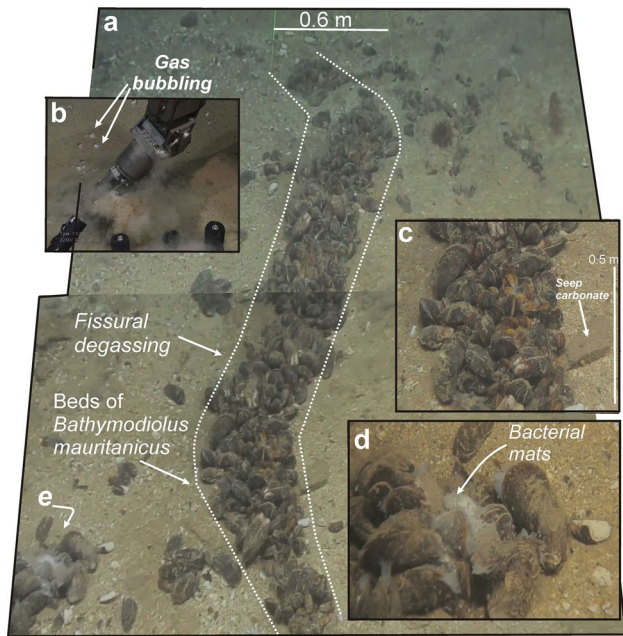
### Methane concentrations derived from cold seeps

The ROV observations of gas bubbles venting from the seafloor at some *Bathymodiolus* beds (Fig. 4b), together with the vigorous release of methane upon push-core recovery collected from this MV confirmed that the interstitial gas concentrations are at (or near to) saturation close to the seafloor. Seafloor bubbling was recorded at *Bathymodiolus* beds and sometimes directly on mussel clumps with bacterial mats (available ROV video footage can be found in Supplementary Material). In these bubbling areas above the *Bathymodiolus* beds, methane concentrations measured in the water sampled with ROV

Niskin bottles reached up to 97.6 nM at 1 m above the seabed.

### Chemistry of interstitial fluids of the extruded muds

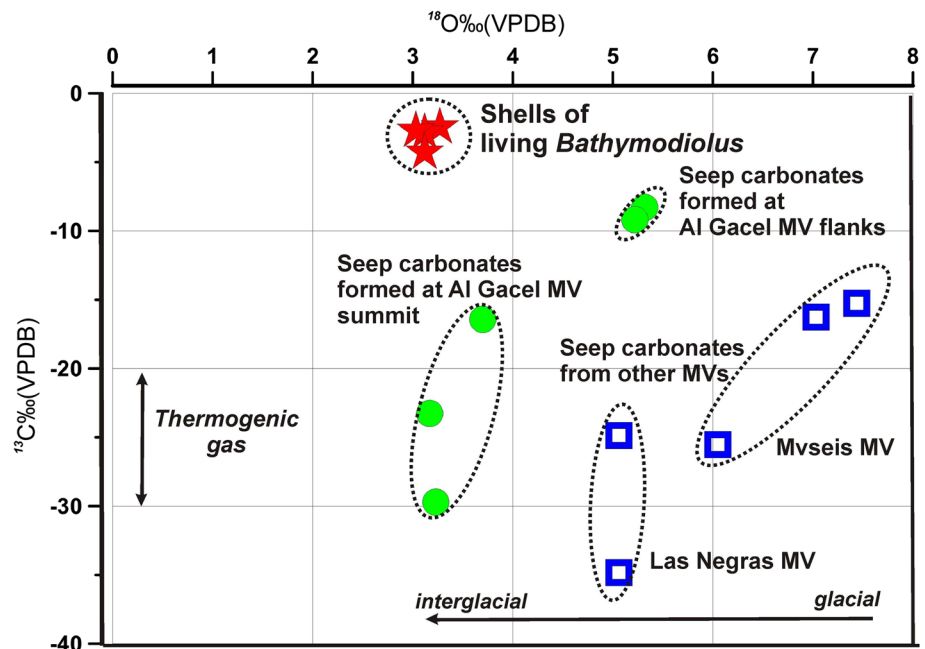
A 137-cm-long gravity core (TG03, location in Fig. 2b) recovered from the summit of Al Gacel MV (762 m depth), displayed a 120-cm lower sediment layer consisting of a mud breccia matrix of dark greenish-grey colour (GLE Y1 4/5GY after Munsell Colour Chart code) with a strong hydrogen sulfide smell and a 14 cm top oxidizing sediment layer with abundant planktonic foraminifera. High contents of elemental organic sulfur and other sulfide compounds (dimethyltrisulfide and 1,4-dimethyltetrasulfide) were detected



**Fig. 4** ROV images of one of the explored *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) beds with close-ups of specific *Bathymodiolus* clumps: **a** The linear *B. mauritanicus* bed may be associated with recent fissures generated on the MV seafloor; **b** Methane bubbling during the collection of the samples by the ROV; **c** A close-up image of the linear *B. mauritanicus* bed and a seep carbonate; **d** Circular clump of *B. mauritanicus* covered with bacterial mats (white) around an active bubbling seep area. Video footage from the ROV can be found in Supplementary Material

in mud-breccia sediments between 80 and 100 cm below seafloor (Table S1, Supplementary Material). Pore-water analyses of the gravity core TG03 displayed higher barium

**Fig. 5** Plot of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopic values for the shells of living *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) and seep carbonates from Al Gacel MV and other MVs (Mvseis and Las Negras) of the Gulf of Cádiz (Location of the MVs in Fig. 1)



(Ba), boron (B) and lithium (Li) concentrations than seawater, with at least one order of magnitude higher at the top of the core, and increasing their concentrations in deeper core sediments (Table S1, Supplementary Material).

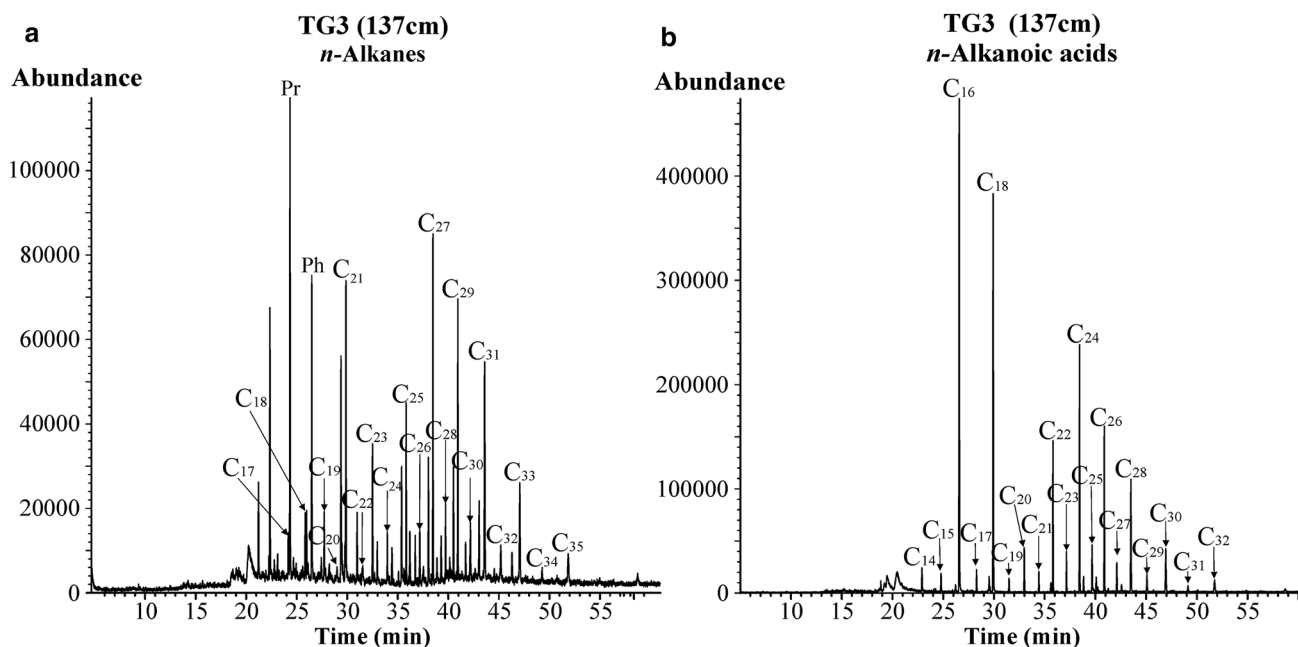
**Carbon and oxygen stable isotopes of *Bathymodiolus* shells and seep carbonates**

Carbon and oxygen stable isotope compositions were analyzed for shells of live *B. mauritanicus* individuals and for seep carbonates (Fig. 5). Thus, shells contained slight negative  $\delta^{13}\text{C}$  values ranging from  $-4.3\text{‰}$  VPDB in large-size individuals to  $-2.4\text{‰}$  VPDB in small-size individuals and low positive  $\delta^{18}\text{O}$  values ( $3.0\text{--}3.3\text{‰}$  VPDB).

Otherwise, carbonates at the summit of Al Gacel MV have high negative  $\delta^{13}\text{C}$  values ranging from  $-29.7$  to  $-16.4\text{‰}$  VPDB but low positive  $\delta^{18}\text{O}$  values ( $3.2\text{--}3.7\text{‰}$  VPDB) similar to those in shells of living *B. mauritanicus* (Fig. 5). In contrast, seep carbonates collected along the flanks of the Al Gacel MV have slighter negative  $\delta^{13}\text{C}$  values ( $-9.2$  to  $-8.3\text{‰}$  VPDB) in comparison with than those seep carbonates collected from the summit but higher positive  $\delta^{18}\text{O}$  values ( $5.0\text{--}5.3\text{‰}$  VPDB) (Fig. 5).

**Biomarkers of the hydrocarbon-enriched extruded muds**

Analyses of *n*-alkanes of sub-seafloor sediments composed of mud breccia collected on the core TG03 at the summit of Al Gacel MV (Fig. 6), showed distribution ranging from *n*-C15 to *n*-C35 with predominance of *n*-C21 and abundant presence at *n*-C29 and minor at *n*-C31 (Fig. 6a). Pristane/



**Fig. 6** Characteristics of the biomarkers of seabed sediments at the summit of Al Gacel MV. Location in Fig. 2a: **a** Distribution of *n*-alkanes, pristane (Pr), phytane/crocetane (Ph); **b** Distribution of fatty acids

phytane ratio ranging from 0.3 to 1.1 is commonly an indicator of anoxia in reducing environments, although as well these values could also be interpreted as indicators of the high input of archaeal microorganisms, potentially methanogens, due to the nature of this type of environments. The carbon preference index (CPI) from 2.2 to 4.6 suggests a deep thermal maturation of the organic matter. Fatty acids were also detected with a distribution ranging from the C<sub>14</sub> isomer to the C<sub>32</sub> homologue with maxima at C<sub>16</sub> and C<sub>18</sub> (Fig. 6b) indicate a bacterial origin and recent participation of microorganisms in the mineralization process.

### Endosymbiotic bacteria of living *Bathymodiolus mauritanicus*

Bacterial symbionts have been identified in most bathymodioline deep-sea mussel species, and they provide some-to-all of the host nutrition (Duperron 2010). At many hydrothermal vents, cold seeps and organic fall habitats, these symbiotic associations can sustain dense aggregations of deep-sea mussels.

Analysis of 16S rRNA gene sequences recovered from 2 different specimens revealed the bacterial community from the *B. mauritanicus* gills collected et al. Gacel MV (Fig. 7). Bacterial DNA was highly abundant in both specimens, obtaining up to 40,000 sequence reads in both cases (see detailed description in Supplementary Material). Methanotrophic bacteria were the most abundant organisms isolated in the gills, but the presence of Verrucomicrobia and

thiotrophic bacteria was also detected. Marine Methylo-trophic Group-1 was the main group of methane-oxidizers, representing 99.99% of the methanotrophs in mussel-3 and 99.94% of the methanotrophs in mussel-4. *Methylotenera* sp., MMG-2 and MMG-3 bacterial taxa were also found within the methanotrophic bacteria.

Light and electron microscopy images revealed the presence of bacteria organized in bacteriocytes inside the gills, as shown in Fig. 8. These bacteriocytes are localized surrounding the blood lacuna of the gills' filaments. The bacteria display a characteristic system of inner membranes typical of methanotrophic bacteria.

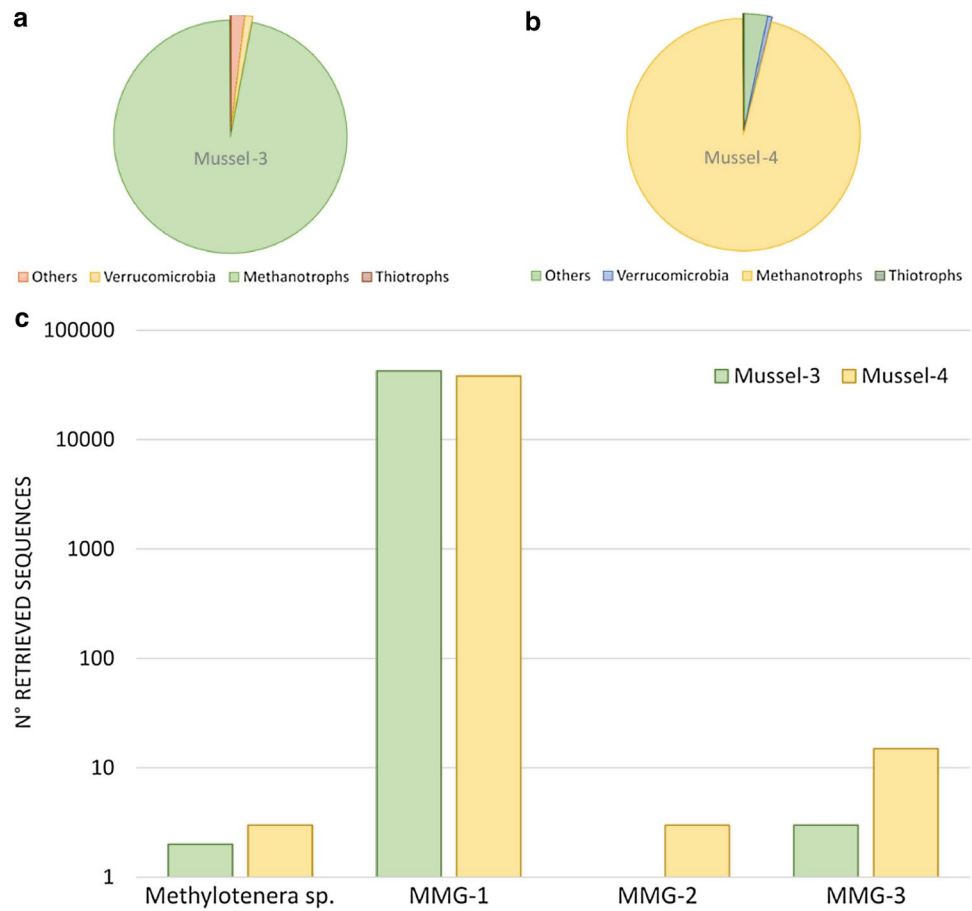
## Discussion

### Influence of the type of hydrocarbon fluids and associated salt brines during the life of fossil and present deep-sea mussels

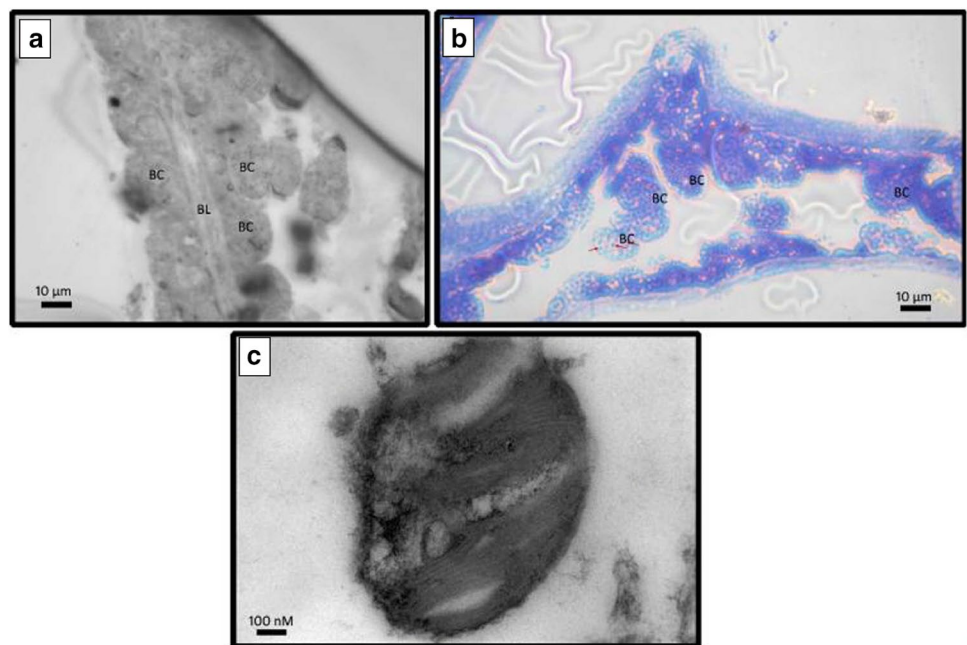
Geochemical, isotopic and seismic data pointed out to thermogenic gas as the origin of cold seeps where *B. mauritanicus* occur at present in Al Gacel MV. In support of this data, the acquired seismic section displayed signals of the occurrence of deep hydrocarbon-enriched fluids migrating upwards throughout hydro-fractures (Fig. 2c). MVs are edifices built up by periodic violent mud-breccia eruptions triggered by overpressured hydrocarbon fluids stored in deep-seated reservoirs. Nevertheless, during dormant



**Fig. 7** Bacterial community isolated from gills of *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) mussels recovered from Al Gacel MV in the Gulf of Cádiz. **a** Relative abundance of bacteria in adult mussel-3. **b** relative abundance of bacteria in juvenile mussel-4. **c** No. of retrieved sequences of methanotrophic bacterial taxa in both mussels. *MMG* Marine Methylotrophic Group



**Fig. 8** Micrographs of *Bathymodiolus mauritanicus* (currently assigned to *Gigantidas mauritanicus*) gills. **a** Gill filament with blood lacuna (BL) surrounded by bacteriocytes (BC). **b** Disrupted gill filament with many bacteriocytes with bacteria inside. Section stained with toluidine-blue. **c** TEM micrograph of a methanotrophic bacterium found inside a gill. Red arrows point to the bacteria inside the bacteriocytes



periods, these conduits favor a continuous leakage of enriched-hydrocarbon fluids and may relict brine waters from the subsurface to the seafloor (Kopf 2002; Dimitrov

2002). Therefore, based on ROV images, it could be possible to support that some linear *B. mauritanicus* beds of Al Gacel MV are located along seafloor fissures formed on the

surface of the MV that favor continuous leakage from sub-surface hydrocarbon reservoirs with diffuse to focused fluid venting along with time and space. Analyses of *n*-alkanes of the mud breccia extruded at the summit of Al Gacel MV showed values from *n*-C<sub>15</sub> to *n*-C<sub>35</sub> with a predominance of C<sub>21</sub> and the low carbon preference index (CPI) indicate the maturity of the organic matter, pointing out the thermogenic origin of the fluids rising from the sub-surface hydrocarbon reservoirs. The concretionary carbonate  $\delta^{13}\text{C}$  values (Fig. 5) most likely indicate varying degrees of mixing between <sup>13</sup>C-depleted bicarbonate produced by thermogenic methane oxidation (predominant at the summit) and <sup>13</sup>C-enriched carbon dioxide produced by methanogenesis (prevailing on the flanks). Carbon isotopes values of the *B. mauritanicus* shells ranging between  $-2.41$  and  $-4.27\text{‰}$  VPDB are interpreted to be results of the mix between several carbon sources: methane anaerobic oxidation, fermentation of organic matter and seawater.

The fact that most depleted carbon isotopes values are found in *B. mauritanicus* suggests the progressive influence of thermogenic gases in the construction of the shells during the life of the deep-sea mussels. Otherwise, high concentrations of Ba, B and Li (up to 18.89 nM, 1170  $\mu\text{M}$  and 46  $\mu\text{M}$  respectively) together with low values of redox potential ( $-200$  mV), at 1.30 m below seafloor, suggest that reducing fluids rising to surface enriched in these elements, probably related to salt brines or as results of the dehydration of smectite in depth and transformation to illite (Hensen et al. 2007). In contrast, pore-water fluids are depleted in Mg and K. These mineralized fluids could explain the origin of circular depression filled by graveyards of deep-sea mussel shells as formerly originated by brine pools surrounded by deep-sea mussels as identified in the Gulf of México (MacDonald et al. 1990), where seep carbonates are enriched in Ba, Sr and Li from the methane seeps (Sun et al. 2021).

### Symbioses as a mechanism for adaptation of deep-sea mussels to cold seeps

Thus, while symbioses may explain why bathymodioline deep-sea mussels are so productive in deep-sea reducing habitats, species survival over successive generations by the repeated colonization of nascent, chemosynthetically active habitat, depends more upon the adaptive characteristics of their lifecycle as a whole (Laming et al. 2018). 16S rRNA gene metabarcoding sequencing and visual analysis of the gills have been used to reveal the presence of methanotrophic symbionts in the gills of two *B. mauritanicus* specimens (Figs. 7, 8). The OTUs recovered from Illumina MiSeq sequencing show the high abundance of Marine Methylo-trophic Group 1 (99.99% and 99.94% of the methanotrophic bacteria detected in mussel-3 and mussel-4, respectively), which has been reported to include many sequences closely

related to bathymodioline deep-sea mussel endosymbionts (Ruff et al. 2013). These deep-sea mussels have reduced digestive systems and their nutrition mostly relies on their chemosynthetic bacteria, organized in bacteriocytes inside their gills (Duperron et al. 2005). Figure 8 shows the localization of these bacteriocytes surrounding the blood lacuna of the gills' filaments. The bacterium from those gills illustrated in Fig. 8C displays inner membranes, a typical feature of type I methanotrophs, like Marine Methylo-trophic Group 1 bacteria (Garrity et al. 2006). Detected sequences related to thiotrophic bacteria can also indicate a dual-symbiosis of the *B. mauritanicus* specimens (Fig. 7), even though their presence was not abundant and no visualization of these bacteria within the gills was possible. Nevertheless, *B. mauritanicus* has been previously identified as dual symbiotic mussel with the dominance of methanotrophic bacteria (Rodrigues et al. 2013).

### Potential influence of the reactivation of seepage activity in the Al Gacel mud volcano: comparison with other cold seeps

Occurrence of hemipelagic sediment overlying mud breccia sediments suggests that Al Gacel MV is now likely in the quiescence phase in terms of mud extrusion. However, the dense populations of *B. mauritanicus* aligned in fractures, indicate an active fluid venting related to the formation or reactivation of seabed fissures connecting with deep-seated reservoirs of thermogenic fluids. At present, methane concentration seems to control the present distribution of benthic habitat types on their surface from chemosynthetic-based fauna living surrounding seeps to non-chemosynthetic species colonizing seep carbonates. Methane concentrations detected in Al Gacel MV are much higher than those registered in other MVs of the GoC such as Captain Arutyunov MV, located northwards of Al Gacel MV, with average values of 2–8 nM in vertical profiles and 20 nM near-bottom (Sommer et al. 2009) or at St Petersburg MV, located northwards of Captain Arutyunov MV, with values ranging from 12.8 nM (seafloor) to 14.29 nM (near-bottom at the MV summit) (Sierra et al. 2017). Other MVs in the Eastern Mediterranean Sea (e.g., Napoli, Milano and Amsterdam) seem to display even higher near-bottom methane concentrations (400–2000 nM), indicating a more intensive degassing related to fluid circulation in fault systems (Charlou et al. 2003).

### Glacial to interglacial variability driven the formation of seep carbonates

Carbon isotope composition from seep carbonates collected in other MVs (e.g. Las Negras and Mvseis MVs) sampled during the SUBVENT cruise (Fig. 1) shows similar depleted  $\delta^{13}\text{C}$  values ranging from  $-30$  and  $-15\text{‰}$ VPDB, similar

to seep carbonates from the summit of Al Gacel MV. The origin of seep carbonates resulting from microbial-mediated oxidation of methane is supported by their negative carbon isotope signatures ranging from  $-56.2$  to  $-8.4\text{‰}$  VPDB, similar to other seep carbonates reported in the GoC (Díaz-del-Río et al. 2003; Magalhães et al. 2012). Otherwise, the oxygen isotope composition of the seep carbonates is controlled by a combination of factors, including the carbonate mineralogy and chemistry, the temperature of precipitation and the pore-water isotopic composition. At present, the near-bottom water temperature measured with the ROV sensor at Al Gacel MV was  $9.9\text{--}10\text{ °C}$  and salinities of  $35.6\text{--}36.0$  psu. According to Magalhães et al. (2012), seep carbonates with heavier isotopic compositions, up to  $5.9\text{‰}$  VPDB (Fig. 5), must have been formed from pore water fluids with an assumed temperature of formation of  $4\text{ °C}$ . Regional palaeoceanographic reconstructions point to  $4\text{ °C}$  as the minimum sea bottom water temperature possible to occur during glacial periods (Voelker et al. 2006). Therefore, we interpret that seep carbonates collected at Al Gacel MV flanks and at other MVs having enriched  $\delta^{18}\text{O}$  values  $> 5\text{‰}$  VPDB were formed during past colder glacial conditions. In contrast, the seep carbonates formed at the summit of Al Gacel MV displaying  $\delta^{18}\text{O}$  values around  $3\text{‰}$  VPDB associated with living *B. mauritanicus* and aggregates of Siboglinids (Fig. 5) are formed at present seabed temperature conditions by active seeps (Rincón-Tomás et al. 2019).

### **Potential causes of the demise of deep-sea mussel populations in the Gulf of Cádiz: variability in tectonic stress, sea-level changes and/or oceanic bottom-current circulation from glacial to interglacial times**

Cold seeps provide habitats that are frequently ephemeral and fragmented as those fueled by active fluid venting, separated sometimes by wide areas with typical deep-sea oligotrophic conditions (Levin et al. 2016). The wide occurrence of *B. mauritanicus* shells reported in the GoC (Fig. 1b) in contrast to live records of this deep-sea mussel suggests a dramatic demise of its populations. Therefore, surrounding Al Gacel MV, small clumps of *B. mauritanicus* have been reported from Darwin MV (1115 m depth) together with extensive mussel graveyards at Ginsburg (910 m), Student (955 m) and Yuma (975 m) MVs by Génio et al. (2008) (Fig. 1). Furthermore, *B. mauritanicus* shells were also detected in Las Negras MV during the SUBVENT expedition (Somoza et al. 2014) and even further north in Almazán MV (at 829 m depth) within the Spanish margin of the GoC (Rueda et al. 2012). Imprints of past extensive cold seeps during glacial times as documented by empty shells of chemosymbiotic bivalves is rather common in MVs from the Mediterranean Sea to the Arctic Ocean (Ivanov et al. 2010;

Taviani et al. 2013; Rovere et al. 2014; Ambrose et al. 2015). In the Mediterranean Sea, large populations of *Bathymodiolus* sensu lato seem to have disappeared from this basin, leaving *Idas modiolaeformis* as the sole bathymodioline species at present (Taviani 2011).

In the Gulf of Cádiz, the fate of large *B. mauritanicus* populations might follow a similar trend to that occurred with the fossil giant cold-water coral reefs associated with the formation of carbonate seeps on MVs (Rincón-Tomás et al. 2019). A progressive decrease in the rate of release of thermogenic hydrocarbon fluids and associated formation of seep carbonates could represent an important factor conspiring in the onset/demise of *B. mauritanicus* populations in the Gulf of Cádiz. The cessation of reducing conditions can cause populations of chemosymbiotic-based bivalves to collapse entirely. Thus, large pool-like structures, ranging 20–50 m in diameter, filled up with large numbers of *B. mauritanicus* shells observed along the southern flanks of Al Gacel MV could be interpreted as relicts of large pockmarks or even brine pools as well as imprints of past intense hydrocarbon seepage. This type of circular aggregations of living deep-sea mussels has been reported in highly active seeps of the Gulf of México (Smith et al. 2000) and the Gabon margin (Ondreas et al. 2005).

Tectonics and global climate changes are the main drivers controlling the activity of seafloor seepage. In the Gulf of Cádiz and Alborán Sea, the release of the huge reservoirs of deep hydrocarbons is tectonically controlled by the convergence between the African-Eurasia plates within the Gibraltar Arc generating extrusion of up to 84 mud volcanoes and mud volcano/diapir complexes (e.g. Somoza et al. 2021a). At global scale, intensifications of seafloor seepage have been related to the increasing destabilization of subsurface hydrates caused by two of these factors: (i) Decrease in hydrostatic pressure during sea-level lowstands (e.g. Teichert et al. 2003 among others) and/or (ii) Warming by incursions of extreme warm bottom waters during deglacial-interglacial times reported into the Arctic by a massive inflow of Atlantic waters since the last glacial (El bani Altuna et al. 2021) and other cases as in the north South China Sea margin reporting intensification of seepage during the last interglacial period (Chen et al. 2019).

In the GoC, the massive outflow of warm Mediterranean waters through the Gibraltar Strait has been related to seafloor morphologies collapses and pockmarks interpreted to be caused by massive destabilization of subsurface hydrates (Gardner et al. 2001). Otherwise, the demise of giant cold water coral reefs along the NW African coast, including the GoC, has been related to the abrupt decrease of bottom-water temperature caused by the onset of inflow of Antarctic-sourced waters along the NW African margin during deglacial (Rincón-Tomás et al. 2019) up to  $6\text{ °C}$  at present conditions between Mediterranean and Antarctic waters (Somoza et al. 2021b).

We suggest that the demise of deep-sea mussel populations in the GoC could be associated with the reduction of seafloor seepage in most of the mud volcanoes. Thus, we hypothesize that this reduction in seafloor seepage might be caused by (i) a decreasing in mud volcanoes activity associated with diminishing in tectonic stress along the African-Eurasian plate boundary; (ii) a reduction in the destabilization of buried subsurface hydrates bearing within the mud volcanoes associated with both global oceanic circulation or by sea-level changes during deglacial and interglacial times i.e. by an abrupt decrease in the bottom water temperature related to the onset of incursions of Antarctic sourced waters into the Northern Hemisphere along the NW African during deglacial times and/or by the increase in hydrostatic pressure by sea-level rise during highstands.

## Conclusion

Based on the available published information, we remark that the extensive beds of *B. mauritanicus* documented in this study could be considered a hot-spot of deep-sea chemosynthesis-based fauna for the NE Atlantic. Moreover, it could represent a relict of formerly widespread deep-sea mussel populations that colonized different cold seeps of the Gulf of Cádiz, but nowadays are restricted to very few cold seeps, with those of Algacel MV harbouring the best and most complex beds of this deep-sea mussel in the GoC and the NE Atlantic.

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