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# Chemosymbiotic species from the Gulf of Cadiz (NE Atlantic): distribution, life styles and nutritional patterns

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## Abstract

Previous work in the mud volcanoes from the Gulf of Cadiz revealed a high number of chemosymbiotic species, namely bivalves and siboglinid polychaetes. In this study we give an overview of the distribution and life styles of those species in the Gulf of Cadiz, determine the role of autotrophic symbionts in the nutrition of selected species using stable isotope analyses ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ ) and investigate the intra-specific variation of isotope signatures within and between study sites. Twenty siboglinid and nine bivalve chemosymbiotic species have been identified and were found living in fifteen mud volcanoes during our studies. Solemyids bivalves and tubeworms of the genus *Siboglinum* are the most widespread, whereas other species were found in a single mud volcano (e.g. *Bathymodiolus mauritanicus*) or restricted to deeper mud volcanoes (e.g. *Polybrachia* sp., *Lamelisabella denticulata*). Species distribution suggests that different species may adjust their position within the sediment according to their particular needs and intensity and variability of the chemical substrata supply. Isotopic values found for selected species are in accordance with values found in other studies, with thiotrophy as the dominant nutritional pathway, and with methanotrophy and mixotrophy emerging as secondary strategies. The heterogeneity in terms of nutrient sources (expressed in the high variance of nitrogen and sulphur values) and the ability to exploit different resources by the different species may explain the high diversity of chemosymbiotic species found in the Gulf of Cadiz. This study increases the knowledge of the chemosymbiotic species in the Gulf of Cadiz, highlight the relevance of seep chemoautolithotrophic production in this area and provide a starting point for future trophic ecology studies.

## 1 Introduction

The finding that hydrothermal vent tubeworms have a significantly depleted  $\delta^{13}\text{C}$  signature, very distinct from the values of other typical deep-sea invertebrates led Rau

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and Hedges (1979) to postulate that these worms rely on some non-photosynthetic food source. Later this finding was repeated for several cold seep invertebrates, and confirmed by the observation that they host symbiotic chemoautotrophic bacteria. In this symbiotic relationship, the invertebrate host facilitates the access to inorganic carbon, oxygen and reduced sulphur-compounds, and in exchange it uptakes the bacterial metabolic byproducts or digests symbiont tissue for its nutrition (Fisher, 1990; MacAvoy et al., 2002). The carbon source for thiotrophic and metanotrophic bacteria is different with the former using pore water dissolved inorganic carbon (DIC) and the latter using CH<sub>4</sub> (Fisher, 1990; Conway et al., 1994; MacAvoy et al., 2002).

The different carbon fixation pathways involve distinct isotopic fractionation (e.g. carbon, sulphur and nitrogen) which makes stable isotope approaches particularly useful for elucidating the nutritional status of organisms in vent and seep environments (Conway et al., 1994; Van Dover and Fry, 1994). Carbon isotopic values have been used to differentiate animals with thiotrophic symbionts from those with methanotrophic symbionts (Brooks et al., 1987; Kennicutt et al., 1992) and to identify the source methane pool as either thermogenic or biogenic (Sassen et al., 1999). Chemoautotrophs tend to have lower  $\delta^{15}\text{N}$  values than heterotrophs or marine phytoplankton and the  $\delta^{34}\text{S}$  values of animals hosting thiotrophic bacteria are depleted relative to those with methanotrophic symbionts and heterotrophs (Brooks et al., 1987). In fact, values of  $\delta^{34}\text{S}$  below 5‰ can be used to infer a thiotrophic mode of nutrition (Vetter and Fry, 1998) reflecting the isotopic signature of the sulphide source (Fisher, 1995). Stable isotope signatures can therefore provide information on food resource use and partitioning, both inter-specifically and intraspecifically (Levesque et al., 2003).

Previous work in the Gulf of Cadiz mud volcanoes (South Iberian Margin) found a high number of chemosymbiotic species, namely bivalves and siboglinid polychaetes (Rodrigues et al., 2008; Hilário and Cunha 2008; Hilário et al., 2010; Oliver et al., 2011); and studies on the faunal assemblages in the area show high biodiversity and high variability in structure, composition and density, which are likely to result from a combination of biogeographic, historical and environmental factors (Cunha et al., 2012).

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Especially relevant for the distribution of chemosynthetic species is the variability in the fluid sources and transport mechanisms determining the intensity of the fluxes and the geochemistry of the porewater and sediments.

The Gulf of Cadiz is an extensive cold seepage area including over 40 mud volcanoes at depths between 200 and 4000 m (Pinheiro et al., 2003; Van Rensbergen et al., 2005). The molecular and isotopic composition of the hydrocarbon gases present in the different mud volcanoes reveal a predominance of thermogenic sources with varying degrees of thermal maturity (Mazurenko et al., 2002, 2003; Stadnitskaia et al., 2006; Hensen et al., 2007; Nuzzo et al., 2008) and, in some cases, partial recycling of thermogenic methane and other hydrocarbons mediated by anaerobic oxidation of methane (AOM)-related methanogenic archaea in the shallow sediments (Nuzzo et al., 2009). Differences in both fluid geochemistry and composition of the microbial assemblages responsible for the AOM activity have major consequences in the quantity and chemical composition of the hydrocarbons reaching near-surface sediments or the water column offering a multitude of physicochemical niches that can be exploited by species with different anatomical features, physiology and symbiotic associations.

Symbiotic bivalves in the Gulf of Cadiz include members of five of the six families known to host chemoautotrophic bacteria: Solemyidae, Lucinidae, Thyasiridae, Vesicomidae, and Mytilidae (Génio et al., 2008; Rodrigues et al., 2008; Oliver et al., 2011). These bivalves have different anatomical organization particularly in relation to the structure of the gill which in all of them are modified to house the symbionts (reviewed by Taylor and Glover, 2010), and also very different life habits ranging from deep sediment burrowers to epifaunal byssate species. Bathymodiolin mussels (family Mytilidae) further differ from the other chemosymbiotic bivalves in the way that they are capable of co-hosting sulphur- and methane-oxidizers symbionts and that they keep their filter feeding ability (Fisher et al., 1993; Duperron et al., 2008).

Siboglinid polychaetes are represented in the Gulf of Cadiz by a high diversity of frenulates (Hilário et al., 2010). Frenulata, the most speciose clade of siboglinids, live in a variety of reducing environments including shelf and slope sediments, cold seeps,

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showed more depleted signatures and much wider  $\delta^{34}\text{S}$  ranges, especially *A. gadirae* (–25.7 and 11.9‰) (Fig. 2, Table 2).

$\delta^{13}\text{C}$  values for Siboglinidae worms varied between –49.8‰ (*Siboglinum* Id from Capt. Arutyunov MV) and –33.1‰ (*Siboglinum* Ib, Gemini MV). Values of  $\delta^{13}\text{C}$  lower than –40‰ were found for *Siboglinum* cf. *poseidoni* and *Siboglinum* Id, both in the Capt. Arutyunov MV, and for one *Lamelisabella denticulata* from Porto MV. Most of the species presented  $\delta^{15}\text{N}$  values lower than 6 ‰ except *Siboglinum* Ib that for the specimens in the shallow Gemini and Lazarillo de Tormes MVs presented values ranging from 8.2 to 12.2‰. Similarly, these two specimens also presented relatively high values of  $\delta^{34}\text{S}$  (6.5 and 5.7‰). The lowest value of  $\delta^{34}\text{S}$  (–16.8‰) was found in the specimen of *Siboglinum* If from Mecnès MV (Fig. 2, Table 2).

### 3.3 Intra-specific variation within and between study sites

The  $\delta^{13}\text{C}$  values for *Acharax gadirae* specimens collected from six different mud volcanoes ranged from –27.2 to –34.7‰, with a clear separation of values from specimens collected above (–27.2 to –29.3‰ in Ginsburg, Yuma, Jesus Baraza and Sagres MVs) and below 2000 m (–31.6 to –34.7‰ in Carlos Ribeiro and Porto MVs). Within the same site variability was much smaller (1.3‰ in four specimens from Yuma MV). *Solemya elarraichensis* presented small intraspecific variation between sites (2.0‰) and negligible variation within the same site (0.2 to 0.6‰). The  $\delta^{13}\text{C}$  signatures for the thyasirid *Thyasira vulcolutre* varied by 2.7‰, between specimens from Sagres and Carlos Ribeiro MVs but most of the variation was observed among specimens from Carlos Ribeiro (range of 2.4‰). “*Bathymodiolus*” *mauritanicus* showed the greatest variability within the same site (7.1‰ in Darwin MV).

The intraspecific range of  $\delta^{15}\text{N}$  values in different sites varied from 3.0‰ (*T. vulcolutre*) to 6.1‰ (*A. gadirae*). Within the same site *S. elarraichensis* showed ranges of 2, 0.5 and 0.4‰ in the Gemini, Mecnès and Darwin MVs, respectively. The four specimens of *A. gadirae* collected in the Yuma MV showed the widest range of  $\delta^{15}\text{N}$  values

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within the same species and site for all studied species (3.8‰). The  $\delta^{15}\text{N}$  ranges were 0.5‰ in Capt. Arutyunov MV and 2.4‰ in Carlos Ribeiro MV for *T. vulcolutre* and 3.0‰ in Darwin MV for “*B.*” *mauritanicus*.

Isotopic signatures of  $\delta^{34}\text{S}$  measured in *A. gadirae* showed a wide range (37.6‰) with high variability within the same site (14.2‰ in Yuma MV) and between different sites (more than 20‰). In *S. elarraichensis* the  $\delta^{34}\text{S}$  overall range was lower: 26.1‰ as well as within the same volcano (2.2, 10.6 and 3.0‰ in Gemini, Mecnès and Darwin MVs respectively). *Thyasira vulcolutre* presented  $\delta^{34}\text{S}$  ranges of 13.4‰ in Capt. Arutyunov MV and 29.8‰ in Carlos Ribeiro MV (comparable to the overall range for the species). The six specimens of “*B.*” *mauritanicus* all from Darwin MV varied by 5.2‰.

Intra-specific variation of isotopic signatures could only be examined for one species of Frenulata, *Siboglinum* Ib. The  $\delta^{13}\text{C}$  values varied from –35.9‰ in the Lazarillo de Tormes MV to –33‰ in the Sagres MV;  $\delta^{15}\text{N}$  values from 0.2‰ (Sagres MV) to 12.2‰ (Gemini MV) and the  $\delta^{34}\text{S}$  from –8.4 (Sagres MV) to 6.5‰ (Gemini MV). Within Yuma MV, ranges of 1.5 and 3.5‰ were registered for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively.

## 4 Discussion

### 4.1 Distribution and life styles

The combination of form and function studies are essential to understand the ways in which chemosymbiotic species have evolved to exploit their environment and to understand their current distribution. Because the association between an eukaryote and its symbionts can be seen as an adaptation to bridge oxic-anoxic interfaces (Cavanaugh, 1994) the chemistry profile of the sediments is expected to play a role in determining which species are able to inhabit a particular site (Dando et al., 2008).

Solemyds, lucinids and thyasirids bivalves are sediment dwellers whose burrows span oxic-anoxic interfaces in the seafloor. Whereas solemyds form “*U*”- or “*Y*”-shaped burrows (Stewart and Cavanaugh, 2006), lucinids and thyasirids maintain an anterior

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inhalant connection to the surface (Taylor and Glover, 2000; Dufour, 2005) and produce long ramified burrows. In some thyasirids the length and number of burrows show a negative relationship with the concentration of hydrogen sulphide in the sediment (Dufour and Felbeck, 2003) and it has been proposed that lucinids and thyasirids are able to “mine” insoluble sulphides (Dando et al., 1994, 2004). Details of sulphur acquisition strategies are available for some lucinid and thyasirid species but both groups exhibit such a diversity of morphologies and live in such a wide variety of habitats that it is likely that they utilize a number of different behaviours and chemical pathways to acquire reduced sulphur from the environment (Taylor and Glover, 2000; Dufour, 2005). Regarding Vesicomidae most species live shallowly burrowed in sediment usually with the posterior half of the shell protruding (Krylova and Sahling, 2010) and the sulphide uptake is through the foot that protrudes into the sediment, while oxygen uptake is through the gill (Goffredi and Barry, 2002; Taylor and Glover, 2010). Finally, bathymodiolin mussels (family Mytilidae) are mostly epibenthic and live attached by byssal threads to hard substrates, sometimes forming tight aggregates. Their reliance on chemosynthetic symbionts located in their gills implies that reduced compounds are needed in the surrounding fluid, as they do not have access to the anoxic sediment (Duperron, 2010). Bathymodiolin mussels occur in a broad range of environments which may be attributed to their nutritional flexibility: they are capable of hosting multiple symbioses obtaining from chemosynthetic symbionts including the co-occurrence of sulphur- and methane-oxidizers in addition to receiving nutrition from filter feeding (Fisher et al., 1993; Duperron et al., 2008).

Frenulata are generally described as tubeworms that extend over oxic-anoxic boundaries in the sediment, with the anterior end on the top layer of the sediment and the posterior tube body buried in the sediment. However this simplified description hides the variety of morphologies of different species that can show wide ranges of sizes (length and width), depth penetration in the sediment and tube thickness, all important features that may influence the ability to exploit different sources of nutrients.

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In the Gulf of Cadiz the methane-sulphate transition zone, where most microbial activity occurs, is in most cases more than 30 cm below the seafloor and often at 80 cm or more. An exception is Capt. Arutyunov MV where high methane concentrations were measured near the surface (Hensen et al., 2007). Burrowing bivalves and frenulates with long bodies were expected in mud volcanoes with wider redox boundaries, living in sediments where high sulphide concentrations are deep below the sediment interface. This holds true for frenulate species, for example the long frenulates *Lamellisabella denticulata*, *Spirobrachia tripeira* and *Bobmarleya gadensis* have only been found in MVs (Porto, Bonjardim and Carlos Ribeiro, respectively) where high sulphide concentrations are deep (~ 50 cm) below the seafloor (Hensen et al., 2007; Nuzzo et al., 2008) and the presence of gas hydrate reservoirs is likely to ensure fluxes capable of sustaining larger biomasses. In Capt. Arutyunov MV geochemical gradients are steep but highly variable; the sediments are frequently disturbed by gas hydrate dissociation and sustain very high densities of smaller frenulates (*Siboglinum* spp.). At shallower MVs, only the slim *Siboglinum* species and also *Polybrachia* sp.1 (at Mercator MV only) are found but always in more modest abundances (Cunha et al. 2012). The presence of small frenulates in MVs with a variety of geochemical settings, suggests that smaller and shorter species may demand less concentration of reduced compounds, allowing them to exploit lower concentrations that diffuse to the upper sediment.

The only epibenthic chemosymbiotic bivalve known in the Gulf of Cadiz, “*Bathymodiolus mauritanicus*” was found in dense aggregations inhabiting the narrow fissures between the large carbonate slabs that pave the crater of Darwin MV; aggregations of small (young) individuals are rare suggesting a strong intraspecific competition for the limited amount of favorable habitat where higher concentrations of reduced compounds reach the seafloor. Unlike many other cold seeps, chemosymbiotic bivalves in the Gulf of Cadiz are predominantly burrowing species that are found in a variety of MVs with very different geochemical settings. Solemyids are widespread but were always found at very low densities. Thyasirids are represented by several species; the juveniles and the smaller mixotrophic species and may occur in relatively high densities and are

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from microbial sulphate reduction coupled to methane oxidation (Scott et al., 2004; Becker et al., 2011). Methane isotopic signature is considerably less depleted at shallower MVs (higher thermal maturity of methane; Nuzzo et al., 2009) where *L. asphaeus* occurs than in the deeper MVs where *T. vulcolutre* is found.

### 5 4.3 Resource partitioning

The high diversity of chemosymbiotic species found in the Gulf of Cadiz may be explained by a combination of historical and contemporary factors (Cunha et al., 2012). The number of species coexisting in a single MV may be as high as nine (e.g. Capt. Arutyunov MV) and in most cases with no apparent zonation pattern, which suggests that there is resource partitioning. This may occur by means of differentiated life styles, differentiated metabolic pathways and strategies or by a combination of these. If chemical resource partitioning occurs, consistent differences in isotope values between species may be expected (Becker et al., 2011). Co-occurrence of phylogenetically close species is particularly interesting and in the Gulf of Cadiz it may be observed both for thyasirids and solemyids.

Overall *Solemya elarraichensis* presented more depleted  $\delta^{13}\text{C}$  and more enriched  $\delta^{15}\text{N}$  signatures than *Acharax gadirae*, which are probably a consequence of metabolic and morphological differences between the two species. Symbionts of co-generics of both species are known to use different forms of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) for  $\text{CO}_2$  fixation. Whereas symbionts of *Solemya* species present RubisCO form I (Scott et al., 2004), symbionts of *Acharax* present the form II of the enzyme (Duperron et al. 2012), which has lower discrimination factors against  $^{13}\text{CO}_2$ . Previous studies have shown that the difference in discrimination against  $^{13}\text{C}$  isotopes by the two forms of RubisCO can explain the disproportion in the  $^{13}\text{C}$  values of the chemoautotrophic symbiosis between two groups of chemosymbiotic invertebrates: the “-30 ‰” group with a form I RubisCO and the “-11 ‰ group” expressing a form II (Robinson and Cavanaugh, 1995).

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When compared with other taxa that also present form II RubisCO (e.g. vent vestimentiferans) the values found for *A. gadirae* are more depleted, but are in accordance with the values found for the bivalves *Calyptogena magnifica* and *Calyptogena pacifica* (Southward et al., 1994; Fisher, 1995), which suggests that bivalves expressing RubisCO form II may have other factors than this enzyme structure and kinetics contributing to the carbon fractionation.

It is worth noticing that within our samples of *A. gadirae*, specimens from deeper MVs presented more depleted  $\delta^{13}\text{C}$  values and in the same range of values found for *S. elarraichensis*. Oliver et al. (2011) ascribed *Acharax* specimens from Porto and Carlos Ribeiro mud volcanoes to the new species *A. gadirae* but because of the small size of the available specimens the same authors raised the possibility of the existence of a different species in these mud volcanoes, which could also explain the difference in the isotopic signatures.

When compared with previous data, the values of  $\delta^{13}\text{C}$  of *S. elarraichensis* and those of *A. gadirae* from the deeper mud volcanoes were similar to those found for other species of the same genera (Table S1). However, several differences were found in the  $\delta^{15}\text{N}$  values between solemyid species, suggesting that they are using different chemical species of nitrogen, tapping different pools of nitrogen, or discriminating differently after acquisition of their nitrogen source. Furthermore, it's likely that the  $\delta^{15}\text{N}$  range reflects a dilution of the very  $^{15}\text{N}$  ammonium values occurring at the sediment water interface (Lee and Childress, 1996; Carlier et al., 2010).

Nitrogen isotope values measured in both chemosymbiotic bivalves and frenulate tubeworms showed a wide range of values, including one species with  $\delta^{15}\text{N}$  values above 6 ‰ (value indicated by Levin and Michener (2002) as upper limit for the presence of chemoautotrophic symbionts). These relatively high values were found in *Siboglinum* lb collected from three of the shallowest mud volcanoes are probably related to the fact that these animals can explore different sources of nitrogen since in addition to the nutrition provided by their symbionts they may be capable of take up and metabolise dissolved organic compounds (Southward and Southward, 1981).

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Furthermore, because of the shallower depth they may have access to organic nitrogen derived from superficial waters. On the contrary, low  $\delta^{15}\text{N}$  values indicate that organic nitrogen is of local origin, presumably resulting from the activity of autotrophic bacteria (Levin, 2005; MacAvoy et al., 2008), and are possible due to assimilation of isotopically light nitrate or ammonium by the symbionts or, in the case of the very negative values, possibly fixation of  $\text{N}_2$  (Fisher, 1995). As proposed by other authors (Carlier et al., 2010) we hypothesize that the inter-specific variations of  $\delta^{15}\text{N}$  found in these study may result from species-specific types of symbionts characterized by different fractionation factor occurring during the assimilation of dissolved inorganic nitrogen, and that in the particular case of frenalate tubeworms, different species may have different degrees of access and abilities to exploit dissolved organic compounds.

In general, there was considerably less intra-specific variation between specimens collected from the same volcano than from different MVs which is plausibly explained by a higher variation in the composition of hydrocarbon pools at larger spatial scales (from one MV to another). These differences maybe lower at shallow MVs as high thermal maturity of the gases is consistent with more limited differences in carbon isotopic composition (Nuzzo et al., 2009). However, there were relevant intra-specific differences in the isotopic signatures of chemosymbiotic species both within and between MVs. The observed intra-specific variation in the  $\delta^{13}\text{C}$  may result from the assimilation of DIC of diverse origins depending on the MV, and in variable proportions, but also from the variable  $\delta^{13}\text{C}$  fractionation occurring during DIC fixation by endosymbionts, with the isotopic fractionation depending on the growth rate, and therefore on the size of each individual as reported for other studies (Carlier et al., 2010).

Similar arguments can be used to explain the differences in the  $\delta^{34}\text{S}$  signatures. There is little fractionation between sulphide and sulphate or organic sulphur as a result of sulphide oxidation by chemoautolithotrophic bacteria (reviewed in Canfield, 2001). Therefore,  $\delta^{34}\text{S}$  values of animals with chemoautolithotrophic sulphur-oxidizing symbionts reflect their reduced sulphur source (MacAvoy et al., 2005) and probably the relative abundance of reduced compounds available.  $\delta^{34}\text{S}$  values were highly variable

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among individuals of the same species either in different MVs or within the same MV reflecting high variability in the sulphide pool on a very small spatial scale. According to Becker et al., (2011) this pattern of high variability is consistent with the cycling of sulphate and sulphide between chemosymbiotic invertebrates and sediment microbial consortia. It is known that in the Gulf of Cadiz, the environmental setting variations and AOM activity are reflected by very diverse microbial community compositions and that the three microbial consortia known to perform this reaction (ANME-1, -2, and -3) are active with different distribution patterns (Vanreusel et al., 2009). It is therefore probable that a diversity of sulphide sources is available locally for exploitation by the chemosymbiotic metazoans.

## 5 Conclusions

The number of chemosymbiotic species found in the Gulf of Cadiz is high when compared with other seep locations such as the mud volcanoes in Eastern Mediterranean Sea and the Gulf of Guinea (Hilário et al., 2010; Oliver et al., 2011). Here we report for the first time on the distribution and life styles of these species and study their nutritional patterns. Our results highlight the relevance of seep chemoautolithotrophic production in this area and provide a starting point for future trophic ecology studies.

In the Gulf of Cadiz the thiotrophy is the dominant nutritional pathway, with methanotrophy and mixotrophy emerging as secondary strategies. We found a wide span of  $\delta^{13}\text{C}$  isotopic signatures that nevertheless are relatively consistent within species resulting from specific metabolic pathways. Contrastingly the highly variable  $\delta^{34}\text{S}$  values are most likely the result of the high variability of the sulphide pool at local and regional scales. The diversity and the distribution of chemosymbiotic species in the Gulf of Cadiz reflect the environmental heterogeneity in terms of nutrient sources, but also an assortment of life-styles that allows resource partitioning at local and regional scales. Future time-series sampling of chemosymbiotic species coupled with geochemical measurements should be undertaken to better understand the interplay between temporal and

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spatial variations of environmental parameters and symbiont dynamics in the studied species.

**Supplementary material related to this article is available online at:**  
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5 **bgd-9-17347-2012-supplement.pdf.**

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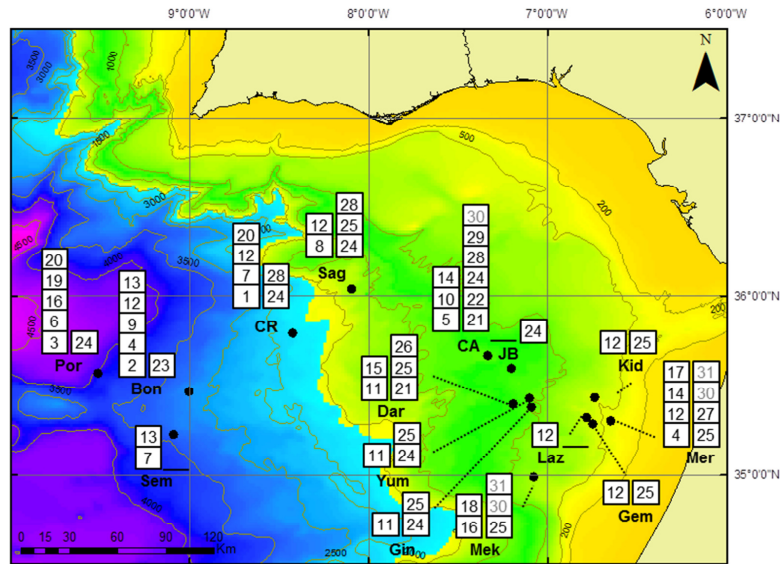
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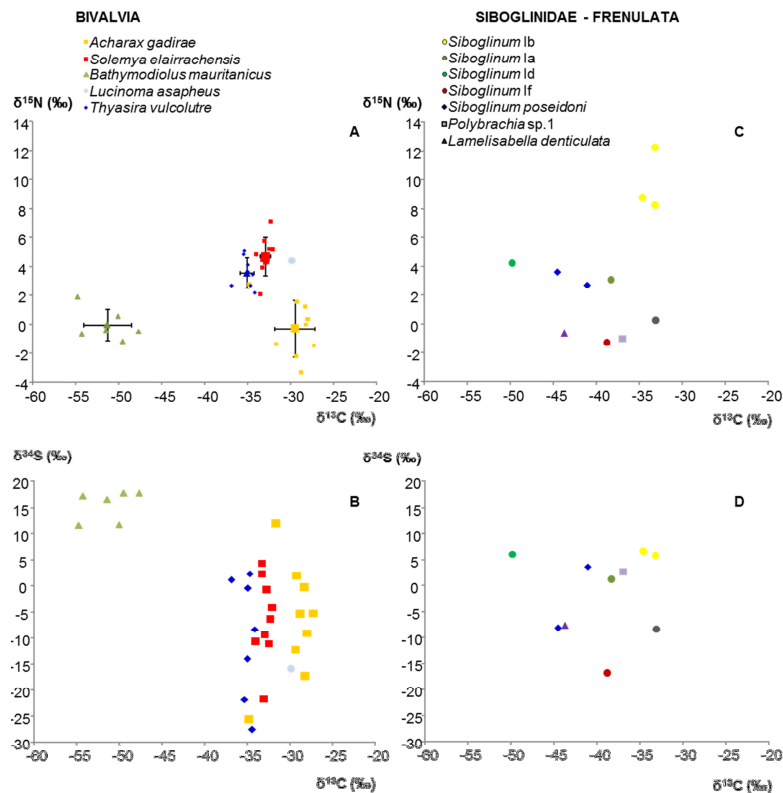
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**Fig. 1.** Map of the Gulf of Cadiz showing the chemosymbiotic species distribution. Siboglinidae species: 1. *Bobmarleya gadensis*; 2. *Lamelisabella* sp.; 3. *Lamelisabella denticulata*; 4. *Polybrachia* sp.1; 5. *Polybrachia* sp.2; 6. *Polybrachia* sp.3; 7. *Polybrachia* Va; 8. *Polybrachia* Vb; 9. *Polybrachia* Vc; 10. *Siboglinum* cf. *poseidoni*; 11. *Siboglinum* la; 12. *Siboglinum* lb; 13. *Siboglinum* lc; 14. *Siboglinum* ld; 15. *Siboglinum* le; 16. *Siboglinum* lf; 17. *Siboglinum* sp.1; 18. *Siboglinum* sp.2; 19. *Spirobrachia tripeira*; 20. Undetermined Ila. Bivalvia species; 21. *Isorropodon megadesmus*; 22. *Isorropodon perplexum*; 23. *Christineconcha regab*; 24. *Acharax gadirae*; 25. *Solemya (Petrasma) elarraichensis*; 26. “*Bathymodiolus*” *mauritanicus*; 27. *Lucinoma asapheus*; 28. *Thyasira vulcolutre*; 29. *Spinaxinus sentosus*; 30. *Axinulus croulinensis* (mixotrophic species); 31. *Thyasira granulosa* (mixotrophic species).

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**Fig. 2.** Mean values (after Table II) of  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  vs.  $\delta^{34}\text{S}$  for Bivalvia (A, B) and Frenulata (C, D) species from the Gulf of Cadiz. Mean values of species and respective standard error are also represented in A.

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