Taxonomy, distribution and community composition of megabenthic non-crustacean invertebrates in the deep Catalan margin

Taxonomía, distribución y composición de la comunidad de invertebrados no crustáceos del megabentos del Mar Catalán profundo

Ariadna Mecho Lausac

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Memoria presentada por Ariadna Mecho Lausac para optar al grado de Doctor por la Universidad de Barcelona

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“*Taxonomy rules*”

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1. General Introduction

1.1. History of Deep-Sea Exploration

The deep ocean is the largest ecosystem on Earth and also the least explored. Only 5% of the deep sea has been explored with remote instruments and less than 0.01% of the deep sea-floor has been sampled and studied (Ramirez-Llodra et al. 2010a). A series of characteristics as low temperatures, relatively constant salinity, light decreasing and pressure increasing with depth and oxygen concentration generally close to saturation make this environment distinct and unique.

Deep-sea scientific explorations began to be accessible during the mid-19th century, when improvements on maritime and oceanographic tools allowed Europe to compile great scientific information around the world. Edward Forbes was considered one of the first's deep-sea researchers, being the first scientist dredging the deep Aegean Sea with the *H.M.S. Beacon* in 1841-42. Based on the results of this cruise, he proposed the Azoic Theory suggesting that no life could exist below 600 m (Forbes 1844).

Although the first record of deep-sea fauna was the ophiuroid *Gorgonocephalus caputmedusae* (as *Astrophyton linckii*), collected by Sir John Ross in 1818 at 1600 m while exploring for the Northwest Passage (Menzies et al. 1973), it was not until 1850, when Michael Sars sampled deep-sea fauna below 800 m depth in the Arctic, that Forbes Azoic Theory was rejected. After the confirmation of deep-sea life, a race of oceanographic surveys began and a number of major cruises were conducted by different nations (i.e. *Galathea Expedition* 1845 to 1847; *H.M.S. Challenger*, 1872–76; Prince Albert of Monaco cruises 1885-1914; *Siboga Expedition* 1899 – 1900 between others). These research expeditions obtained data of an increasing number of new species and new bathymetric and biogeographic information, some of which has not been updated until today (Fig. 1).

In the Mediterranean Sea, description of bathyal fauna began at the end of the 19th century. Scientific reports of the cruises carried out by the *R.N. Washington* (1881-1882) and *S.M.S. Pola* (1890-1898) (Fig. 1) provided the first extensive taxonomical descriptions of bathyal and abyssal Mediterranean fauna (Marenzeller 1893; Bartolini Baldelli 1914), including many new species of non-crustacean invertebrates. The majority of the species sampled in these cruises were archived in several museum collections (e.g. Muséum National d'Histoire Naturelle in Paris, Naturhistorische Museum in Wien, Stazione Zoologica Anton Dohrn di Napoli and the Musée Océanographique de Monaco) where they are stored still today. From the late 1920s to 1960s the number of deep-sea Mediterranean research cruises decreased, resulting in limited new information of deep-sea species (Pérès and Picard 1956a).

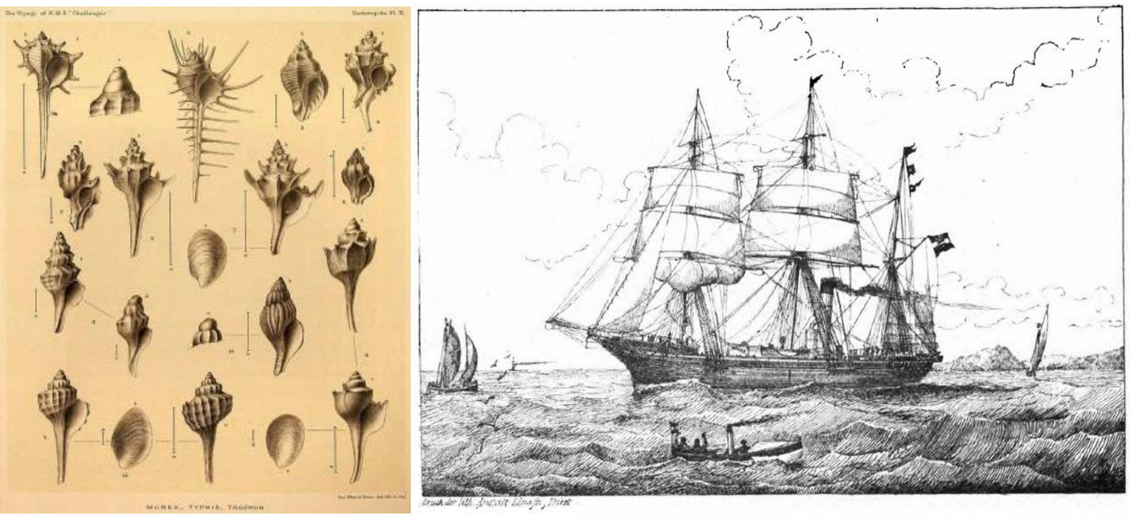

Figure 1. Table from Challenger Expedition. Illustration of the *S.M.S. Pola*

In 1960s and 1970s, deep-sea research evolved to an ecological approach, with the introduction of new sampling equipment that provided, for the first time, quantitative samples of deep-sea communities, allowing to move from descriptive to analytical studies (Hessler and Sanders 1967; Grassle and Sanders 1973; Grassle 1977). These improvements in sampling methods and equipment promoted also a second period of deep-sea scientific exploration in the deep Mediterranean Sea, conducted by ships such as the '*Bambu*', '*Mango*' and '*Ruth Ann*' in Italian waters, as well as the R.V. '*Jean Charcot*' in the Alboran Sea or the R.V. '*Garcia del Cid'* in the Balearic Sea. In the last decades of the 20th century, several studies were conducted in the deep Mediterranean Sea, from the western (Alvà 1987a; Villanueva 1992; Cartes and Sardà 1993; Stefanescu et al. 1993; Sardà et al. 1994) to the eastern (Galil and Goren 1995) basins, resulting in a considerable increase in data and information (Tyler 2003; Sardà et al. 2004c; Danovaro et al. 2010)

Finally, in the second half of the 20th century and first decade of 21th, the '*in situ*' studies of the deep-sea floor have been possible by means of modern technology such as submersibles, Remotely Operated Vehicles (ROVs) (Grassle et al. 1975; Stoner et al. 2008; Dumser and Türkay 2008), Autonomous Underwater Vehicles (AUVs) and deep-sea permanent observatories (Momma et al. 1998; Pfannkuche and Linke 2003; Howe and McGinnis 2004). These in situ observation, sampling and experimentation methods have been applied also in the deep Mediterranean Sea, from early 1950s (Pérès and Picard 1956b; Pérès 1958; Taviani et al. 2004; Bahamon et al. 2011; Aguzzi et al. 2012), allowing new observations from the cold-water coral reefs to the behaviour of fisheries target species.

1.2. Importance of Taxonomy

The word *Taxonomy* comes from the Greek ταξις, *taxis*, 'order', and νομος, *nomos*, 'method', and in biology, it has been described as "*A field of science (and major component of systematics) that encompasses description, identification, nomenclature, and classification*" (Simpson 2010). Linnaean taxonomy classifications persist mostly unchanged since the 1700s. This binomial nomenclature allows the classification of species in a consensus for all the scientific community. Morphological taxonomy has been largely improved by new technologies from electron microscopy to DNA sequencing (Edgecombe et al. 2011; Telford 2013) allowing new ordination of the groups and identification of cryptic species (Struck et al. 2007). However, molecular taxonomy has to be considered as a tool and it could not be a total substitutive of the morphological taxonomy (Ebach and Holdrege 2005; Gregory 2005).

With the use of new molecular tools, some phylogenetic hypotheses have been exposed in recent years, resulting in the same number of new phylogenetic trees. The most relevant of these trees were summarized in recent studies (Giribet et al. 2007; Edgecombe et al. 2011), nonetheless no consensus has been acquired. For this reason the present study follows the classical phylogeny of the Tree of Life Web Project (http://tolweb.org/Animals/2374).

Meanwhile, although morphological taxonomy is at the base of many other biological sciences (i.e. phylogeny, genetic populations, biodiversity, ecology between others), a significant decrease of expert taxonomists has been signaled since the beginning of the 21th century (Godfray 2002). This lack of expert taxonomists can be attributed to different factors (Godfray 2002; Giangrande 2003), from the difficulty to attract large-scale funding for taxonomical projects, to the problematic of recovering and checking old reported information. One of the most significant examples of this problem was described in a study estimating the time elapsed between the discovery and description of a new species, which could be 21 years depending of the Phyla (Fontaine et al. 2012). Las decades of 20th century, taxonomy becomes a marginal science and that derived in a considerable gap of information, and wrong classifications, which results in negative effect on associated science (biodiversity, biogeography, studies of genetic fluxes between populations, phylogenetic studies...) and diminishing the quality and accuracy of these studies (Giangrande 2003; Khuroo et al. 2007).

When exploring large and remote ecosystems, such as the deep sea, new species are collected continuously, reaching in some cases between 50% and 100% of the total sampled species (Martinez Arbizu and Brix 2008). Nonetheless, not only the descriptions of these species are required, but also the re-description of old species is necessary in order to provide new information (Brökeland and Kai Horst 2009) and make this information more accessible for the whole scientific community.

Currently, many initiatives have been developed in order to regroup, standardize and unify all the available information online (Fig. 2). This includes web sites publishing openly the original descriptions and information of species (*The Biodiversity Heritage Library*, **BHL**: http://www.biodiversitylibrary.org/), authoritative and comprehensive list of valid names (*World Register of Marine Species* ,**WoRMS**: http://www.marinespecies.org/index.php) and its deep-sea component **WoRDSS** (http://www.marinespecies.org/deepsea/), biogeographic references (*The Ocean Biogeographic information System* ,**OBIS:** http://iobis.org/), DNA barcoding (*Barcode of Life Database*, **BOLD**: http://www.barcodeoflife.org/), global web sites with all accessible data (*Encyclopedia of Life*, **eol:** http://eol.org/ or the *Tree of Life Web Project*

Figure 2. Logos of some of open access collaborative webs.

ToL: http://tolweb.org/tree/), and the INDEEP **Deep-Sea Id** app for iPhones (http://www.indeep-project.org/wg/taxonomy-evolution). All these databases and online interfaces are helping to make the existing taxonomical data more easily accessible and unified internationally.

1.3. The deep Mediterranean Sea

Situated between the African and European plates, the Mediterranean Sea covers a total area of 2.966.000 km^2 (Bass 2002). It has a mean water depth of 2500 m and a maximum depth of 5121 m at the North Matapan-Vavilov Trench (Central Mediterranean). Geographically, the Mediterranean Sea can be divided in two major basins, the western and the central-eastern basins. These two basins are separated by the shallow Sicilian Strait, with a mean depth of 400 m. The western basin is linked to the Atlantic Ocean by the Gibraltar Strait, while the eastern basin opens to the Black Sea by the Bosfor Strait, and with the Red Sea by the Suez Chanel.

The surface waters come from the Atlantic and turn into intermediate waters in the eastern Mediterranean Sea. Low-salinity Atlantic waters enter into the Mediterranean by the Gibraltar Strait, while denser deep-Mediterranean waters flow beneath the Atlantic waters in the opposite direction (Bass 2002). Deep and bottom currents are largely unexplored, but episodic intensification of high current speed has been documented in western Mediterranean Sea (Canals et al. 2006).

The Mediterranean Sea presents some unique characteristics in the deep sea. Firstly, it is homoeothermic below 200–300 m depth, with deep waters at temperatures of 13–14°C (Tyler 2003). Consequently, the thermal barrier for the vertical distribution of the fauna observed in other areas disappears in the deep Mediterranean Sea. It also promotes, a high decomposition rate of sinking organic matter, resulting in a lower quality of the major food source that reaches the bathyal and abyssal seafloor (Sardà et al. 2004c; Ramirez-Llodra et al. 2008). The deep Mediterranean Sea is also characterized by its oligotrophy, which increases also from west to east, causing a food limitation for the deep-sea communities that depend on the input of organic matter produced in the euphotic zone (Danovaro et al. 1999; Danovaro et al. 2010). Meanwhile high oxygen concentrations are present across the water column down to the seafloor (Sardà et al. 2004c).

The deep Mediterranean seafloor presents a complex assemblage of markedly different habitats (Sardà et al. 2004a; Coll et al. 2010; Danovaro et al. 2010) that include sedimentary slopes, submarine canyons and sea hills, deep basins, cold-water coral ecosystems, hydrothermal vents, cold seeps and deep anoxic basins (Danovaro et al. 2010; Company et al. 2012). The presence of these different systems results in a large-scale heterogeneity of the continental margin and the deep basin (Carpine 1970; Emig 1997; Buhl-Mortensen et al. 2010). The specific geomorphological characteristics of these habitats (e.g. the elevation of hills, the walls and axes of the submarine canyons, the inclination of the continental slopes, etc.) and the associated abiotic processes (e.g. variation in oceanographic currents, hard vs. soft substratum, food availability) facilitate the development and maintenance of diverse faunal communities (Levin et al. 2010).

In the Mediterranean, the bathyal fauna is composed by communities that include prokaryotes, foraminifera, meiofauna, macrofauna and megafauna (Danovaro et al. 2010). From all these groups, the megafauna is the best known, especially vertebrate species such as cetaceans and fishes. Meanwhile, invertebrate species are poorly known. These invertebrate faunal communities are, to date, largely unexplored (Tyler 2003), and new faunistic records, new species and first records are constantly described. As an example, within the framework of sampling operations that provided data for the present Thesis, a new species of Galatheid crab (*Munidopsis ariadne* Macpherson, 2011) was collected (Macpherson 2011). Also a new record of sipunculid has been described in a publicaton co-authored by the author of this Thesis (Saiz et al. 2014).

1.4. Invertebrates in the deep Mediterranean Sea

It is commonly known that the term “invertebrates” is not properly a taxon ”*per se*” but a term of convenience largely used, allowing us to regroup a high number of Phyla. This group includes the entire species of animals without vertebral column derived from the notochord (accordingly to that the Subphylum Tunicata belonging the Phylum Chordata could also been considered as invertebrate). Currently, 95% - 98% of the 1.4 million known animal species are invertebrates distributed into 30 Phyla (Ramirez-Llodra et al. 2010a), thus representing the majority of species of the Kingdom Animalia. Of these 30 Phyla, 29 are present in marine habitats, most of them with some species inhabiting deep-sea areas.

In the deep Mediterranean Sea, the benthic and benthopelagic megafauna are largely dominated by fish and decapod crustaceans. Thus, the Subphylum Crustacea has been the invertebrates group that has received most research attention, both because of their main ecological role (high abundance and biomass) as well as the commercial value of certain species (e.g. *Aristeus antennatus* (Risso, 1816), *Nephrops norvegicus* (Linnaeus, 1758)) (Sardà et al. 1994; Company et al. 2004; Sardà et al. 2004b; Aguzzi et al. 2009b; Bahamon et al. 2009). For these reasons, crustaceans are well known taxonomically in comparison to other megafaunal groups, such as ascidians, sponges, echinoderms, sipunculans and echiurans (Monniot and Monniot 1975; Alvà 1987a; Uriz and Rosell 1990; Villanueva 1992; Pancucci-Papadopoulou et al. 1999; Quetglas et al. 2000). Although some biological and ecological studies on deep Mediterranean invertebrates exist (Fredj 1974; Laubier and Emig 1993; Pons-Moya and Pons 2000; D'Onghia et al. 2003; Ramirez-Llodra et al. 2008; Cartes et al. 2009; Ramirez-Llodra et al. 2010b), our knowledge of the composition, distribution and function of the bathyal and abyssal Mediterranean invertebrate communities is still limited and further research is necessary.

In the present Thesis, crustaceans were not included, since their taxonomy, biology and ecology have been extensively studied in the deep Mediterranean (Company and Sardà 1997; Abelló et al. 2002). My research efforts have focused, instead, in the less-well known deep-Mediterranean, non-crustacean invertebrates, for which, in many instances, information is non-existent, scarce or out-dated.

The Phylum Echinodermata is one of the most representative taxa in terms of abundance and biomass in the deep Mediterranean Sea (once the crustaceans have been excluded). This group presents a wide bathymetric range of distribution, covering all studied depths, from 400 to 2850, m and plays a major role in the benthic community (Pérès and Picard 1956a; Fredj 1974; Cartes et al. 2009). Accordingly, the echinoderms have received special attention in this study, in the Mediterranean but also in the

Cantabrian Sea. The latter study was a by-product of parallel investigations conducted on similar habitats and depths and using comparable methods to those used in the Mediterranean study projects that form the base of this Thesis. As a result, several deep-sea echinoderms (in particular those belonging to the Class Holothuroidea) from the Cantabrian Sea have been identified and described (Appendix I).

1.4.1. *Phylum Echinodermata*

The Phylum Echinodermata is a well-defined taxonomical group divided into five Classes (Fig. 3): Crinoidea (sea lilies and feather stars), Ophiuroidea (basket stars and brittle stars), Asteroidea (starfishes), Echinoidea (sea urchins, sand dollars, and sea biscuits), and Holothuroidea (sea cucumbers). They were grouped into four synapomorphies (a shared trait derived from a common ancestor, not present before it, in the ancestor lineage) defining the Phylum and present in all specimens:

- A calcitic skeleton composed of many ossicles.
- The presence of a water vascular system.
- Mutable collagenous tissue.
- Pentaradial body organization in adults.

Figure 3. Five echinoderm classes. From left to right: Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea and Crinoidea

Echinoderms are composed only by marine species that have colonized all habitats, from the tropics to the poles and from the inter-tidal to the abyssal plains, and usually represent a large proportion of the fauna (Hyman 1955). In the deep Atlantic Ocean, for example, the echinoderms are the most important group in terms of abundance, biomass and ecosystem function (Billett 1991; Billett et al. 2001a) and have, thus, been largely studied at the taxonomic level (e.g. Hérouard 1923; Koehler 1927; Mortensen 1927; Sibuet 1977; Borrero Perez et al. 2003, amongst others). Comparatively, Mediterranean echinoderms inhabiting middle and lower slopes have been poorly studied (Marenzeller 1893; Bartolini Baldelli 1914; Tortonese 1965; Sibuet 1974; Alvà 1987a) and most reports provide only species lists without further taxonomical information (Tortonese 1979; Pérez-Ruzafa and López-Ibor 1988; Rinelli 1998; Coll et al. 2010).

Although the deep-sea Echinoderms reported in this Thesis represent a large proportion of the total abundance and biomass of non-crustacean invertebrates in the Mediterranean, this group remains largely unknown. In this study, we have addressed this knowledge gap and have conducted an update of taxonomical descriptions, bathymetric distribution ranges and a study of the biological cycle of a key species from the middle and lower Mediterranean slope. Information about life–history strategies of deep-sea invertebrates has largely improved in the past few years, showing a great variability in reproductive patterns and traits (reviewed in Young 2003). Although the reproductive patterns of many deep Atlantic echinoderms have been well described since the early 1980s (Tyler 1983; Gage et al. 1986; Young et al. 1992; Galley et al. 2008; Benítez-Villalobos and Díaz-Martínez 2010; Ross et al. 2013), these types of studies remain scarce in the Mediterranean Sea (Ferrand et al. 1988), with fishes and crustaceans receiving most of the research focus (Company and Sardà 1997; Puig et al. 2001; Company et al. 2003; D'Onghia et al. 2004; Follesa et al. 2007; Fernandez-Arcaya et al. 2012; Fernandez-Arcaya et al. 2013a; Fernandez-Arcaya et al. 2013b).

1.5. New observation and sampling tools

Because of its remoteness, sampling the deep sea is both expensive and technologically difficult and, thus, the deep seafloor and water column remain largely unexplored. Additionally, certain habitats such as hydrothermal vents, cold seeps, submarine canyons, seamounts and cold-water corals, amongst others, have complex and in some cases fragile geomorphological and biological structures that increases the difficulty in their study and sampling (Clark and Rowden 2009; Orejas et al. 2009). In these habitats, the use of novel non-intrusive observation methods and directed sampling technology with submersibles, remote operated vehicles (ROVs), autonomous underwater vehicles

(AUVs), cable observatories and landers has been introduced, replacing other traditional sampling methods such as trawling.

ROVs allow precision in samples acquisition; they can be maneuvered precisely and used to select and recover small, delicate samples more precisely than any other sampling system. It provides an undisturbed vision of the studied ecosystem *in situ*. Sample acquisition can be achieved maintaining the environmental conditions and minimizing habitat damage as well as providing information of small-scale heterogeneity that contrasts with the samples of traditional samplers (e.g. trawls) that integrate information over the studied area. ROVs are linked to the ship by an umbilical cable with, often, a tether management system (TMS) and operated by a crew onboard a vessel. ROVs are equipped with different video/still cameras and lights and depending on the work to be conducted, additional equipment such as laser points, hydraulic manipulators and accurate subsea navigation systems can be used.

In situ visual observations are adequate to study not only environments with complex topography or fragile structures, but also the faunistic ethology and the interaction between species and their environment (Trenkel 2003; Doya et al. 2014). The use of submersibles, ROVs, AUVs and cabled observatories has provided a wealth of novel deep-sea data, such as species compositions and distribution (Buhl-Mortensen et al. 2005; Fonseca et al. 2013), ethology (Aguzzi and Company 2010; Aguzzi et al. 2012) and anthropogenic impacts (Palanques et al. 2006; Miyake et al. 2011; Ramirez-Llodra et al. 2011) that could not have been acquired by traditional sampling methods.

The use of video-imaging surveys by means of ROV has been used increasingly in the Mediterranean Sea in recent years (Galgani et al. 2000; Bo et al. 2011; Fabri et al. 2013). These studies have shown the usefulness ROVs for the study of geomorphological or structurally complex habitats such as cold coral corals, seamounts and canyons, providing an alternative novel approach to the study of deep Mediterranean ecosystems.

1.6. Knowledge framework for the project

For decades, the deep-sea research group at the Marine Sciences Institute (ICM-CSIC) in Barcelona, as well as research groups from other institutions (IEO, CEAB-CSIC, IMEDEA-UIB) have studied the deep benthic ecosystems in the Mediterranean, with a main focus on fishes and crustaceans (Sardà et al. 2004a; Company et al. 2012). The species log-books of many of these cruises used a number of incorrect or non-scientific names for the least abundant or poorly known invertebrates. More recently, a few studies have included species list of non-crustacean invertebrates (Ramirez-Llodra et al. 2008; Cartes et al. 2009; Ramirez-Llodra et al. 2010b), but most of these lists were based on species names from previous publications and not supported by a real taxonomical description. It is in this context of limited taxonomical and, in some cases, biological information of non-crustacean invertebrates from the deep Mediterranean Sea that I undertook this PhD thesis.

The specific objectives of this PhD are described in Section 2. Below, we summarize the general material and methods used in this project, while detailed methodologies for the different analyses are found in the corresponding section of each publication.

1.7. General Material and Methods

1.7.1 *Studied area*

Ten multidisciplinary oceanographic cruises were conducted between October 2008 and April 2013 to sample the deep seafloor of the western Mediterranean Sea. The sampling areas included the Blanes Canyon and its adjacent open slope, the Palamós (also named La Fonera) and Cap de Creus Canyons, sea hills in the Gulf of Valencia and Eivissa Channel, and landslides on the Eivissa Channel (Fig. 4). These cruises took place in the framework of four Spanish and European research projects (PROMETEO, DOSMARES, PROMARES and EUROLEON), sampling at depths between 850 and 2845 m and acquiring video footage from 60 m to 2000 m depth. Additionally, a trans-Mediterranean cruise took place in the context of the European project BIOFUN (EuroDEEP Eurocores, European Science Foundation) during July 2009.

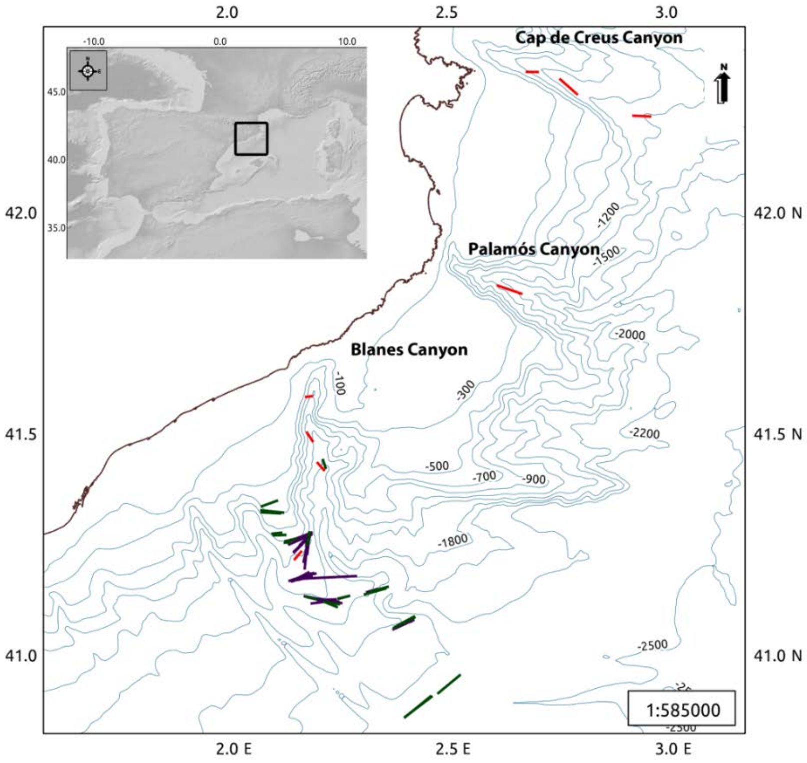

Figure. 4. Studied area by traditional methods

This cruise sampled the western, central, and eastern Mediterranean basins at 1200, 2000 and 3000 m depth. In addition, a 4000 m depth station was sampled in the central basin. Because of the low number of non-crustacean invertebrates species collected on the central and eastern basins (n = 11), only the western Mediterranean samples were used in the analyses of the present study. Nonetheless, in order to avoid the loss of this scarce information, the species sampled on the central and eastern basins were reported in the species table.

1.7.2. *Traditional sampling methods*

A total of 223 benthic trawls were completed resulting in a total swept area of 10.3 km². Of these hauls, 119 samples were obtained by a single warp otter-trawl Maireta system (OTMS, Sardà et al. 1998) with a net length of 25 m and a cod-end mesh size of 12 mm. A SCANMAR system was used to estimate the width of the mouth of the net. An average horizontal opening of 12.7 ± 1.4 m was calculated. As the SCANMAR system can only operate down to 1200 m depth, the same value for the mouth’s width of the net was used also for deployments deeper than 1200 m. The height of the trawl mouth was estimated to be 1.4 m (Sardà et al. 1998) (Fig. 5).

Figure 5. Otter-trawl Maireta system

In addition, 49 hauls were conducted with an Agassiz dredge, made of a square steel frame with a mouth width of 2.5 m and a mouth height of 1.2 m, and fitted with a 12 mm mesh net (Fig. 6A). Further, 55 samples were obtained with an epibenthic sledge, which consisted of a rectangular steel frame with three nets attached at different heights (10-50 cm, 55-95 cm and 100-140 cm above the bottom) with a mesh size of 300 µm (Fig. 6B).

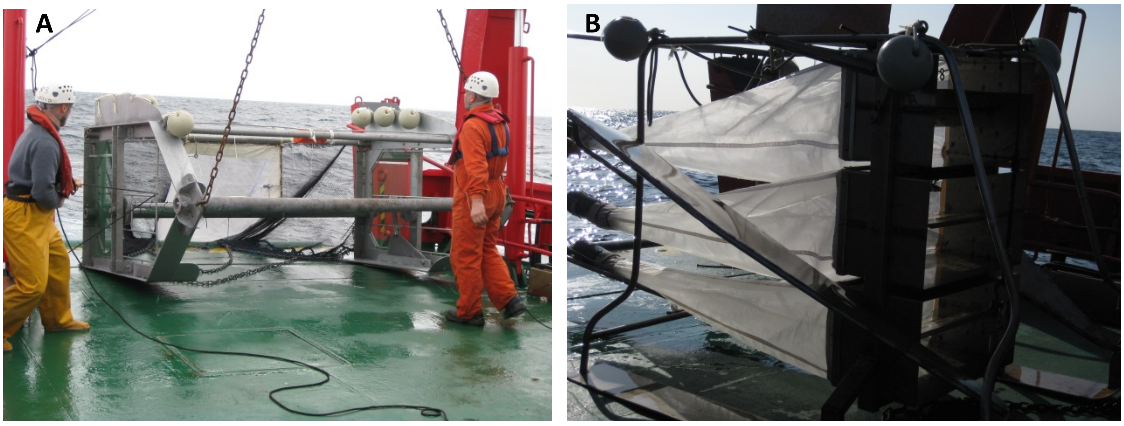

Figure 6. A) Agassiz dredge. B) Epibenthic sledge.

Faunal samples were obtained also from 15 bottles in 5 different sediment traps (Fig. 7) deployed in the Blanes Canyon axis from November 2008 to February 2009, four of them at 1200 m and one at 1500 m depth.

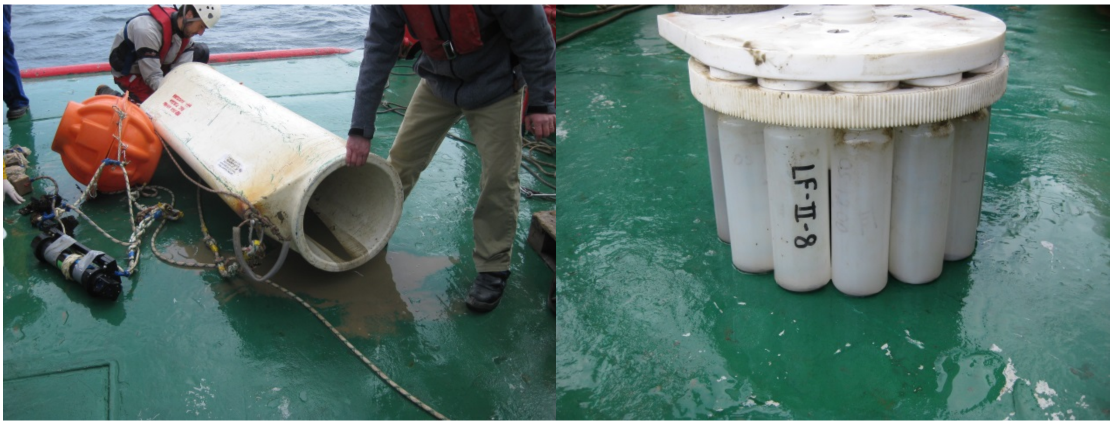

Figure 7. Sediment trap and bottles

All sediment traps were deployed at 22 m above the bottom. These samples, although not typically benthonic, have been included because a relatively significant number of individuals of a benthonic holothurian were collected (see Chapter 2).

1.7.3. *New technologies and sampling methods*

The ROV Max Rover II (Fig. 8A) of the Hellenic Centre of Marine Research (HCMR, Greece) was used to conduct seven seafloor surveys of different geomorphological habitats during the oceanographic cruise EUROLEON, which took place in October 2007 onboard R/V Hespérides. The ROV collected a total of 30 h of video over 14.74 km of surveyed seafloor. The Max Rover II was equipped with two wide-angle colour CCD cameras, offering a frontal and a lateral view, plus a third camera with a macro-zoom. Lighting asset was composed by 2 x 100 W HID lights and 4 x 150 W Quartz lights. The survey was conducted at an ROV speed of approximately 2.3 knots at 2 m above the seafloor.

Three areas of the north-western Mediterranean were video-surveyed (Fig. 8B): the Blanes Canyon, the Gulf of Valencia and the Eivissa Channel. A total of seven dives were performed in the three areas, providing data from 3 distinct geomorphological zones: Blanes Canyon-head, an unreported sea hill in the Gulf of Valencia, a small flat topped sea hill in Eivissa Channel, and the escarpments of two submarine landslides also in Eivissa Channel.

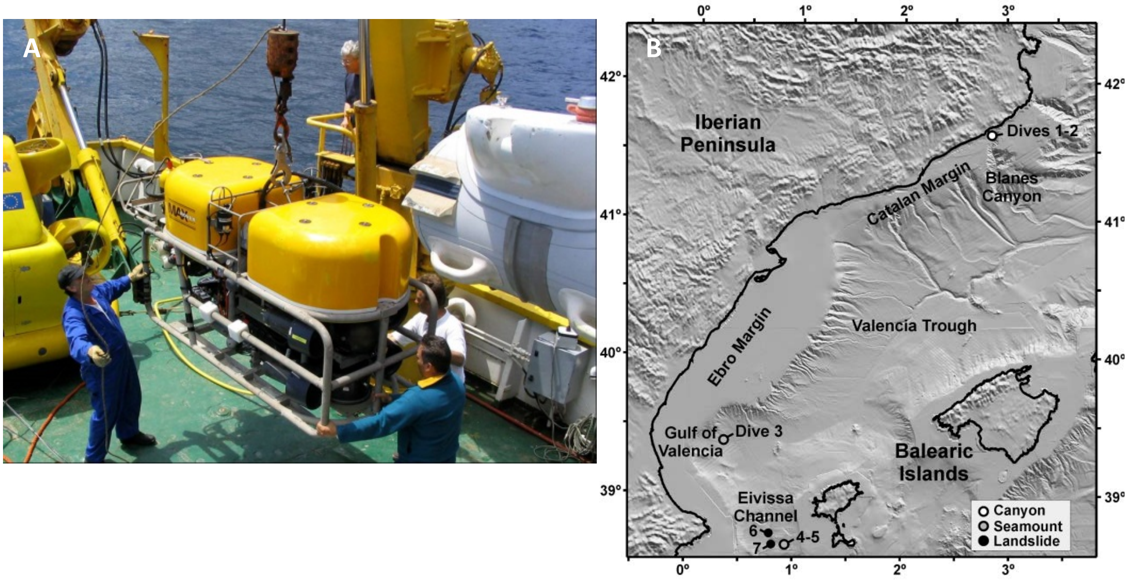

Figure 8.A) ROV Max Rover II B) Studied area.

Finally, video surveys were made during the PROMARES cruise using the ROV Liropus 2000 from the Spanish Oceanographic Institute (IEO), from 29 June to 15 July 2011. Seabed exploration was conducted from R/V Sarmiento de Gamboa on the Catalan margin and its deeply incised canyons (Cap de Creus, La Fonera and Blanes) (see Fig. 4). Footage was obtained with the front-pointing video camera (HD Kongsberg OE14-502) and two small cameras (OE14376) on the extensible arm (Fig. 9). Thirty six video transects were conducted along the axes of the Blanes, Palamós and Cap de Creus canyons between depths of 300 and 1800 m.

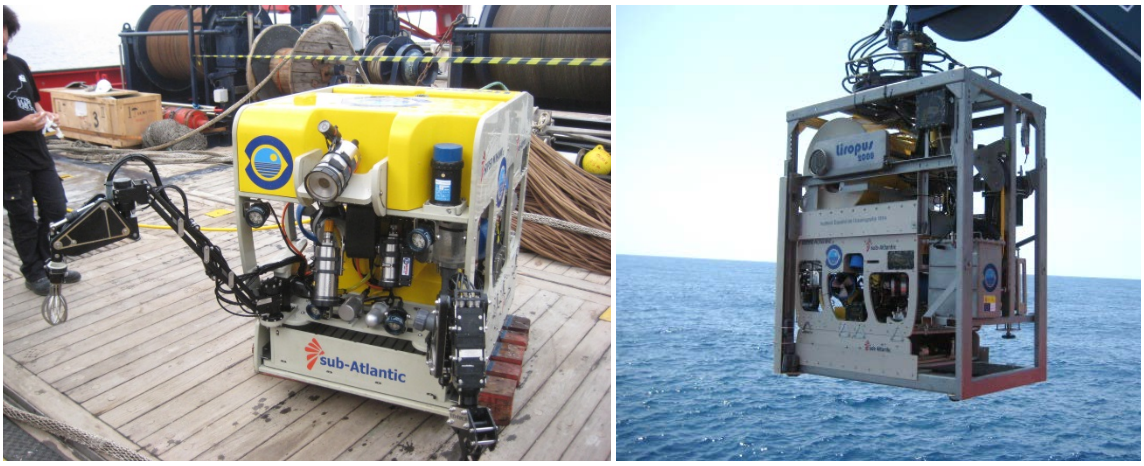

Figure 9. ROV Liropus 2000

2. Aims and objectives

The overall aim of the present thesis was to describe and characterize the populations of non-crustaceans invertebrates from the bathyal north-western Mediterranean continental margin and deep basin. It was believed that, with previous information of these taxonomic groups being scarce and dispersed, a study that will compile and synthesize old available data (bibliography review) and newly acquired data (present study), would be extremely useful for future biological and ecological studies of deep benthic Mediterranean communities. Nonetheless, it was considered that the study and description of all Phyla was a task too broad to be conducted in the timeframe of a single PhD. Thus, this project is a ‘first step’ to improve our knowledge of the deep Mediterranean fauna. To achieve this aim, a number of specific objectives were addressed:

1. To classify taxonomically and designate a valid name for all the non-crustacean invertebrates sampled in the cruises of the present study.
2. To report bathymetric and geographic distribution for all species analyzed.
3. To survey *in situ* different geomorphological habitats and relate habitat characteristics to invertebrate species.
4. To review all available taxonomical data and describe re-discoveries and new bathymetric ranges of deep Mediterranean echinoderms through newly acquired data.
5. To study the reproductive biology of poorly known species of ecological relevance such as the asteroid *Ceramaster grenadensis*.

The results of this doctoral thesis are presented as a compendium of the following per-reviewed scientific publications:

1. **Mecho, A**., Ramirez-Llodra, E., Aguzzi, J., Segura, M., Sardà, F., Company, J.B. Community structure and distribution of non-crustacean invertebrates in bathyal areas of the north-western Mediterranean Sea. (submitted Marine Biology)
2. **Mecho, A.**, Billett, D.S.M., Ramirez-Llodra, E., Aguzzi, J., Tyler, P.A., Company, J.B. (2014). First records, rediscovery and compilation of deep-sea

echinoderms in the middle and lower continental slope in the Mediterranean Sea. *Scientia Marina*, 78 (2): doi: 10.3989/scimar.03983.30C.

3. **Mecho, A**., Fernández -Arcaya, U., Ramirez-Llodra, E., Aguzzi, J., Company, J.B. (*submitted*). Reproductive biology of the deep-sea asteroid *Ceramaster grenadensis* (Perrier, 1881) from the north-western Mediterranean Sea. (Submitted to *Mediterranean Marine Science*).
4. **Mecho, A**., Aguzzi, J., De Mol, B., Ramirez-Llodra, E., Company, J.B., Bahamon, N., Canals, M., Lastras, G. (*submitted*). Visual faunistic exploration of geomorphological human-impacted deep-sea habitats of the north-western Mediterranean Sea. (Submitted to *PLoS ONE*).
5. **Mecho, A.**, Aguzzi, J., Company, J.B., Canals, M., Lastras, G., Turon, X. (2014). First in situ observation of the deep-sea carnivorous ascidian *Dicopia antirrhinum* C. Monniot, 1972 in the western Mediterranean Sea. *Deep Sea Research I*, 83: 51-56. doi: 10.1016/j.dsr.2013.09.007.

An additional relevant publication resulting from parallel studies conducted in the framework of this PhD project are included in the annexes:

Saiz, J.I., Cartes, J., Mamouridis, V., **Mecho, A**., Pancucci-Papadopoulou, M.A. (2014). New records of *Phascolosoma turnerae* (Sipuncula: Phascolosomatidae) from the Balearic Basin, Mediterranean Sea. *Marine Biodiversity Records*, 7: 1-5. doi: 10.1017/S1755267214000153.

Results have been discussed in the context of the improved taxonomical knowledge now available for deep Mediterranean non-crustacean invertebrates, their habitats and bathymetric and geographic distributions, as well as biological data of one of the key groups, the echinoderms. The results of this study and ensuing papers (both published and under review) provide a central source of information on deep Mediterranean invertebrate taxonomy.

3. Impact factor of published articles

Los abajo firmantes, el Dr. Joan B. Company, la Dra. Eva Ramirez-Llodra y el Dr Jacopo Aguzzi, como director y codirectores de la tesis doctoral con título: "*Taxonomy, distribution and community composition of megabenthic non-crustacean invertebrates in the deep Catalan margin*" presentada por Ariadna Mecho, certifican que los trabajos que se presentan a continuación han sido o están en proceso de publicación en las revistas científicas que se detallan. Todas ellas se encuentran catalogadas en el *Institute for Scientific Information* (ISI) y su índice de impacto, según el *Journal Citation Reports* (JCR) 2012, es el indicado en cada caso.

1. **Mecho, A.**, Ramirez-Llodra, E., Aguzzi, J., Segura, M., Sardà, F., Company, J.B. *Community composition and distribution of non-crustacean invertebrates in bathyal areas of the north-western Mediterranean Sea*. (submitted to Marine Biology). **Índice de Impacto: 2.46, 1er cuartil en el área de biología marina y de agua dulce.**

Mediante el estudio de este trabajo se pretende realizar una exhaustiva descripción de la distribución de todas las especies muestreadas desde los años 2008 a 2013 a profundidades comprendidas entre los 850 m y los 2850 m de profundidad en el Mediterraneo occidental. Igualmente se describen los patrones batimétricos de densidad y biomasa por Clases de los invertebrados no-crustáceos y se compara su distribución dentro-fuera de zonas de cañones submarinos. El enfoque del estudio, diseño de los análisis, recolección y clasificación taxonómica de las muestras y los análisis de patrones de densidad y biomasa corrieron a cargo de la doctoranda. La Dra. Eva Ramirez-Llodra se encargó de los análisis de biodiversidad y todos los co-autores participaron en el la estructuración, discusión y corrección del artículo.

2. **Mecho, A**., Aguzzi, J., De Mol, B., Ramirez-Llodra, E., Company, J.B., Bahamon, N., Canals, M., Lastras, G. (submitted). *Visual faunistic exploration of geomorphological human-impacted deep-sea habitats of the north-western Mediterranean Sea* (Submitted to PLoS ONE). **Índice de Impacto: 3.730, 1er cuartil en el área de ciencias multidisciplinares.**

Este estudio se integra en un marco multidisciplinar con el fin de obtener una visión en conjunto de los diversos hábitats señalados en el Mediterráneo nor-occidental, como son colinas submarinas, cañones y deslizamientos submarinos mediante el uso de un ROV. El artículo describe la fauna asociada a dichos hábitats en función de diversas características ambientales tales como el tipo de sedimento, la inclinación del fondo o las corrientes, entre otros. Asimismo, se detalla el efecto antropogénico en estas zonas y su impacto en la fauna circundante. Como información adicional, se proponen patrones de comportamiento en varias de las especies observadas. La batimetría de las zonas de estudio fueron proporcionados por el Dr. Miquel Canals y el Dr. Galderic Lastras del Departamento de Geología de la UB. El geoposicionamiento de la fauna vía GIS corrió a cargo del Dr. Ben de Mol, actualmente en Senergy, Noruega. Los análisis estadísticos correspondieron al Dr. Nixon Bahamón. La doctoranda fue responsable del diseño y análisis del estudio faunístico, en colaboración con los co-autores del ICM-CSIC Todos los co-autores participaron en la del estudio, interrelacionando las múltiples disciplinas con el fin de obtener una visión general.

3. **Mecho, A.**, Fernández-Arcaya, U., Ramirez-Llodra, E., Aguzzi, J., Company, J.B. (submitted). *Reproductive biology of the deep-sea asteroid* Ceramaster grenadensis *(Perrier, 1881) from the north-western Mediterranean Sea*. (Submitted to Mediterranean Marine Science). **Índice de Impacto: 1.574, 1er cuartil en el área de la biología marina y de agua dulce.**

En este trabajo se describe, por primera vez, la distribución poblacional y la biología reproductiva de la especie de asteroideo batial más común del margen continental catalán: *Ceramaster grenadensis* (Perrier, 1881). Debido a los escasos trabajos referentes a dicha especie, el presente artículo aporta una información que amplía considerablemente el conocimiento de *C. grenadensis* a nivel global. El diseño del estudio, la recolección y preparación de las muestras y análisis histológicos y de imagen corrieron a cargo de la doctoranda. Todos los co-autores participaron en la discusión y revisión del artículo.

4. **Mecho, A.**, Billett, D.S.M., Ramirez-Llodra, E., Aguzzi, J., Tyler, P.A., Company, J.B. (2014). *First records, rediscovery and compilation of deep-sea echinoderms in the middle and lower continental slope in the Mediterranean Sea.* Scientia Marina, 78 (2): doi: 10.3989/scimar.03983.30C. **Índice de Impacto: 1.006, 1er cuartil en el área de la biología marina y de agua dulce.**

El objetivo de este trabajo consiste en ser una fuente de información sobre todos los equinodermos presentes en el Mar Mediterráneo profundo (por debajo de los 850 m). Para este fin, se recopiló, revisó y sintetizó toda la información bibliográfica disponible sobre taxonomía y distribución de equinodermos de profundidad en el Mediterráneo. Adicionalmente, con el fin de aportar nuevos datos, se identificaron y describieron en detalle todas las especies recogidas en las campañas oceanográficas de la tesis doctoral, analizando sus rangos batimétricos y comparándolos a los anteriormente descritos. La doctoranda fue responsable única de la idea y enfoque de este estudio. Asimismo, se ocupó de recolectar y preservar todos los especímenes durante las campañas oceanográficas. Posteriormente realizó su clasificación y descripción taxonómica. A continuación se encargó de recopilar toda la información bibliográfica disponible en una tabla compilatoria con el fin de reagrupar en un solo artículo tanto la información existente como los nuevos datos adquiridos. El Dr. David Billett y el Prof. Paul Tyler participaron en la confirmación de dos especies en las que podía haber dudas sobre su clasificación. Todos los co-autores participaron en la discusión y corrección del artículo.

5. **Mecho, A.**, Aguzzi, J., Company, J.B., Canals, M., Lastras, G., Turon, X. (2014). *First in situ observation of the deep-sea carnivorous ascidian* Dicopia antirrhinum *C. Monniot, 1972 in the western Mediterranean Sea.* Deep Sea Research I, 83: 51-56. doi: 10.1016/j.dsr.2013.09.007. **Índice de Impacto: 2.816, 1er cuartil en el área de oceanografía.**

En el presente estudio, se confirma por primera vez la presencia de la especie *Dicopia antirrhinum* C. Monniot, 1972 en el Mar Mediterráneo profundo así como la primera observación de esta especie en vivo a nivel global. La publicación del presente artículo revela el limitado conocimiento de la fauna asociada a zonas profundas de cañones submarinos en el margen continental catalán y la valiosa contribución de metodologías de alta tecnología como los ROVs. La contribución de la doctoranda en el presente estudio incluye la participación en la campaña donde se observó el espécimen, la primera constatación de la especie como sujeto de interés, la búsqueda bibliográfica asociada a dicha especie y la redacción del artículo. Todos los co-autores participaron activamente en la discusión y corrección del manuscrito. El Dr. Turón se encargó, como taxónomo especializado en ascidias, de la disección y descripción interna del espécimen. El Dr. Miquel Canals y Dr. Galderic Lastras aportaron los datos referidos al área geográfica y sus características mediante mapas batimétricos.

Barcelona 24 de Julio de 2014

Fd: Joan Baptista Company   Fd: Jacopo Aguzzi   Fd: Eva Ramirez-Llodra

4. Summary of results and general discussion

Taken together, the present results account for the importance of increase taxonomical studies of continental margin in non-crustacean invertebrate communities, not only directly related with fisheries, but likely being of elevated value for integrated approaches to ecosystem managements. Taxonomy alone should be considered as the cornerstone of faunistic exploration, being the base for biodiversity evaluation and population distribution-connectivity studies with traditional sampling methods as well as new molecular technology.

On the present study, a total of 5877 individuals were reported. Of them, 5565 were classified to species level. Individuals from eight phyla were identified, comprising a total of 18 classes, and 69 species (see Paper 1). Most of these individuals were preserved, bio-sampled for tissue molecular analysis and stored in the Biological Reference Collection of the Institute of Marine Science, Barcelona (Spain) for future species comparison. A byproduct of the present work is the large collection of video-imaging material, including the production of a future field guide for the practical classification of those faunal components often discharged by ecological and faunistic studies that are mostly focused on few targeted fish and crustacean decapod species.

Two species have been reported as first records in the Mediterranean: the regular echinoid *Gracilechinus elegans* (Düben & Koren, 1844) (see Paper 2) and the carnivore ascidian *Dicopia antirrhinum* C. Monniot, 1972 (see Paper 5). Three other species were considered as ‘rare species’: the endemic holothurians *Hedingia mediterranea* (Bartolini Baldelli, 1914) and *Penilpidia ludwigi* (von Marenzeller, 1893) (see Paper 2) and the wood boring sipuncula *Phascolosoma (Phascolosoma) turnerae* Rice, 1985 (see Appendix 9.2). Because there is no consensus regarding what determines a ‘rare species’, in this study “rare” species are considered as those taxa that have been reported less than five times in the whole Mediterranean Sea (Cunningham and Lindenmayer 2005). For each of the 2 new faunistic records and each of the 3 “rare” species, at least 3 individuals were collected (or observed). Thus, the results suggest that these species are probably more abundant than expected (Wheeler 2007; Fontaine et al. 2012).

In this PhD Thesis, the bathymetrical range of distribution was extended for 11 species: the Porifera *Polymastia tissieri* (Vacelet, 1961) from 2211 m to 2850 m; the cold water corals *Desmophyllum dianthus* (Esper, 1794) and *Madrepora oculata* Linnaeus, 1758, down to 2250 m and 1450 m depth, respectively; the Echiurans *Bonellia viridis* Rolando, 1821 and *Echiurus abyssalis* Skorikow, 1906, from 1621 m and 837 m down to 2000 m and 1750 m depth, respectively; the Sipuncula *Sipunculus (Sipunculus) norvegicus* Danielssen, 1869 from 1927 to 2000 m depth; the echinoderms *Ceramaster grenadensis* (Perrier, 1881) from 2500 m to 2845 m, *Brissopsis lyrifera* (Forbes, 1841) from 1650 m to 2250 m, *Hedingia mediterranea* and *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823, from 1100 m to 1500 m and 300 m to 850 m respectively; and, finally, the carnivore ascidian *D. antirrhinum* for the first time at 1100 m depth. The bathymetric distributions previously reported for these species were probably a result of the lack of a systematic and intensive sampling in the lower bathyal slope and deep basins in the north-western Mediterranean Sea. Furthermore, in some cases (i.e. *C. grenadensis* and *B. lyrifera*) (see Paper 2), the new bathymetric range reported here represents an increase of the maximum depth of its global distribution, not only for the Mediterranean Sea (Clark and Downey 1992; Koukouras et al. 2007).

Prior to this Thesis, the faunal studies in the Mediterranean deep waters mostly focused on fishes and crustacean decapods (Moranta et al. 1998; Sardà et al. 2003; Company et al. 2004; D'Onghia et al. 2004). The few faunal or species community/assemblages studies that included non-crustacean invertebrates, were rarely analyzed at species level (Sardà et al. 1994; Ramirez-Llodra et al. 2010b; Tecchio et al. 2013). The results of the present PhD Thesis provide a completely new and validated faunal list of non-crustacean invertebrates from bathyal and abyssal Mediterranean waters, providing at the same time, new elements to assess the structure of their populations and, in some case, data of the seasonal reproductive cycle of invertebrate non-crustacean is provided.

The present Thesis reported the presence of a high number of Classes of suspension and sediment feeders in the submarine canyons areas (see Paper 1): the Class Anthozoa of the Phylum Cnidaria, both Classes belonging to the Phylum Sipuncula, and the Class Echinoidea for the Echinodermata. A significantly higher individual density and total biomass per species of non-crustacean invertebrates in canyon areas compared to the adjacent open slope was also reported (see Paper 1). These data confirm from an ecological point of view, the role of certain habitats (with geomorphological characteristics of the seabed) in driving oceanographic processes that affects species composition and assemblages (Clark et al. 2010; Tecchio et al. 2013; Schlacher et al. 2014). Canyons possess particular topographic and hydrodynamic characteristics that act as conduits of organic matter from the shelf to deeper areas conditioning the community structure (Canals et al. 2006; Arzola et al. 2008; Company et al. 2012). This concentration of organic matter and the associated presence of zooplankton aggregations in the head of some canyons (Macquart-Moulin and Patriti 1996) may result in higher biodiversity of resident benthic fauna in these habitats. Differences in megafauna density and biomass between canyons and the adjacent sedimentary slopes have been reported in previous studies (Genin 2004; Coll et al. 2010; Company et al. 2012; Tecchio et al. 2013). Increased food availability may explain the presence of large aggregations of *B. lyrifera* sampled with trawls and observed by ROV in the canyon axis (see Paper 2) and also described in other canyons (Carpine 1970; Laubier and Emig 1993; Ramirez-Llodra et al. 2008; Martín et al. 2014). Another potential explanation for the lack of this burrowing echinoid above 1000 m depth is the high impact of commercial trawling in the area, as this echinoid was previously reported abundantly from the slope and canyon in the upper slope, both by scientific studies (Sardà et al. 1994; Sanchez et al. 2004) and the fishermen themselves (Mecho, pers. com.).

Total density of non-crustacean invertebrates increased with depth (see Paper 1), with a maxima of 1386 individuals per km^2 at 2250 m depth. The peak of density at 2250 m depth was caused by the high number of the Brachiopod *Gryphus vitreus* sampled at these depths. The presence of the patching holothurian *Pseudostichopus occultatus* (see Paper 2), together with high densities of *G. vitreus,* were responsible for the drastic increase in total density at this depth (i.e. 2250 m). The high numbers of *G. vitreus* coincide with an elevated quantity of “clinker” (burnt residue of charcoal from steam ships) signaled at this depth that provides an ideal solid substrate for this species (Ramirez-Llodra et al. 2013). In terms of total biomass, a maximum was observed at 1200 m and 1500 m depth. Most of this biomass is constituted by specimens sampled inside canyons and the presence of large cephalopods. The observed peaks coincide with the general biomass depth-related pattern reported for all megafauna (fishes and crustaceans) studied in the area (Tecchio et al. 2011; Tecchio et al. 2013). A significant decrease in individual density and species biomass was observed at 1350 m depth (see Paper 1).

Of all sampled Phyla, the echinoderms were one of the most important in terms of individual density and biomass, representing 57% of the total density and a 41% of the total biomass. Echinoderms were present along the whole bathymetric range sampled, both inside canyons and on adjacent open slopes. This is in accordance with related studies of the Atlantic Ocean, where echinoderms are also a key group in terms of abundance, biomass and ecosystem function (Billett 1991; Billett et al. 2001b). The large number of investigations conducted in the Atlantic Ocean have resulted in a good taxonomic and population biology knowledge of this group in the Atlantic (Hyman 1955; Clark and Downey 1992; Ameziane and Roux 1997; Rogacheva et al. 2013). This contrasts with the knowledge gap for the same group in the deep Mediterranean. Thus, and considering the high number of individuals of different species collected during the thesis project, it was considered essential to undertake a detailed study of this Phylum, both in terms of taxonomy and population biology.

The first approach of the study of deep Mediterranean echinoderms was taxonomic; with the objective to review, synthesize and update all existing information and to include new data (see Paper 2). The results were a full compilation of available information and plus new observations, describing in detail all echinoderms specimens sampled below 850 m depth. The first mention of the regular echinoid *Gracilechinus elegans* (Düben & Koren, 1844) was cited, and also the presence of two rare endemic holothurians *Hedingia mediterranea* (Bartolini Baldelli, 1914) and *Penilpidia ludwigi* (von Marenzeller, 1893) (see Paper 2).

The only echinoderm species sampled at all depths was the asteroid *C. grenadensis*. Because of its wide bathymetric distribution and relative high abundance, as well as the lack of previous information on the reproductive processes of this species, a detailed population structure and reproductive survey on that species was conducted (see Paper 3). The density pattern of this *C. grenadensis* was similar at all sampled depths, with a slight, but not statistically significant, increase at 1500-1750 m. This patterns is similar to what has been observed in other invertebrate groups sampled in the same area, such as the crustacean *Aristeus antennatus* (Risso, 1816) (Sardà et al. 2003). On the other hand, the mean biomass distribution showed a significant decrease at increasing depths (for statistical details see Paper 3). This distribution patterns coincides with the observed biomass trend as function of depth for the whole megafauna from the same area (Tecchio et al. 2013). Juveniles and small adult specimens were found at the deepest range of their bathymetric distribution (see Paper 3). This bathymetric trend in size structure is in accordance with patterns observed for other species as, for example, the irregular echinoid *Brissopsis lyrifera* (Forbes, 1841) in the Atlantic Ocean (Harvey et al. 1988). This pattern was also found in the populations of *B. lyrifera* and *Molpadia musculus* Risso, 1826, with the smallest individuals found at the deepest zones of their bathymetric range of distribution (see Paper 2). The decrease in size with depth has been related to the low food availability in the deeper areas compared with more productive shelf and upper slope habitats (Rex et al. 2006). Seasonally, the mean individual size decreased from summer, to spring when the smallest individuals were captured (for statistical details see Paper 3).

Results contained in the present Thesis indicate that in *C. grenadensis*, individuals living at greater depths increase their reproductive effort by reducing the somatic investment (small sizes) and devoting a higher amount of energy to oocyte production (i.e. higher GI values) (see Paper 3). Apparently, females may be storing nutrients in the pyloric caeca in order to maintain a constant production of gametes in a food-limited environment (Benítez-Villalobos and Díaz-Martínez 2010), allowing a constant transfer of energy from the pyloric caeca to the gonads (Mcclintock et al. 1995). Seasonally, the results point to the same process of transfer of nutrients from the pyloric caeca to the gonads during periods of gonadal growth (summer–autumn). Higher reproductive activities in autumn have also been described for deep-sea fishes in the same study area (Fernandez-Arcaya et al. 2013a; Fernandez-Arcaya et al. 2013b) and were also described for other echinoderms (Ferrand et al. 1988; Mcclintock et al. 1995). This could be the season in which energy reserves are accumulated providing from the fluxes of organic matter sunken from the photic zone (Herring 2002; Company et al. 2003).

A high amount of high quality information can be obtained with traditional benthic sampling methods (i.e. trawling, dredging, and sledge). New species for science can be caught and specimens can be identified to species level, supporting information for biodiversity analyses and for describing patterns in biomass and abundance. However, all data from the trawled transect are integrated within a single sample and these sampling methods do not provide small-scale information on heterogeneity, patchiness or behaviour. On the other hand, *in situ* observations and sampling become essential in certain habitats of complex geomorphology or fragile faunal communities, where these "blind" traditional sampling methods could not be used. ROVs are not destructive; they provide *in situ* observation of communities, behaviour, and information on small-scale heterogeneity and the relations between individuals/species and their habitat (McClain and Barry 2010; Bo et al. 2011). However, ROVs do not provide data on burrowing species and there is a decreased accuracy in taxonomic classification of species. For these reasons, a combination of both sampling methods (trawling and in situ observations) highly enhances our understanding of the community composition, relationships to the habitat and amongst individuals and species and ecological processes of the deep-sea benthos.

The ROV study conducted during this thesis provided a total of 4519 faunistic observations (see Paper 4) from three different geomorphologic habitats (i.e. canyon, sea hill and landslide). The fauna belonging to the Phyla Chordata, Cnidaria and the Subphylum Crustacea was the most abundant. The Phyla Porifera, Echinodermata and the miscellaneous group "Other" (i.e. Brachiopoda, Annelida, Ctenophora and Echiura) were less representative (see Paper 4). A high level of anthropogenic impact in all studied zones was reported, with 158 artificial artefacts of different kinds observed.

In the canyon area, the majority of the reported video-observations refers to suspension and sediment feeders species, commonly described at similar depths in canyons of the Balearic Sea (Ramirez-Llodra et al. 2008; Ramirez-Llodra et al. 2010b; Company et al. 2012). This data also coincide with the data obtained in the present study with trawling methods (see Paper 1). As described above, the high abundance of suspension feeders and detritus feeders is probably the result of the specific topographic and physical characteristics of the canyon (Genin 2004; Coll et al. 2010; Company et al. 2012; Tecchio et al. 2013).

On hill areas, the presence of an elevated number of benthic Phyla in comparison to the adjacent muddy seafloor was video-reported (see Paper 4). Hills and seamounts are considered zones of high biodiversity sustained by the particular geomorphologic structures that strongly conditions local hydrography, with the consequent increase of water column nutrients retention (Rogers 1994; Rowden et al. 2010). The rocky substrate on sea hills are particularly suitable for colonization by sessile filter-feeding organisms contributing to the establishment of high diversity faunal communities (Buhl-Mortensen et al. 2005; Roberts et al. 2006). However recent studies have provided data from seamounts that presented similar benthic diversity and endemism patterns than those reported on adjacent continental margins, which are traditionally considered poorer in overall biodiversity (Howell et al. 2010; Rowden et al. 2010; Schlacher et al. 2010). This is not the case of video-surveys conducted in this Thesis, where significant differences in the density of fishes between the rocky hill and the surrounding muddy area were reported.

In the video-surveyed landslides, the motile fauna was the dominant group (see Paper 4). These results corroborate other studies that used traditional sampling methods (i.e. trawl) (Stefanescu et al. 1993; Sardà et al. 1994; Abelló et al. 2002), that signaled these groups as the most abundant in terms of biomass. An interesting annotation was that when crustacean decapods were dominant, fishes were rarely seen, and *vice versa*. This suggests a possible trophic competitive exclusion, based on the capacity of certain taxonomical groups to occupy the ecological role of others, hence substituting them.

Video-observations provide the unique opportunity to describe behavioural patterns and the interactions amongst species/individuals and between individuals and their habitat. The ethological characterization is important in order to explain the observed species distributions based on the type of animal displacement, as well as to understand potential biases occurring with trawling caused by the reaction of animals to invasive collection procedures (Aguzzi and Sardà 2007; Bahamon et al. 2009). In non-crustacean invertebrates, jellyfishes such as *Pelagia noctiluca* (Forsskål, 1775) and specimens of the Order Coronatae, were observed swimming a few centimetres over the seabed. In the case of *P. noctiluca*, small groups were observed touching the seafloor over the top of the flat sea hill in the Eivissa Channel (see Paper 4) at 200 m depth.

The use of ROVs also allowed for serendipitous discoveries, such as the accounted first faunal report of the deep-sea carnivore ascidian *Dicopia antirrhinum*. During a ROV dive at 1100 m depth in the La Fonera canyon, a rare ascidian species was reported by the author of this thesis. A total of five adult individuals were observed in two different locations of the northern canyon wall always at around 1100 m depth (see Paper 5). Subsequently, one specimen was collected by the ROV. The direct collection of a single individual provided a valuable specimen in perfect conditions for preservation and subsequent taxonomical studies. This is the first time that this species has been observed in its natural habitat, being discovered in the late 70s (Monniot 1972) in Atlantic waters. Carnivore ascidians are commonly known by the species *Megalodicopia hians* Oka, 1918, a species sampled in Monterey Bay (California, USA) and Japanese waters. This species has been studied (Okuyama et al. 2002; Havenhand et al. 2006) and exhibit in aquariums, nonetheless all the other species remains largely unknown (Tatián et al. 2011) and have been rarely observed *in situ* (Monniot and Monniot 2003; Sanamyan and Sanamyan 2006).

In summary, this Thesis has addressed a major gap in knowledge in deep-sea Mediterranean non-crustacean invertebrate taxonomy and biology, providing a central source of validated old and new faunistic and population biology data. This research effort represents the first step towards a full understanding of the community composition, bathymetric and geographic distribution, habitat-fauna relationships and biology of deep Mediterranean invertebrates, setting the bases for further sampling efforts and subsequent taxonomic, biological and ecological studies. With the help of the present data, future studies will be conducted to fully understand the biodiversity and ecosystem function of this group, in previously unexpected depths and areas.

5. Conclusions

The study of the non-crustacean invertebrates dwelling on the deep Mediterranean Sea is of interest for different marine science disciplines, being the diversity of species and their distributions largely unexplored. In fact, the present Thesis conducted an effort in order to increase valid scientific information not only at taxonomical level, but also at the level of community composition and distribution. Detailed data on the reproductive biology and ethology was required for some group of ecological relevance in order to solve some relevant gaps of information.

Based on the study of the non-crustacean invertebrates collected along the deep continental margin of the north-western Mediterranean Sea (from 400 m to 2850 m depth), this study concluded what follows:

1. This work evidenced and updated species bathymetrical and geographical distributions ranges of non-crustacean invertebrate species by different methodologies (i.e. traditional and technologically advanced sampling). As a result of that effort, new faunistic reports were described: two first records (i.e. *Gracilechinus elegans* (Düben & Koren, 1844) *Dicopia antirrhinum* C. Monniot, 1972), three rare species (i.e. *Hedingia mediterranea* (Bartolini Baldelli, 1914), *Penilpidia ludwigi* (von Marenzeller, 1893) and *Phascolosoma (Phascolosoma) turnerae* Rice, 1985), and eleven extensions of maximum depth range of distribution.

2. Densities and biomass of non-crustacean invertebrates were pointed to be significantly higher in submarine canyons than in surrounding open slopes. These findings suggest submarine canyon habitats of the north-western Mediterranean Sea as hotspots of benthic megafauna communities.

3. When all the Phyla were plotted together along a wide bathymetric range (i.e. 400 – 2250 m), an increasing pattern of density with depth was found. The increasing observed pattern is explained by the high number of patching species collected at deepest areas (i.e. 1500 – 2250 m).

4. The most abundant group in terms of density was the Phylum Echinodermata. A Phylum largely undescribed in deep Mediterranean waters, when compared with

the faunistic data already available for the Atlantic. All specimen sampled were classified, described and their bathymetrical ranges re-defined. These results were compared and extended with the previous published literature, checking, validating (or discarding) all available information.

5. The size pattern distribution of the most abundant species of Asteroidea in the present study, *Ceramaster grenadensis* show a depth related trend showing smaller sizes at greater depths (dwarfism), as observed in other species of Atlantic Ocean. Additionally, juveniles were sampled at the deepest range of distribution of the species (i.e. 2250 m).

6. Histological analysis of *C. grenadensis* gonads, revealed a semicontinous reproductive patterns, with a maximum of reproductive activity in autumn (maximum values of Gonadal Index). This finding coincides with some already published study for other deep-sea Mediterranean Echinoderm species such as *B. lyrifera*.

7. The *in situ* faunal sampling with video technologies (i.e. Remotely Operated Vehicles; ROVs) showed the occurrence of different species assemblage compositions in different geomorphological habitats such as, submarine canyons, sea hills and landslides. Benthic cnidarian communities were observed in seamount rocky areas while submarine landslides fauna were mainly composed by vagil fauna as fishes and crustaceans. The description of characteristics seascape units might help to estimate taxonomical group in other similar but presently unexplored deep-sea areas.

8. Anthropogenic impact was shown in different zones of the deep north-western Mediterranean Sea. In different sampled zones (by traditional or technologically-new methods), a high number of human artefacts was reported. On each zone different kind of impact were observed, being canyon areas mostly affected by litter artifacts, hills by longlines and finally landslides by trawl marks.

9. This new record increased the lower depth limit of this genus in the Mediterranean Sea from 500 to 1100m depth. This finding contributes to the

knowledge of the poorly investigated Mediterranean deep ascidian fauna, in sharp contrast with the relatively well- known shallow-water Mediterranean ascidians.

10. Finally, this study suggests that an increasing effort combining traditional and technologically-new sampling methods is advisable in order to obtain a more complete overview of species inhabiting the different deep-sea continental margins.

6. Resumen

6.1. Historia del Mar profundo

El mar profundo se podría describir como el ecosistema más angosto del planeta, así como el más desconocido. Hoy en día tan sólo el 5% ha sido observado y únicamente un 0.01% del fondo marino ha sido realmente muestreado y estudiado (Ramirez-Llodra et al. 2010a). Las profundidades marinas se caracterizan por tener unas propiedades que las hacen únicas en el mundo. Sus bajas temperaturas, una salinidad relativamente constante, poca o ninguna luz, concentraciones de oxígeno cercanas a la saturación y una presión que aumenta conforme se va incrementando la profundidad hacen de este entorno un lugar diferente y único.

El estudio de las profundidades marinas comenzó relativamente tarde en la historia de la ciencia debido a la problemática que implica muestrear a profundidades tan elevadas. La exploración del mar profundo se inició a mediados del siglo IXX, cuando las mejoras del instrumental marítimo y oceanográfico permitieron acceder a estos remotos lugares. Edward Forbes fue considerado como el primer científico en trabajar en ellas al muestrear las profundidades del Mar Egeo con el *H.M.S. Beacon* en 1841-42. En base a sus desafortunados resultados (sus muestreos se realizaron en una zona de anoxia en la que la fauna era prácticamente inexistente (Danovaro et al. 2010) propuso la famosa Teoría de la zona Azoica, según la cual no podía existir vida por debajo de los 600 m (Forbes 1844). Esta teoría fue finalmente descartada en 1850, cuando Michael Sars recolectó gran cantidad de fauna profunda alrededor de los 800 m de profundidad en aguas del Ártico. A partir de este momento empezó una carrera oceanográfica enfocada a muestrear las profundidades marinas. Las grandes expediciones que acontecieron, (e.g. *Galathea Expedition* 1845-1847; *H.M.S. Challenger*, 1872-76; *Prince Albert of Monaco cruises* 1885-1914; *Siboga Expedition* 1899-1900 entre otras) aportaron una serie de datos nuevos relacionados con la fauna batial y abisal que en algunos casos, a día de hoy todavía no ha sido actualizados al no tener datos nuevos para contrastar.

En el Mediterráneo, el estudio de la fauna profunda comenzó a finales del siglo XIX, con campañas como las realizadas mediante el *R.N. Washington* (1881-1882) y el *S.M.S. Pola* (1890-1898). Entre los años 20 y los 60 del siglo XX, el número de campañas de muestreos en profundidad disminuyó considerablemente debido a las 2 guerras mundiales, limitando la información sobre este hábitat (Pérès and Picard

1956a). A partir de finales de los 60 y principios de los 70 las mejoras en las herramientas de muestro permitieron una nueva época dorada para la ciencia marina a nivel global. Igualmente permitió el cambio de enfoque de los datos; de ser puramente descriptivo se pasó a un estudio más analítico y ecológico (Hessler and Sanders 1967; Grassle and Sanders 1973). En el Mediterráneo también se realizaron importantes campañas oceanográficas durante esta época, como las realizadas mediante el ‘*Bambu*’, el ‘*Mango*’ y el ‘*Ruth Ann*’ en aguas italianas, el R.V. ‘*Jean Charcot*’ por parte de los franceses en aguas de Alborán y el R.V. ‘*García del Cid*’ en las Islas Baleares.

A finales del siglo XX, se realizaron varios estudios del Mediterráneo profundo, desde las zonas occidentales (Alvà 1987b; Villanueva 1992; Cartes and Sardà 1993; Stefanescu et al. 1993) a las orientales (Galil and Goren 1995) incrementando considerablemente los conocimientos sobre las profundidades del Mar Mediterráneo. En esta época reciente, la aparición de nuevas tecnologías como vehículos dirigidos por control remoto (ROVs) y observatorios permanentes, permitió el estudio de las comunidades *in situ*, (Stoner et al. 2008; Aguzzi et al. 2012) aportando datos complementarios imposibles de obtener con los muestreos tradicionales.

6.2. La Importancia de la Taxonomía

La palabra taxonomía proviene del griego ταξις, *taxis*, ‘ordenamiento’, y νομος, *nomos*, ‘norma’ o ‘regla’, y en biología se describió como “*El campo de científico (y mayor componente de la Sistemática) que se encarga de describir, identificar, nombrar y clasificar los seres vivos existentes*” (Simpson 2010). La taxonomía Lineana, basada en una nomenclatura binomial (*Genero especie*), posibilita un consenso global sobre como nombrar a una especies determinada, por ello esta nomenclatura sigue prácticamente inalterada desde el siglo XVI. Las nuevas tecnologías tales como los microscopios electrónicos o la secuenciación del ADN (Edgecombe et al. 2011; Telford 2013), aportaron grades mejoras a la taxonomía morfológica, permitiendo la reorganización de los organismos y la identificación de especies crípticas (muy similares morfológicamente entre sí pero que no se pueden reproducir entre ellas) (Ebach and Holdrege 2005; Gregory 2005).

A pesar de su importancia y de ser la base de otras disciplinas (e.g. filogenia, ecología de poblaciones, biodiversidad y ecología entre otros), se observó un alarmante descenso de taxónomos a finales del siglo XX y principios del XXI (Godfray 2002). Esta pérdida de expertos taxónomos se atribuye a varios factores (Giangrande 2003), desde la dificultad de atraer financiación a largo plazo para proyectos puramente taxonómicos como el problema de recolección y validación de información antigua, en muchas ocasiones dispersa e inaccesible. Uno de los ejemplos más claros de este problema se describió en un estudio que estimó que el tiempo transcurrido entre el descubrimiento y la descripción de una especie nueva podía llegar a los 21 años, en función del Filo al que pertenecía dichas especie (Fontaine et al. 2012).

Esta disminución de expertos taxónomos en los últimos años derivó en una considerable falta de información, así como la aparición de clasificaciones erróneas que repercutieron en las ciencias asociadas (biodiversidad, biogeografía, genética de poblaciones etc...) disminuyendo la calidad y precisión de estos estudios (Giangrande 2003; Khuroo et al. 2007). Actualmente y con el fin de paliar estos problemas, se han desarrollado varias iniciativas con el fin de agrupar, estandarizar y unificar toda la información disponible en la red, ayudar a hacer más accesible los datos taxonómicos existentes así como unificarlos de manera internacional.

6.3. El Mediterráneo Profundo

El Mediterráneo profundo se caracteriza por una serie de particularidades que lo hacen único. Presenta una homeotermia constante por debajo de los 200-300 m de profundidad con temperaturas de 13-14ºC, mientras en otros océanos como el Atlántico la temperatura suele bajar con la profundidad alcanzando hasta los 0ºC (Tyler 2003). La consecuencia de esta elevada temperatura en profundidad resulta en la pérdida de la llamada “barrera térmica” con lo que la fauna puede moverse verticalmente sin ninguna frontera física. Estas altas temperaturas también implican una mayor velocidad de descomposición en las partículas que caen al fondo desde la zona fótica, por lo que la calidad de los nutrientes es peor de lo que serían en aguas más frías (Sardà et al. 2004c; Ramirez-Llodra et al. 2010a). Esto nos lleva a la segunda característica del Mediterráneo profundo, su oligotrofia. El Mediterráneo profundo es altamente oligotrófico, y esta particularidad se incrementa de oeste a este, causando una limitación de nutrientes en las comunidades batiales que dependen de los aportes de materia orgánica de zonas más superficiales (Danovaro et al. 1999; Danovaro et al. 2010).

El lecho marino del Mediterráneo presenta una compleja colección de hábitats, como taludes sedimentarios, cañones y montes submarinos, llanuras batiales, arrecifes de corales profundos, surgencias frías entre otros (Danovaro et al. 2010; Company et al. 2012). Las características geomorfológicas de estos hábitats junto con los procesos abióticos que en ellas se generan, facilitan el desarrollo y la permanencia de una elevada diversidad de comunidades faunísticas diferentes (Levin et al. 2010), muchas de ellas con especies todavía desconocidas.

6.4. Los invertebrados en el Mediterráneo profundo

El término invertebrados, no es un taxón *per se*, sino más bien una manera comúnmente aceptada de agrupar juntos todos los organismos carentes de columna vertebral. Actualmente, entre un 95% - 98% de los 1.4 millones de especies conocidas son invertebrados y se reparten entre 30 Filos (*Phyla*)(Ramirez-Llodra et al. 2010a). De los 30 Filos, 29 están presentes en ecosistemas marinos, y prácticamente todos presentan especies en hábitats de mar profundo.

En el Mediterráneo profundo, la megafauna bentónica y bentopelágica se encuentra ampliamente dominada por peces y crustáceos. Debido a esto, el Subfilo Crustacea es uno de los grupos de invertebrados más ampliamente estudiado, tanto por su importancia a nivel ecológico (abundancia y biomasa), como a nivel comercial y de pesquerías (e.j. *Aristeus antennatus* (Risso, 1816), *Nephrops norvegicus* (Linnaeus, 1758) (Sardà et al. 1994; Company et al. 2004; Aguzzi et al. 2009a). Comparativamente, este grupo de invertebrados es mucho más conocido a todos los niveles que otros grupos de megafauna como podría ser ascidias, esponjas, equinodermos, equiúridos o sipuncúlidos (Monniot and Monniot 1975; Alvà 1987a; Uriz and Rosell 1990; Villanueva 1992; Pancucci-Papadopoulou et al. 1999). Este elevado conocimiento de los crustáceos es la razón por la cual no se considerarán en la presente Tesis, donde se estudiaron otros grupos más desconocidos.

Entre estos grupos el más abundante en la presente Tesis fue el Filo Echinodermata. Este filo se divide en cinco Clases, Crinoidea (lirios de mar), Ophiuroidea (ofiuras), Asteroidea (estrellas de mar), Echinoidea (erizos de mar), y Holothuroidea (pepinos de mar). Los equinodermos están compuestos por especies exclusivamente marinas y han colonizado con éxito todos los hábitats, desde zonas tropicales hasta los polos pasando por las grandes profundidades, en todos ellos representando una gran proporción de la fauna local (Hyman 1955). Es un grupo ampliamente estudiado en el Atlántico profundo, donde son los más importantes en términos de abundancia y biomasa (Billett 1991; Billett et al. 2001a). Debido a esto, su estudio a nivel taxonómico es muy amplio y antiguo (Hérouard 1902; Koehler 1927; Mortensen 1927; Sibuet 1977; Borrero Perez et al. 2003), sin embargo incluso a día de hoy se siguen encontrando especies nuevas (Gebruk 2008; Gebruk et al. 2013). En el Mediterráneo, sin embargo, los estudios realizados en equinodermos residentes en el talud medio y profundo son más bien escasos (Marenzeller 1893; Bartolini Baldelli 1914; Tortonese 1965; Sibuet 1974; Alvà 1987a), y generalmente se limitan a aportar un listado de especies sin descripción taxonómica (Tortonese 1979; Pérez-Ruzafa and López-Ibor 1988; Rinelli 1998; Coll et al. 2010). A nivel biológico como por ejemplo a nivel de ciclos reproductivos también son escasos los trabajos realizados en el Mediterráneo profundo (Ferrand et al. 1988).

En este contexto de información dispersa, de difícil acceso o directamente inexistente, se consideró de primer orden en esta Tesis el recopilar, confirmar, describir y estudiar toda la información existente sobre los equinodermos de profundidad (entre 850 m y 2850 m) presentes en el Mediterráneo. Asimismo se creyó conveniente estudiar el ciclo reproductivo de una de las especies más significativas, el asteroideo *Ceramaster grenadensis* (Perrier, 1881).

6.5 Nuevas herramientas de observación y muestreo

Algunas zonas del fondo marino tales como las fuentes hidrotermales, las surgencias frías, los cañones submarinos o los arrecifes de corales de aguas frías entre otros, resulta especialmente difíciles de muestrear mediante los métodos tradicionales (ej. arrastres) debido a la complejidad y fragilidad de estos hábitats (Orejas et al. 2009; Clark et al. 2010). En estos casos las nuevas técnicas de muestreo no-intrusivas como son los vehículos dirigidos por control remoto (ROV), los vehículos autónomos submarinos (AUV), landers y estaciones permanentes, permiten adquirir datos de estas zonas problemáticas, reemplazando o complementando los métodos de muestreo tradicionales.

En el caso de la presente Tesis, estas nuevas tecnologías nos permiten, no solamente obtener datos de zonas de difícil acceso, sino adquirir una visión *in situ* del lecho marino, estudiar las comunidades faunísticas y el comportamiento de las especies en su hábitat natural.

6.6 Área y metodología de estudio

El material utilizado en la presente tesis fue recopilado a lo largo de 10 campañas oceanográficas realizadas entre octubre 2008 y abril 2013 en el Mediterráneo occidental en el marco de los Proyecto Nacionales y Europeos PROMETEO, DOSMARES, PROMARES y EUROLEON. Las áreas de muestreo incluyeron el Cañón de Blanes y su talud adyacente, los cañones de la Fonera y Cap de Creus, dos montañas submarinas ubicadas en el Golfo de Valencia y el Canal de Ibiza, y dos deslizamientos submarinos del Canal de Ibiza. Las profundidades muestreadas mediante ROV fueron de entre los 60 m y los 1100 m de profundidad; y entre los 850 m y los 2850 m mediante muestreos tradicionales. Adicionalmente se realizó una campaña transmediterránea (BIOFUN) en la cual se muestrearon tres áreas del Mediterráneo, oeste, central y este, pero debido al bajo número de especies recogidas, no se utilizaron estos datos en los análisis de composición faunística, sin embargo las especies fueron añadidas a la tabla de especies del Artículo 1.

Se realizaron un total de 223 pescas de arrastre. De ellas, 119 fueron realizados mediante un arte de arrastre bentónico Maireta (OTMS)(Sardà et al. 1998), esta posee una red de arrastre de 25 m de largo, 1.4 m de anchura de boca y un copo de malla de 12 mm. Se realizaron también 49 muestreos mediante una draga Agassiz, constituido por un marco de hierro forjado de 2.5 m de ancho por 1.2 m de alto, y un copo de malla de 12 mm. Finalmente en 55 pescas se utilizó un patín suprabentónico de tres estratos (entre 10-50 cm, 55-95 cm and 100-140 cm del suelo) con mallas de 300 µm, sin embargo, debido al bajo número de especies recolectadas y los errores que implica este tipo de muestreo, no se utilizaron para los análisis, aunque las especies sí son mencionadas en la tabla de especies del Artículo 1 (para detalles concretos de los muestreos, almacenamiento y procesado de las muestras ver apartado de Material y Métodos particular de cada artículo).

Adicionalmente y con la intención de obtener una visión global de las comunidades bentónicas, se analizaron los videos obtenidos mediante dos ROV. La campaña EUROLEON se realizó en octubre 2007 abordo del R/V Hespérides mediante el ROV Max Rover II del Hellenic Centre of Marine Research (HCMR, Grecia). El área de estudio comprendía tres estructuras geomorfológicas clave del Mediterráneo occidental como son un cañón, dos montañas submarinas y dos deslizamientos submarinos situados en el margen continental catalán, el golfo de Valencia y el Canal de Ibiza. La campaña PROMARES, se realizó a borde del R/V Sarmiento de Gamboa en Junio 2011 y los datos se obtuvieron mediante el ROV Liropus 2000, propiedad del Instituto Oceanográfico Español (IEO). En este caso el área de estudio se centró en tres cañones del margen catalán profundo (Cap de Creus, La Fonera y Blanes).

6.7 Resultados generales y discusión

En el presente estudio se obtuvieron 5877 individuos mediante las técnicas de muestreos tradicionales. De estos, 5565 fueron clasificados hasta género o especies. Estos especímenes se clasificaron en 8 Filos, 18 Clases y 69 especies (ver Artículo 1). Gran parte de los especímenes recogidos están en proceso de numeración para ser almacenados en la Colección Biológica de Referencia del Instituto de Ciencias del Mar de Barcelona, con el fin de ser accesible para posteriores estudios.

En la presente Tesis se describe por primera vez en el Mediterráneo la presencia de dos especies: el equinoideo regular *Gracilechinus elegans* (Düben & Koren, 1844) (ver Artículo 2) y la ascidia carnívora *Dicopia antirrhinum* C. Monniot, 1972 (ver Artículo 5). Igualmente se describió la presencia de tres especies consideradas ‘raras’: las holoturias endémicas *Hedingia mediterranea* (Bartolini Baldelli, 1914) muestreada únicamente una vez, previamente a este estudio, y *Penilpidia ludwigi* (von Marenzeller, 1893) muestreada tres veces con anterioridad (ver Artículo 2) y el sipúnculido *Phascolosoma (Phascolosoma) turnerae* Rice, 1985 citado previamente una vez (ver Appendix 9.2). A falta de un consenso que designe una especie como rara y teniendo en cuenta los estudios sobre esta designación (Cunningham and Lindenmayer 2005), consideramos rara una especie cuando su presencia ha sido citada menos de 5 veces en todo el Mediterráneo a lo largo de la historia. Teniendo en cuenta que todas estas especies raras fueron muestreadas más de tres veces en el transcurso de esta Tesis, podemos suponer en base a otros estudios (Wheeler 2007; Fontaine et al. 2012) que la presencia de estas especies es mayor de lo esperado.

Asimismo en la presente Tesis se amplió el rango de distribución batimétrica de 11 especies: el Porifero *Polymastia tissieri* (Vacelet, 1961) hasta los 2850 m; el de los corales *Desmophyllum dianthus* (Esper, 1794) y *Madrepora oculata* Linnaeus, 1758, hasta 2250 m y 1450 m respectivamente; los equiúridos *Bonellia viridis* Rolando, 1821 y *Echiurus abyssalis* Skorikow, 1906, hasta 2000 m y 1750 m respectivamente; el sipuncúlido *Sipunculus (Sipunculus) norvegicus* Danielssen, 1869 hasta los 2000 m; entre los equinodermos, el asteroideo *Ceramaster grenadensis* (Perrier, 1881) hasta los 2845 m, *Brissopsis lyrifera* (Forbes, 1841) a 2250 m, *Hedingia mediterranea* y *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823, hasta los 1500 m y 850 m respectivamente; y finalmente se amplió el máximo rango de profundidad para la ascidia carnívora *D. antirrhinum* hasta los 1100 m. Este elevado número de ampliaciones de rango batimétrico se debe probablemente a la falta de muestreos profundos en el talud inferior y las llanuras batiales del Mediterráneo nororiental. Sin embargo, en algunos casos concretos como *C. grenadensis* y *B. lyrifera* el rango batimétrico descrito en la presente tesis es el máximo descrito a nivel global para estas especies (Clark and Downey 1992; Koukouras et al. 2007) (ver Artículo 2).

Los estudios sobre invertebrados no-crustáceos son pocos en el Mediterráneo profundo (Ramirez-Llodra et al. 2008; Cartes et al. 2009), siendo más bien una parte remanente de los estudios de peces y crustáceos (Sardà et al. 1994; Tecchio et al. 2013). En la presente Tesis, se ofrece una lista completa de los invertebrados no-crustáceos de las profundidades batiales y abisales recolectadas en las campañas descritas previamente.

Los datos se analizaron además de a nivel puramente taxonómico, a nivel poblacional, revelando mayores densidades (individuos · km $^{-2}$) y biomasas (kg · km $^{-2}$) en zonas de cañones submarinos (ver Artículo 1). Esto se debe a una mayor cantidad de nutrientes presentes en estas zonas, debido a sus características físicas y geomorfológicas que suelen actuar como transporte de nutrientes de zonas someras a zonas más profundidad dentro de cañón (Genin 2004; Company et al. 2012).

A nivel de patrones batimétricos, se observó un incremento de densidad conforme se incrementa la profundidad, con máximos a 2250 m (ver Artículo 1). Esto se debe a la elevada presencia de *Gryphus vitreus*, una especies de Braquiópodo que se ancla a sustratos duros. Estas profundidades coinciden en donde se citaron mayores cantidades de *clinker* (Ramirez-Llodra et al. 2013), un nombre utilizado para los restos de carbón fósil utilizado durante años por los barcos de vapor que posteriormente se tiraban al mar. Estos trozos de *clinker* confieren un sustrato ideal para las especies que necesitan de un anclaje duro para asentarse como por ejemplo *G. vitreus*. También a estas profundidades se recogieron numerosos ejemplares de *Pseudostichopus occultatus* (ver Artículo 2) una especie de holoturia con tendencia a presentarse en grandes agregaciones (Morgan and Neal 2012), lo cual, junto con la especie anterior aumenta considerablemente la densidad estimada a 2250 m. En términos de biomasa, los máximos se encontraron entre 1200 m y 1500 m de profundidad, coincidiendo con los máximos obtenidos en otros grupos de megafauna en la misma área (Tecchio et al. 2011; Tecchio et al. 2013).

A nivel faunístico el grupo más abundante fueron los equinodermos, representando el 57% del total de especímenes recolectados. Debido a esto y a su desconocimiento se estudiaron en mayor detalle en el Artículo 2. La única especie de equinodermos recogida a todas las profundidades y estaciones del año fue el asteroideo *C. grenadensis*, lo que la convertía en especie idónea para el estudio de su biología y ciclo reproductivo. Uno de los resultados más significativos de este estudio (ver Articulo 3), fue la confirmación de una disminución del tamaño de los especímenes adulto conforme aumentaba la profundidad, encontrándose los individuos más pequeños a más profundidad. Esto coincidía con estudios realizados en otros equinodermos muestreados en el Atlántico (e.g. *B. lyrifera*) (Harvey et al. 1988) y con observaciones de otras especies recogidas en la presente tesis como son *B. lyrifera* y *Molpadia musculus* (Mecho, pers. obs). Esta disminución de tamaño se ha asociado con la menor disponibilidad de alimento a mayores profundidades (Rex et al. 2006). También se demostró que los especímenes presentes a mayores profundidades presentaban unos índices gonadales superiores, sugiriendo así que los individuos de mayor profundidad preferían invertir la energía en el esfuerzo reproductivo antes que en el crecimiento somático. A nivel estacional, se observó la presencia de actividad reproductiva durante todo el año, con un mayor incremento en otoño. Estos datos coinciden con estudio realizados en otros grupos de megafauna como peces u otros equinodermos (Ferrand et al. 1988; Fernandez-Arcaya et al. 2013a; Fernandez-Arcaya et al. 2013b) y se relaciona con una mayor reserva de nutrientes en dicha estación del año (Herring 2002; Sardà et al. 2003).

Si pasamos a considerar los datos obtenido mediante imágenes de ROV, se recopilaron un total de 4520 observaciones faunísticas (ver Artículo 4) de los tres hábitats geomorfológicos descritos anteriormente (un cañón, dos montes y dos deslizamientos submarinos). Los datos que se obtuvieron describieron una mayor presencia de especies filtradoras y detritívoras en áreas de cañón, entre ellas la ascidia carnívora *Dicopia antirrhinum*, (ver Artículo 5) situada a 1100 m de profundidad en las inmediaciones del cañón de La Fonera. Esta elevada presencia de especies se debe como se ha comentado anteriormente a la mayor presencia de nutrientes en zonas de cañón que en los alrededores de estos (Company et al. 2012). En las zonas de montes submarinos, se describió una mayor presencia de especies sésiles, como corales y gorgonias. Esto se debe a las características específicas de estas áreas tanto a nivel geomorfológico como hidrográfico además de la presencia de un sustrato duro que permite que se establezcan complejas comunidades faunísticas (Rogers 1994; Rowden et al. 2010). Finalmente en zonas de deslizamientos submarinos, normalmente los grupos de fauna más observados fueron aquellos que presentan una elevada movilidad, como son peces y crustáceos. Esto se corresponde con los datos obtenidos en zonas semejantes mediante muestreos tradicionales, los cuales mencionan una mayoría de estos grupos a nivel de abundancias y biomasas a profundidades similares (Stefanescu et al. 1993; Abelló et al. 2002).

Finalmente, tanto mediante observaciones de ROV como por medio de los muestreos tradicionales se estudió la presencia del impacto antropogénico en la zona de estudio. Los datos recogidos con estos últimos, se recopilaron en un estudio englobando material de noroeste Mediterráneo (Ramirez-Llodra et al. 2013). Mediante las imágenes recogidas se constató una mayor presencia de palangres en zonas rocosas como los montes submarinos, mientras que las zonas de cañón lo más observado eran restos de broza (plásticos, botellas, telas etc...). En las zonas más llanas de los deslizamiento, los más observados fueron las marcas de arrastre, las cuales deformaban totalmente el lecho marino.

En resumen podemos concluir que la presente tesis aporta nuevos y reveladores datos sobre un grupo tan desconocido como son los invertebrados no-crustáceos del Mediterráneo profundo a todos los niveles (taxonómico, poblacional y biológico), tanto por medios tradicionales como nuevas tecnología. Con esta Tesis se pretende reagrupar, validar y simplificar los datos ya existentes así como aportar datos nuevos con el fin de que en un futuro otros investigadores obtengan la información de una manera más sencilla y directa de la que se encontró la presente doctoranda.

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8. Publications

Community composition and distribution of non-crustacean invertebrates in bathyal areas of the north-western Mediterranean Sea

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Resumen

Los estudios realizados sobre composiciones faunísticas referentes a invertebrados en el noroeste Mediterráneo profundo, suelen ir enfocados principalmente al grupo de los crustáceos decápodos. Debido a ello, los otros grupos de invertebrados no-crustáceos, han sido considerablemente menos estudiados y la información disponible suele estar dispersa o anticuada. En este contexto de escaso conocimiento, este estudio recoge nuevos datos faunísticos en relación a rangos batimétricos, distribuciones, densidades, y biomasas de las poblaciones de los invertebrados no-crustáceos presentes en las zonas del margen continental profundo entre los 400 y los 2850 m de profundidad. Asimismo se estudiaron los patrones de composición y distribución faunística en zonas de cañones submarinos. Se recogieron 5876 individuos pertenecientes a 68 especies. De estas, una fue descrita como primera cita en el Mar Mediterráneo y tres se consideraron especies raras. Igualmente se amplió el rango máximo de distribución batimétrica para 11 especies. Finalmente se observó una mayor abundancia y biomasa de invertebrados no-crustáceos en las zonas de cañón submarino, confirmando estas áreas como “hotspots” para las comunidades bentónicas.

Abstract

Invertebrate communities' composition of western Mediterranean deep sea is mostly centred on crustacean decapods, being other groups of megafauna mostly unknown. For these non-crustacean invertebrates groups, poor information is presently available, being often dispersed and out-dated. In this context of low information, present study reports new faunistic data in relation to bathymetrical ranges of distributions as well as conducted a comparative study along the deep-sea continental margin (from 400 to 2850 m depth) on density, biomass and biodiversity bathymetrical pattern of non-crustacean invertebrate community. We also report differences in community composition between inside and outside canyon areas. A total of 5876 individuals were collected. From them, a first record in the Mediterranean Sea, three rare species and eleven bathymetrical ranges of distribution were extended. Depth related patterns were observed on density distribution, showing their maximum of abundance at deeper areas (i.e 1500-2250 m). The higher concentration of patchy species at deeper range could be driven the observed increasing pattern. Additionally, higher densities and biomasses were observed in canyon areas than in its adjacent open slope, confirming them as hotspots for faunal communities.

KEY-WORDS: Non-crustacean invertebrates, taxonomy, distribution, community, canyon.

Introduction

The shallow Mediterranean Sea could be considered a ‘*well studied*’ environment, when compared with other oceans (Coll et al. 2010), however, when this comparison is referred to the deeper areas of the Mediterranean (deeper than 500 m depth) the information remains scarce (Company et al. 2004; Danovaro et al. 2010; Ramirez-Llodra et al. 2010). Published data are partly available for specific zones such as the ‘hot spots’ habitats (e.g. cold coral reefs, cold seeps, carbonate muds, canyon areas or submarine hills (Mastrototaro et al. 2010; Bo et al. 2011; Bienhold et al. 2013). The north-western Mediterranean represents an exception, being presently one of the most studied regions at all faunistic levels (i.e. population distributions, biodiversity and communities zonation). This affirmation is especially true for the deep-sea continental margin, the fauna of which has been studied for the half of the past century (Pérès and Picard 1956; Carpine 1970; Alvà 1987; Stefanescu et al. 1993). The specific geomorphological characteristics of the local margin (i.e., high number of submarine canyons and the steep inclination of the continental slope) and associated biotic and abiotic processes (i.e. variation in oceanographic currents, the mixing in hard and soft substrata and higher food availability compared with other Mediterranean areas), result in a high habitat heterogeneity of the north-western continental margin (Carpine 1970; Company et al. 2012; Tecchio et al. 2013). That conjunct of highly diversified habitats promotes the establishment and maintenance of diverse faunal communities, the composition and functioning of them are just starting now to be described and understood (Levin et al. 2010).

The faunistic studies carried out in continental margin areas deeper than 500 m in the north-western Mediterranean basin were mainly focused on the two most abundant groups, fishes and crustaceans (Abelló et al. 2002; Sardà et al. 2003; Fernandez-Arcaya et al. 2012; Fernandez-Arcaya et al. 2013). That interest was primarily motivated by the need to understand heavily exploited ecosystems by local fisheries (Stefanescu et al. 1993; Company et al. 2004; Sardà et al. 2004) and their fishery related studies (Sardà et al. 1994; Moranta et al. 1998; Aguzzi et al. 2009). The number of studies oriented to other groups of megafauna are comparatively lower (Turon 1988; Quetglas et al. 2000; Ramirez-Llodra et al. 2008; Cartes et al. 2009). Among these, there are the non-crustacean invertebrates, which are presently at the centre of an important scientific sampling effort (Ramirez-Llodra et al. 2008; Cartes et al. 2009; Mecho et al. 2014b), which for example, is providing an entirely new set of faunal data and is resulting in the detection of new species and records (Mecho et al. 2014a; Mecho et al. 2014b; Saiz et al. 2014).

The importance of taxonomical studies on non-crustacean invertebrates resides not only in the description of species and their taxonomical classification, but in the fact that several secondary studies that take advantage of this knowledge (Godfray 2002; Khuroo et al. 2007). Giangrande (2003), describes that a '*taxonomic sufficiency*' (consisting in suggest that working at a taxonomic level higher than species does not result in an important loss of information) had been signalled these last years, and how it could have an effect of inaccuracy of biodiversity evaluation and an exclusion of some entities before understanding their role in ecology.

Presently, the invertebrate species composition of north-western deep-sea communities is mostly centred on crustacean decapods, leaving several gaps of knowledge on other ecologically relevant and apparently abundant invertebrate groups. For these groups, poor information is presently available, being samples often disregarded at sampling since their taxonomical status is of difficult determination. The present study reports new faunistic data in relation to the depth of distributions, densities, and biomasses of populations of several deep-sea continental margin non-crustacean invertebrates into canyons. Species taxonomic determination was carried out in these geomorphologic areas of difficult sampling access, in order to achieve more reliable faunal lists to be used for future ecological studies.

Materials and Methods

Study area

Ten oceanographic cruises were conducted between October 2008 and April 2013 to sample the deep seafloor of the western Mediterranean Sea. The sampling areas included the Blanes Canyon and its adjacent open slope, the Palamós Canyon (also named La Fonera) and the Cap de Creus Canyon (Fig. 1). These cruises took place in the framework of three Spanish research projects (PROMETEO, DOSMARES, and

PROMARES), sampling at depths between 400 and 2845 m (Table 1). Additionally, a trans-Mediterranean cruise took place in the context of the European project BIOFUN (EuroDEEP Eurocores, European Science Foundation) during July 2009. This cruise sampled the western, central and eastern Mediterranean basins at 1200, 2000 and 3000 m depth. Considering the low number of trawls conducted on central (n= 6) and eastern basins (n= 10) during BIOFUN cruise and the low number of obtained species (n=11), these samples were exclude from the analyses. However, the specimens were integrated in the general species list (Table 2).

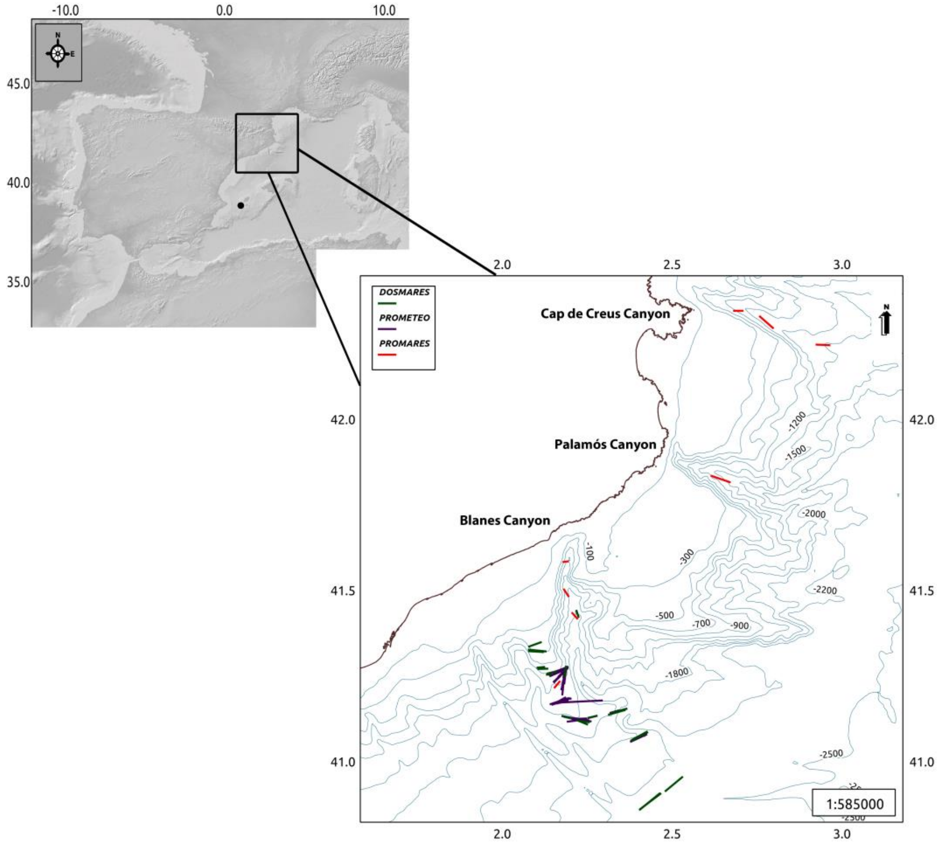
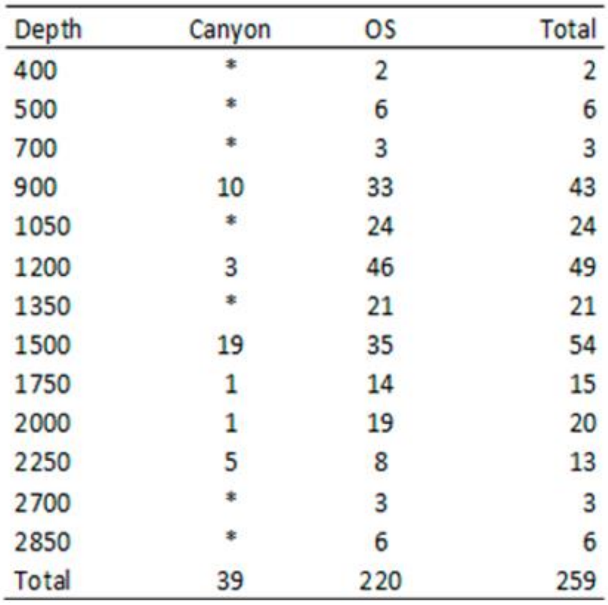


Figure 1. Study area representing the three sampled canyons and adjacent open slopes in the north-western Mediterranean Sea. Colour lines represent trawl stations. Red: PROMARES trawls; Blue: PROMETEO trawls; Green: DOSMARES trawls. Note: the black spot south Balearic Islands shows the western Mediterranean trawl at 2850 m depth from BIOFUN project.

A total of 259 benthic trawls were completed (Table 1) resulting in a total swept area of 10.6 km². Of these hauls, 129 samples were obtained by a single warp otter-trawl Maireta system (OTMS, Sardà et al. 1998) with a net length of 25 m and a cod-end mesh size of 12 mm. A SCANMAR system was used to estimate the width of the mouth of the net. An average horizontal opening of 12.7±1.4 m was calculated. As the

SCANMAR system can only operate down to 1200 m depth, the same value for the mouth’s width of the net was used also for deployments deeper than 1200 m. The height of the trawl mouth was estimated to be 1.4 m (Sardà et al. 1998). In addition, 45 hauls were conducted with an Agassiz benthic dredge, made of a square steel frame with a mouth width of 2.5 m and a mouth height of 1.2 m, and fitted with a 12 mm mesh size net. Bathymetric range sampled by the Agassiz dredge is from 900 m to 2850 m depth, no Agassiz data is available from 400 to 900 m depth. Further, 55 samples were obtained with an epibenthic sledge which consisted of a rectangular steel frame (mouth width of 0.8 m and mouth height of 0.4 m), three nets attached at different heights (10-50 cm, 55-95 cm and 100-140 cm above the bottom) and a mesh size of 300 μm (only the frame closer to bottom was considered in the present study).



Depth	Canyon	OS	Total
400	*	2	2
500	*	6	6
700	*	3	3
900	10	33	43
1050	*	24	24
1200	3	46	49
1350	*	21	21
1500	19	35	54
1750	1	14	15
2000	1	19	20
2250	5	8	13
2700	*	3	3
2850	*	6	6
Total	39	220	259

Table 1. Number of benthic trawls used in the present study by depth and geomorphological area. Canyon area included La Fonera, Cap de Creus and Blanes canyons. OS: open slope area.

Specimen identification

The individuals of non-crustacean invertebrate species were sorted, weighed, counted and fixed with 40% formalin diluted with seawater and neutralized with borax on board ship. After 30 days, the non-gelatinous specimens were transferred to 70% alcohol in the laboratory for further examination. Individuals of relevant species were fixed in absolute ethanol on board to allow for molecular analyses (not included in this study).

Back to laboratory, all specimens were classified to the lowest taxonomic level. The identification of some groups was confirmed with the help of several taxonomic specialists (i.e. Class Echiura and Phylum Sipuncula by Dr. J.I. Saiz-Salinas, Class Cephalopoda by Dr. R. Villanueva, Phylum Porifera by Dr. M.J. Uriz, Class Holothuria by Dr. David Billett and Class Echinoidea by Prof. Paul Tyler). The nomenclature was checked against the World Register of Marine Species (WoRMS) and the Phylogenetic classification of the species was based on the Tree of Life Web Project (ToL, http://tolweb.org/tree/). All specimens available would be stored in the Biological Reference Collections to provide information for future researchers. Species were compiled in a complete list (Table 2) with checked valid names, depth of occurrence, presence-absence in open slope and canyon area, and also were classified as pelagic, benthopelagic or benthic species, and vagil or sessile species.

Statistical data treatment

Density and biomass per phyla and classes (ind·km^{-2} and kg·km^{-2}, respectively) were calculated and standardized per km^{2}, bathymetrically and per area (open slope vs submarine canyon). In the present study, species considered strictly pelagic were excluded from the distribution and community composition analysis to avoid any bias of the data. Although, these species were also listed in Table 2 (i.e, *Chrysaora hysoscella* (Linnaeus, 1767), *Pelagia noctiluca* (Forsskål, 1775), *Pterotrachea scutata* Gegenbaur, 1855, *Cymbulia peronii* Blainville, 1818, *Pyrosoma atlanticum* Péron, 1804 and Salpa spp).

The Hurlbert's rarefaction index ES(25) was calculated for α-diversity. Species accumulation curves were calculated for the open slope and the submarine canyon, separately for OTMS and Agassiz samples.

Due to the differences in the sampled fraction of the megafauna and considering other studies (Tecchio et al. 2011a), samples from Agassiz dredge and the benthic otter trawl (OTMs) were considered separately. Epibenthic sledge samples were discarded for analyses due to the punctual reported specimens and the bias that produced this sampling method. As in the case of strictly pelagic species, all collected species were mentioned in the species table.

Results

New distribution records

A total of 5876 individuals of non-crustacean invertebrates were collected along the deep Catalan margin. Of them, 5564 were classified down to the taxonomic level of species. Individuals belonged to eight phyla, for a total of 18 classes and 68 species (Table 2).

Among relevant species, we sampled the regular echinoid *Gracilechinus elegans* (Düben & Koren, 1844) at 1500 m depth, being hence the first record in the Mediterranean Sea (Mecho et al. 2014b). Three other 'rare species' were also reported: the endemic holothurians *Hedingia mediterranea* (Bartolini Baldelli, 1914) and *Penilpidia ludwigi* (von Marenzeller, 1893) and the wood boring sipuncula *Phascolosoma (Phascolosoma) turnerae* Rice, 1985 (Saiz et al. 2014).
Because there is no consensus regarding what determines a 'rare species', in this study "rare" are considered as those taxa that have been reported less than five times in the whole Mediterranean Sea. For each of the previous species, at least 3 individuals were collected.

Bathymetrical range of distribution was extended for 11 species: the Porifera *Polymastia tissieri* (Vacelet, 1961) from 2211 to 2850 m; the cold water corals *Madrepora oculata* Linnaeus, 1758 and *Desmophyllum dianthus* (Esper, 1794) from 1100 m for both species to 1450 and 2250 m, respectively. The Echiurans *Echiurus abyssalis* Skorikow, 1906 and *Bonellia viridis* Rolando, 1821, from 837 and 1621 m down to 1750 and 2000 m depth, respectively; the Sipuncula *Sipunculus (Sipunculus) norvegicus* Danielssen, 1869 from 1927 to 2000 m depth; the echinoderms *Hedingia mediterranea* and *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823, from 1100 m to 1500 m and from 300 m to 850 m respectively, *Brissopsis lyrifera* (Forbes, 1841) from 1650 m to 2250 m and *Ceramaster grenadensis* (Perrier, 1881) from 2500 m to 2845m.

Table 2. Table of species sampled in western Mediterranean Sea. Depth range: depth of distribution in present study. OS: open slope; C: canyon area. * Species were maximum depth of distribution has been extended.

Taxa	n. sampled	Depth range (m)	area (OS/C)	Bottom relation	vagil-sessile
PORIFERA					
Calcarea					
Sycon sp. Risso, 1827	1	2000	OS	benthic	sessile
Demospongiae					
Thenea muricata(Bowerbank, 1858)	26	900-1050	OS	benthic	sessile
Polymastia tissieri (Vacelet, 1961)*	18	1200-2850	OS	benthic	sessile
Radiella sarsi (Ridley & Dendy, 1886)	45	1050-2000	OS-C	benthic	sessile
CNIDARIA					
Anthozoa					
Isidella elongata (Esper, 1788)	11	1500 -2250	OS	benthic	sessile
Actinauge richardi (Marion, 1882)	4	1200	OS	benthic	sessile
Calliactis parasitica (Couch, 1842)	1	400	OS	benthic	epifauna
Epizoanthus incrustatus Düben & Koren, 1847	2	400	OS	benthic	epifauna
Unidentified Anthozoa	50				
Actinia spp	94	1200	OS	benthic	sessile
Epizoanthus spp	64	500/1750	OS-C	benthic	epifauna
Desmophyllum dianthus (Esper, 1794)*	537	900-2250	OS-C		
Lophelia pertusa (Linnaeus, 1758)	13	900	C	benthic	sessile
Madrepora oculata Linnaeus, 1758*	120	1200-1450	C	benthic	sessile
Scyphozoa					
Chrysaora hysoscella (Linnaeus, 1767)	20	2000	OS-C	pelagic	vagil
Pelagia noctiluca (Forsskål, 1775)	200	500-2700	OS-C	pelagic	vagil
O. Coronatae (Black jellyfish)	12	1050-2700	OS-C	benthopelagic	vagil
ANNELIDA					
ECHIURA					

Taxa	n. sampled	Depth range (m)	area (OS/C)	Bottom relation	vagil-sessile
Bonellia viridis Rolando, 1821*	4	1200-2000	OS	benthic	infauna
Bonelliopsis minutus Datta-Gupta, 1981	5	1350-1500	OS -C	benthic	infauna
Nellobia eusoma Fisher, 1946	4	1500	C	benthic	infauna
Bonellidae spp	4	1200/1500	OS -C	benthic	infauna
Echiurus abyssalis Skorikow, 1906*	12	1350-1750	OS -C	benthic	infauna
Polychaeta	22				
SIPUNCULA					
Sipunculidea					
Sipunculus (Sipunculus) norvegicus Danielssen, 1869*	16	900-2000	OS	benthic	low mobility
Golfingia (Golfingia) vulgaris vulgaris (de Blainville, 1827)	9	900	OS -C	benthic	infauna
Nephasoma (Nephasoma) diaphanes corrugatum Cutler & Cutler, 1986	1	1500	OS	benthic	infauna
Nephasoma spp	1	2000	OS	benthic	
Unidentified sipuncula	3	500/1200	OS	benthic	
Phascolosomatidea					
Phascolosoma (Phascolosoma) turnerae Rice, 1985	1	1500	OS	benthic	wood
Aspidosiphon (Aspidosiphon) muelleri muelleri Diesing, 1851	13	900	OS -C	benthic	empty shells
MOLLUSCA					
Bivalvia					
Delectopecten vitreus (Gmelin, 1791)	17	1200-2000	OS	benthic	epifauna
Limaria hians (Gmelin, 1791)	2	1500	OS	benthic	infauna
Abra longicallus (Scacchi, 1835)	96	700-1500	OS -C	benthic	infauna
Unidentified bivalvia	2	900-1050	OS -C	benthic	
Scaphopoda					

Taxa	n. sampled	Depth range (m)	area (OS/C)	Bottom relation	vagil-sessile
Antalis spp	49	900-2000	OS -C	benthic	infauna
Scaphopoda spp	17	900	OS	benthic	infauna
Gastropoda					
Pterotrachea scutata Gegenbaur, 1855	14	900-1500	OS		
Pagodula echinata (Kiener, 1840)	5	900-1500	C	pelagic	vagil
Euspira fusca (Blainville, 1825)	73	500-1500	OS -C	benthic	crawler
Aporrhais serresianus (Michaud, 1828)	22	900-2000	OS -C	benthic	crawler
Cymbulia peronii Blainville, 1818	404	500-2850	OS -C	pelagic	vagil
Cephalopoda					
Galiteuthis armata Joubin, 1898	8	1200-2000	OS -C	bentho-pelagic	vagil
Histioteuthis bonnellii (Férussac, 1835)	3	500-2000	OS	bentho-pelagic	vagil
Histioteuthis reversa (Verrill, 1880)	13	500-2000	OS -C	bentho-pelagic	vagil
Todarodes sagittatus (Lamarck, 1798)	2	400-1050	OS	bentho-pelagic	vagil
Heteroteuthis dispar (Rüppell, 1844)	3	1050-1350	OS	bentho-pelagic	vagil
Neorossia caroli (Joubin, 1902)	8	900-1500	OS -C	bentho-pelagic	vagil
Sepietta oweniana (d'Orbigny, 1841)	1	500	OS	bentho-pelagic	vagil
Sepiolidae spp	2	400-500	OS	bentho-pelagic	vagil
Unidentified squid	2	2000	OS	bentho-pelagic	vagil
Opisthoteuthis calypso Villanueva, Collins, Sánchez & Voss, 2002	32	900-2000	OS	bentho-pelagic	vagil
Eledone cirrhosa (Lamarck, 1798)	4	400-500	OS	bentho-pelagic	vagil
Octopus salutii Vérany, 1836	1	400	OS	bentho-pelagic	vagil
Bathypolypus sponsalis (P. Fischer & H. Fischer, 1892)	65	500-1500	OS -C	bentho-pelagic	vagil
BRACHIOPODA					
Gryphus vitreus (Born, 1778)	1472	500-2250	OS -C	benthic	sessile

Taxa	**n. sampled**	**Depth range (m)**	**area (OS/C)**	**Bottom relation**	**vagil-sessile**
ECHINODERMATA					
Asteroidea					
Ceramaster grenadensis (Perrier, 1881)*	150	700-2845	OS -C	benthic	crawler
Hymenodiscus coronata (G.O. Sars, 1872)	47	1500 - 2250	OS -C	benthic	crawler
Echinoidea					
Gracilechinus elegans (Danielssen and Koren, 1883)	6	1500	C	benthic	crawler
Brissopsis lyrifera (Forbes, 1841)*	556	400 - 2250	OS -C	benthic	crawler
Holothuroidea					
Mesothuria (Allantis) intestinalis (Ascanius, 1805) Östergren, 1896	56	900 - 1750	OS -C	benthic	crawler
Pseudostichopus occultatus von Marenzeller 1893	474	2000 - 2250	OS -C	benthic	crawler
Holothuria (Panningothuria) forskali Delle Chiaje, 1823	1	850	C	benthic	crawler
Molpadia musculus Risso, 1826	27	500 - 1050	OS	benthic	infauna
Hedingia mediterranea (Bartolini Baldelli, 1914) Tortonese, 1965*	11	900 - 1500	OS -C	benthic	infauna
Penilpidia ludwigi (von Marenzeller, 1893)	200	900 -1500	OS	bentho-pelagic	crawler
Ypsilothuria bitentaculata (Ludwig, 1893)	27	900 - 1350	OS	benthic	infauna
Leptosynapta sp	1	500	OS	benthic	infauna
CHORDATA					
Thaliacea					
Pyrosoma atlanticum Péron, 1804	320	400-2850	OS -C	pelagic	sessile
Salpa spp	265	500-2850	OS -C	pelagic	vagil

Bathymetrical patterns of density and biomass distribution

Density and biomass of non-crustacean invertebrate species were plotted for the three sampling systems (i.e. epibenthic sledge, the Agassiz and the OTMS trawls) (Fig. 2A, B). Density presented two trends, showing low values from 400 to 1050 m depth, and a decreasing pattern of higher values from 1200 to 2850 m depth (Fig. 2A). These trends were mainly influenced by the samplings conducted by the Agassiz trawl that usually presented higher values than OTMS. This latter one, presented similar density values along all the bathymetrical sampling depths. The epibenthic sledge presented a peak of densities at 900 m as result of a catch with more than 200 patching holothurians; this peak was discarded from the graphics due to the bias that produced. Biomasses, as observed in density showed higher values in Agassiz and a peak of biomass was also observed at 1200 – 1500 m depth (Fig. 2B).

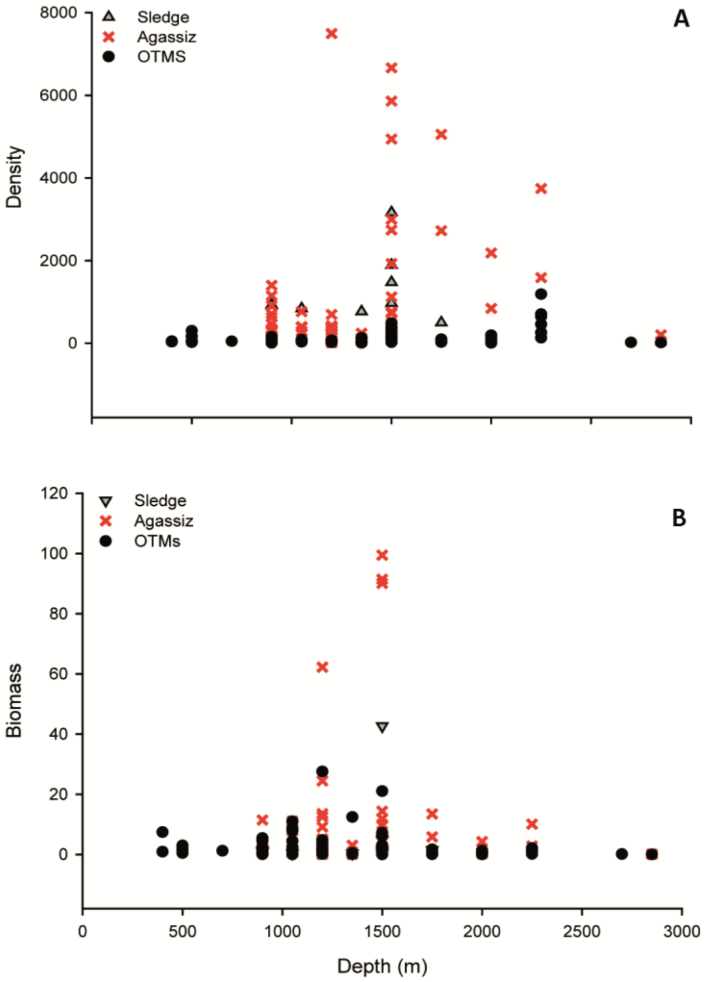


Figure 2. Bathymetrical trends of A) density and B) biomass of non-crustacean invertebrates sampled in Epibenthic sledge, Agassiz and OTMS trawl

In order to better understand the results described below, we analysed bathymetrically the density and biomass by class. The classes of the Phyla Brachiopoda and

Echinodermata were the more abundant with higher biomasses at the deepest bathymetric range of this study (below 1500 m) (Figs. 3 and 4). These two phyla were the most abundant non-crustacean invertebrates along the Catalan margin (Fig. 5). Species of the Phylum Mollusca were also present at all sampled depths, but with higher densities and biomasses found down to 1750 m. The densities of the 4 classes of Mollusca were different depending on the sampler used, with more strictly benthic classes of Mollusca found in Agassiz trawl (i.e. Scaphopoda and Bivalvia), compared with OTMS samples that mostly contained Cephalopoda and Gastropoda. Taking into account the bias resulting from epibenthic sledge data, they are not studied in these analyses

Community composition

Of all sampled Phyla, the echinoderms were one of the most important, representing 57% of the total density and a 41% of the total biomass (Fig. 5A, B). Differences in faunistic composition were also observed for the 3 different samplers used (Fig. 5). The more abundant phyla in Agassiz trawl samples were Brachiopoda, Echinodermata, and Cnidaria, representing 32%, 31%, and 19% respectively of the total of all sampled species (Fig. 5A). For OTMS samples, the higher densities were for Echinodermata, Brachiopoda, and Mollusca, with 46%, 27%, and 13% respectively of the total sampled species (Fig. 5A). Epibenthic sledge mostly collected specimens within the Phylum Echinodermata, which therefore made up the 91% by the resulting from one single trawl (Fig. 5A).

The Agassiz sampler collected high biomasses of Echinodermata, Cnidaria, and Brachiopoda (48%, 26% and 13%, respectively) (Fig. 5B), while OTMS mostly captured individuals within the Phylum Mollusca, the biomass of which represented the 69% of the total, followed by Echinodermata (23%) and by a minority of Cnidaria (5%) (Fig.5B).

Biomasses of epibenthic sledge samples were constituted by 87% of Mollusca, resulting from the sample of a single large individual of the cephalopod *Bathypolypus sponsalis* (P. Fischer & H. Fischer, 1892).

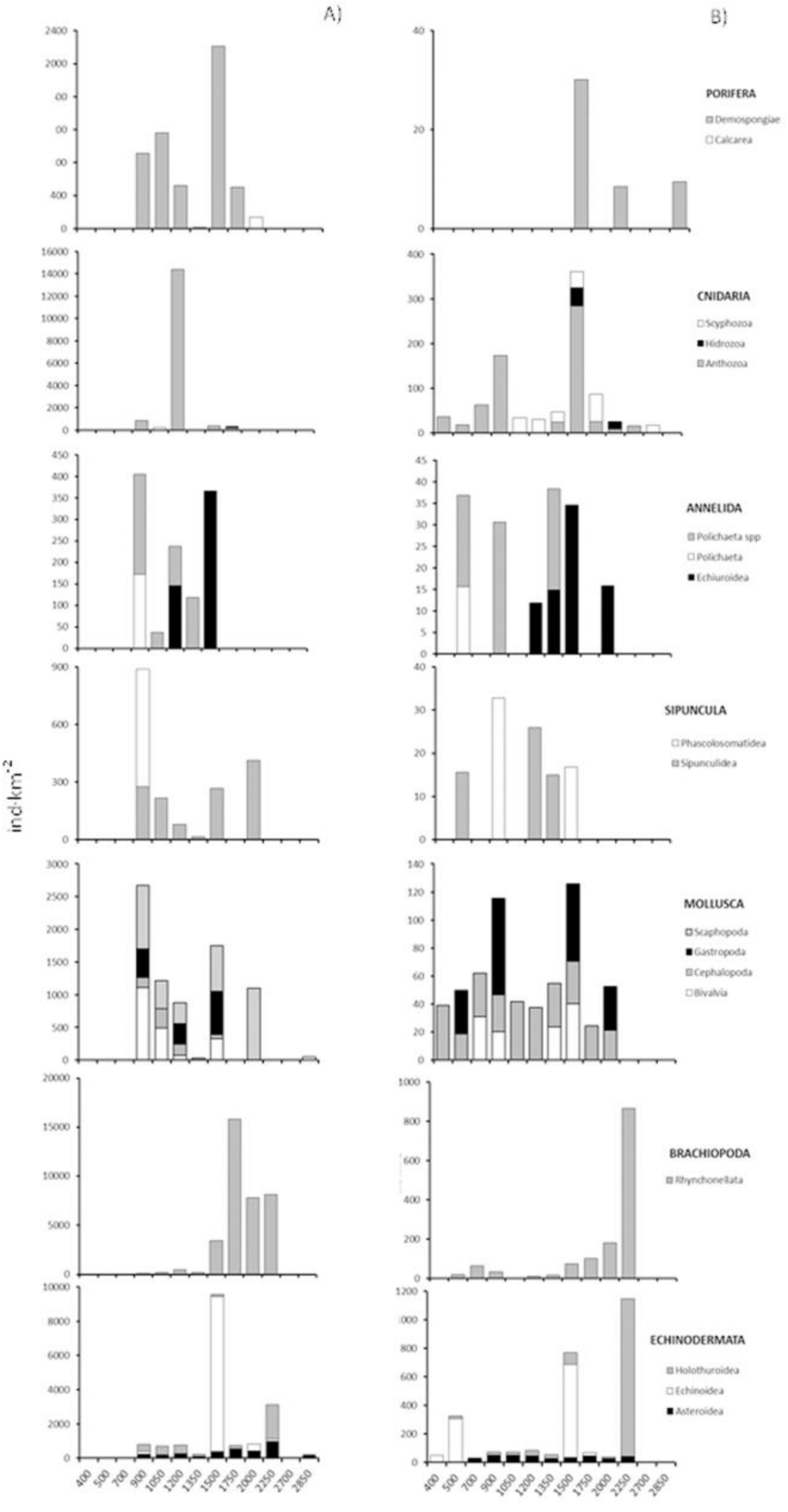


Figure 3. Density bathymetric distribution by Class for A) Agassiz trawl and B) OTMS

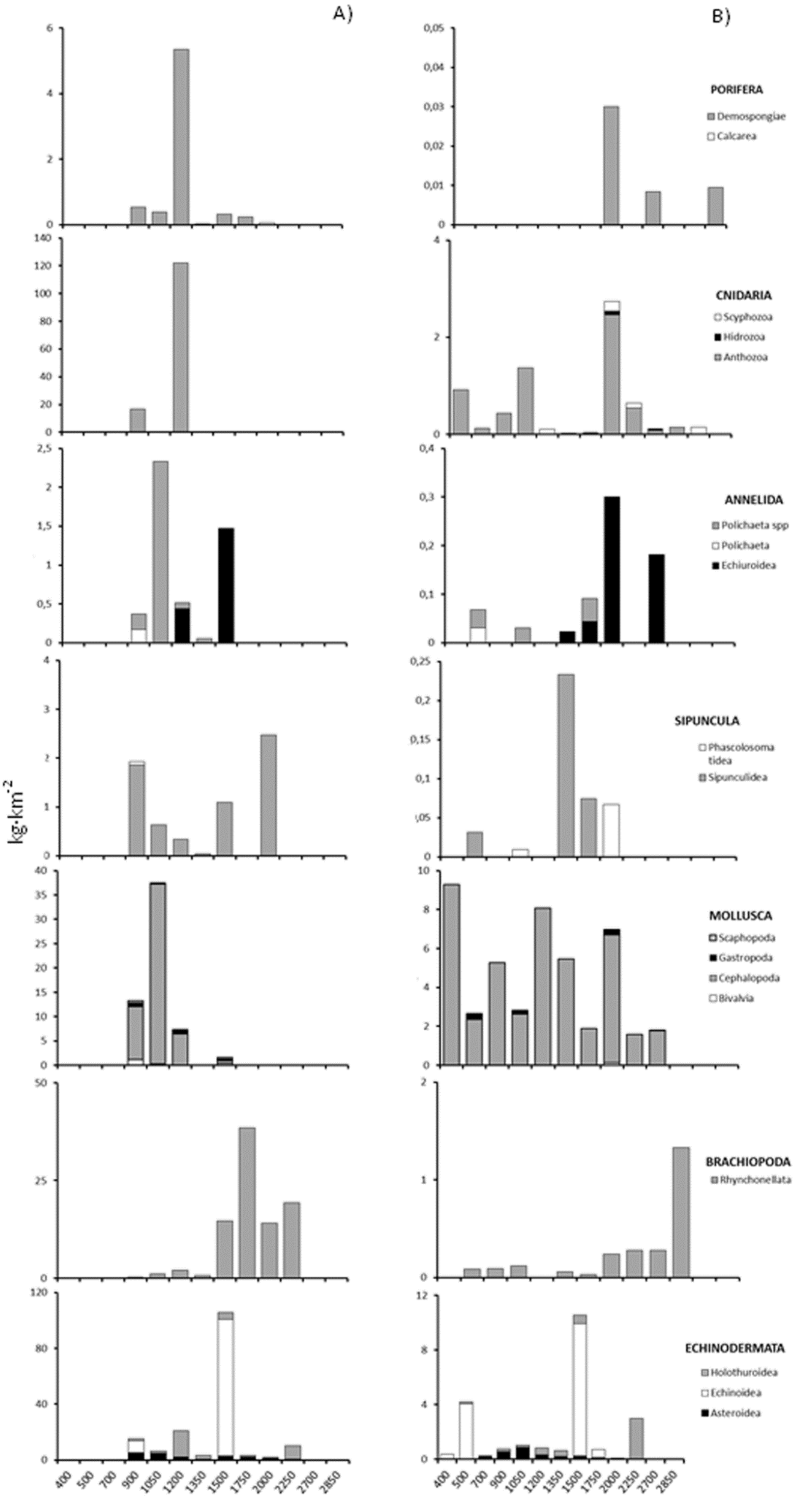


Figure 4. Biomass bathymetric distribution by Class for A) Agassiz trawl and B) OTMS

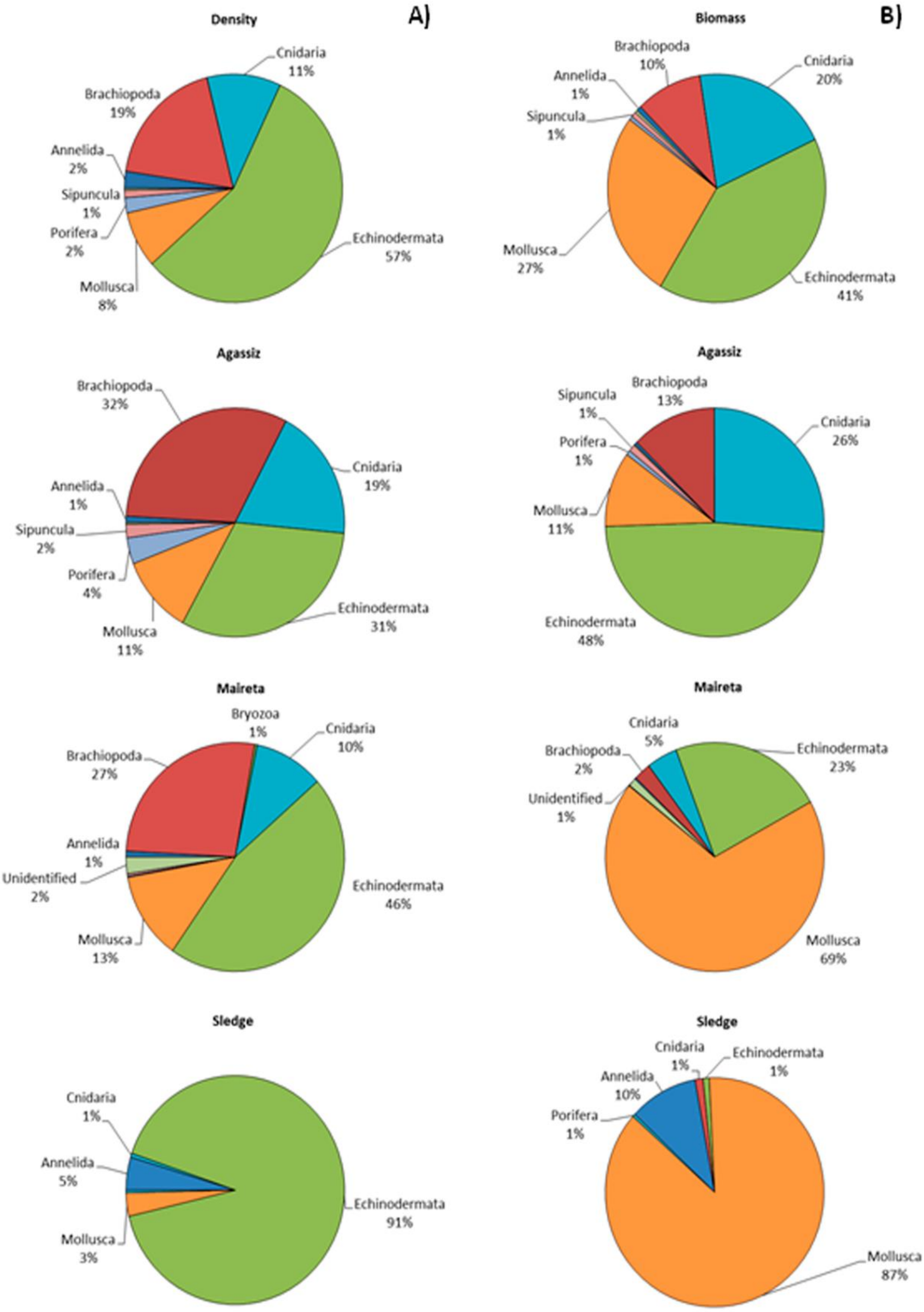


Figure 5. Faunistic composition by gear for A) total density and B) biomass of non-crustacean invertebrates. Epibenthic sledge data was not used on posterior analyses (for further information see section Material and Methods).

Biodiversity patterns

Biodiversity rarefaction index ES(25) showed different patterns with depth for both sampling methods (OTMS and Agassiz trawl). For OTMS biodiversity a bi-modal shape was observed, decreasing from 400 to 1050 m and increasing after until 2000 m depth (Fig. 6A). The Agassiz rarefaction index presented a decreasing trend with depth (Fig. 6B), showing a higher number of species sampled at shawoller depths.

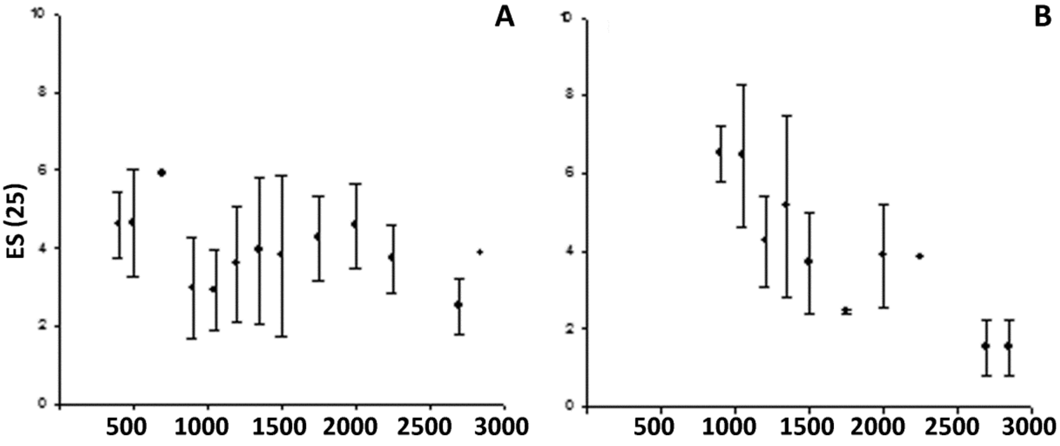


Figure 6. Hulbert's Rarefaction ES(25) for Open Slope with sampled with A) OTMS and B) Agassiz

Submarine canyon versus *open slope*

When bathymetrical patterns of density and biomass were studied by geomorphological areas (opens slope or canyon) (Fig 7A, B), the higher values reported at 1200 m – 1500 m depth coincide with trawls conducted inside canyon areas (Fig 7A, B). Although, when canyon samples were discarded bathymetrical patterns maintain similar distributions with low values.

Densities and biomasses of non-crustacean invertebrates were higher at submarine canyon area compared with adjacent open slope (Fig. 8). Mean density in the former zone were of 1940 ind·km^{-2} for Agassiz trawl and 221 ind·km^{-2} for OTMS, while in the open slope the density for Agassiz was of 872 and 77 ind·km^{-2} for OTMS (Fig. 8).

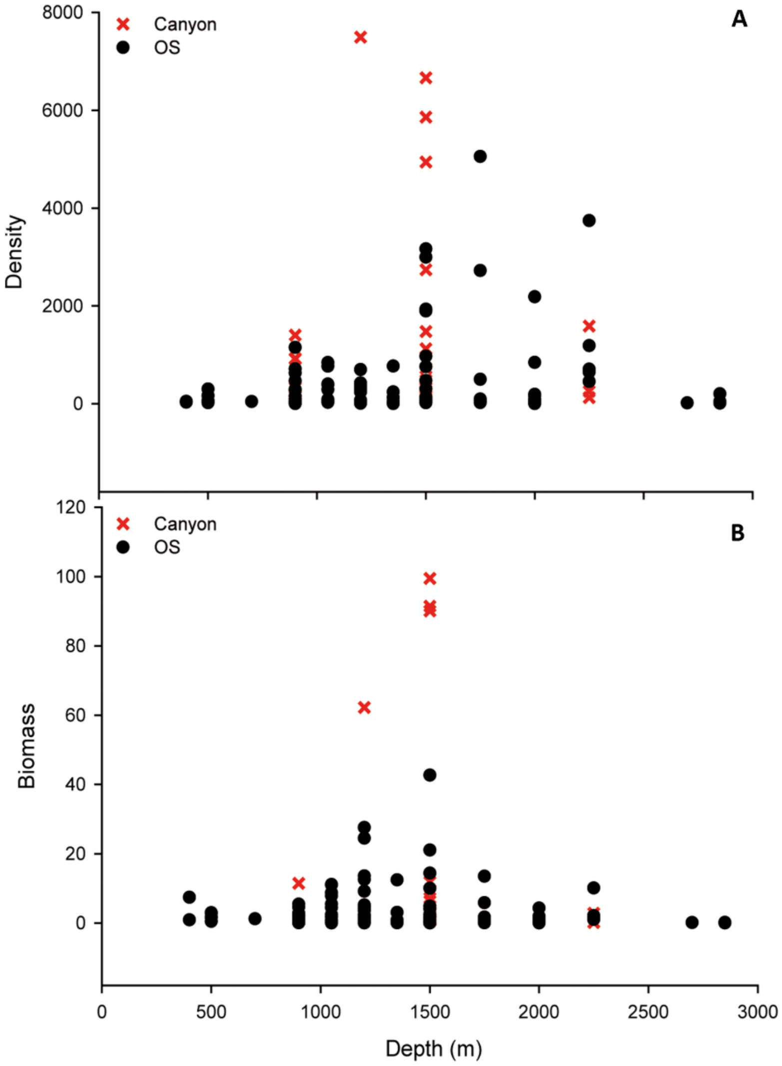


Figure 7. Bathymetrical trends of A) density and B) biomass of non-crustacean invertebrate sampled in open slope (OS) and canyon areas.

The same trend was observed for biomass with 17.2 kg·km^{-2} for Agassiz and 2.79 kg·km^{-2} for OTMS in canyon areas, while in the adjacent open slope the Agassiz reported 4.7 kg·km^{-2} and OTMS 1.76 kg·km^{-2} (Fig. 8).

Faunistic composition in terms of densities was different between the two areas, canyon and open slope, (Fig. 9) and these differences were observed in samples of both sampling methods used (Agassiz and OTMS).

In open slope the three most abundant Phyla were similar for both trawls, with just slight differences in their proportion.

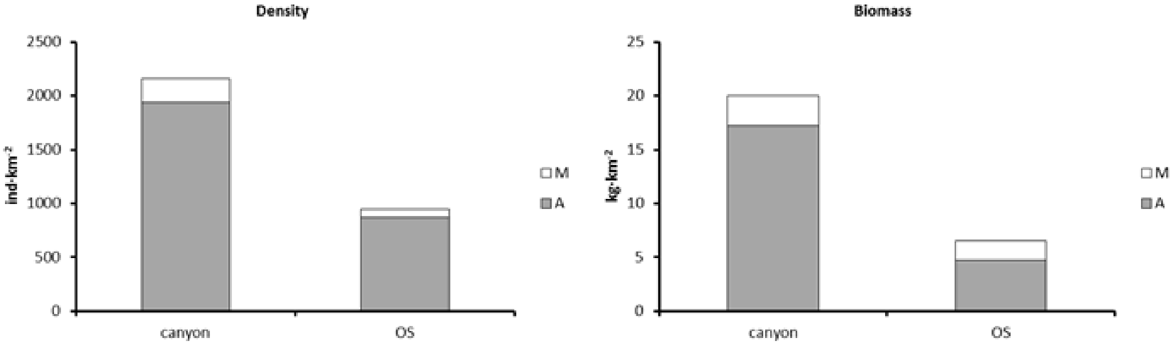


Figure 8. Total density and biomass of non-crustacean invertebrates sampled in open slope and canyon areas.

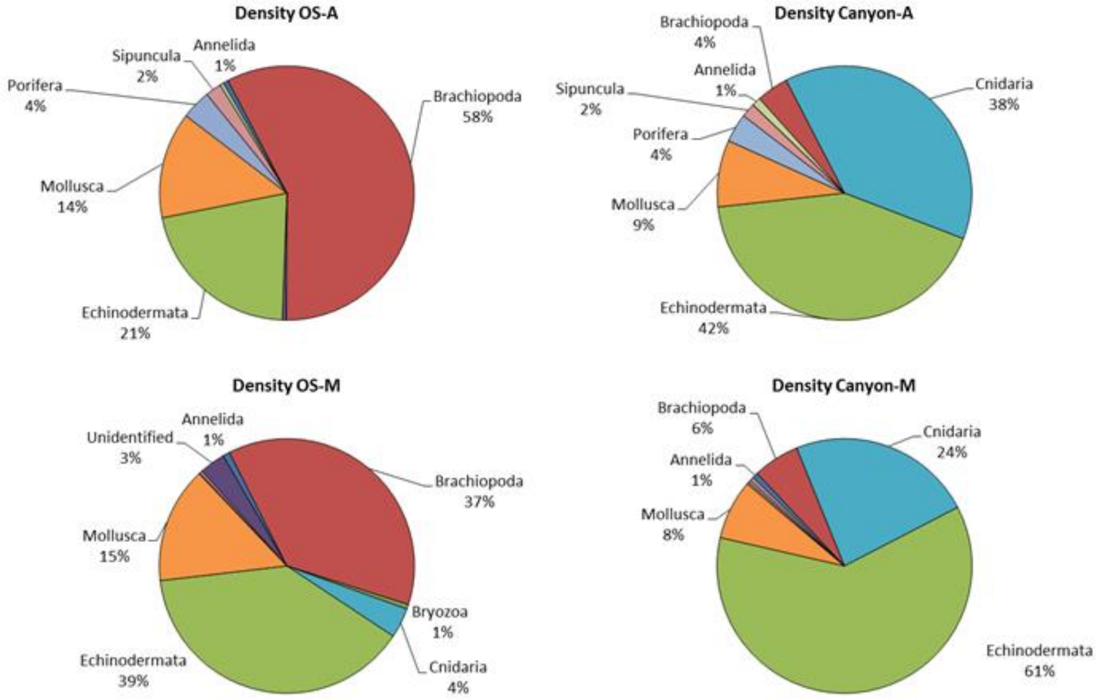


Figure 9. Faunistic densities composition sampled by gear in canyon and open slope (OS) areas. A: Agassiz; M: OTMS.

Instead, biomasses showed differences in faunistic composition both by area and trawl method (Fig. 10). The higher biomasses on canyon samples were represented by cnidarians and echinoderms for both samplers. For open slope, the phyla composition of samples were different depending on the used method, being Agassiz more equally represented (Echinodermata represented 42% of the total biomass, followed by Brachiopoda, 31%, Mollusca, 24% and all the other Phyla less than a 2%) (Fig. 10). On open slope, the biomass classes caught by OTMS were mainly composed by the Phylum Mollusca, representing an 84% of the total biomass, Echinodermata a 10% and Brachiopoda only a 3% of the biomass (Fig. 10).

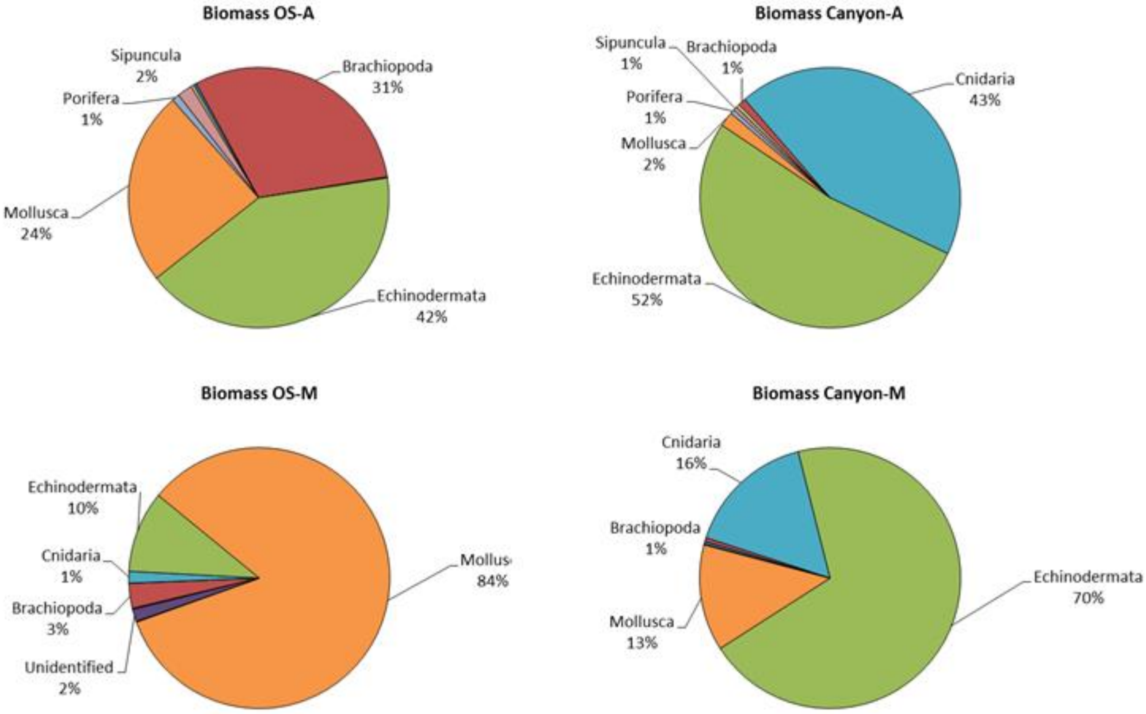


Figure 10. Faunistic densities composition sampled by gear in canyon and open slope (OS) areas. A: Agassiz; M: OTMS

In canyon samples obtained by Agassiz trawl only the class Brachiopoda was the most abundant in the open slope than in canyon area (Fig. 11). The 4 classes of the Phylum Mollusca were instead more abundant in the open slope but more equally distributed. All the other Phyla presented higher densities inside than outside canyon (Fig. 11A). With OTMS, these differences were less obvious, and only Phyla Porifera, Cnidaria and Echinodermata were more abundant inside canyons (Fig. 11B). Biomasses from Agassiz presented similar patterns of distribution (Fig. 12A). In OTMS, biomasses were distributed more equally than in Agassiz samples (Fig. 12B).

Seven species were reported only in canyon areas, while 27 were collected exclusively in open slope areas (Table 2). All the other species were present on both areas. The species were considered as belonging to the canyon when animals were sampled inside

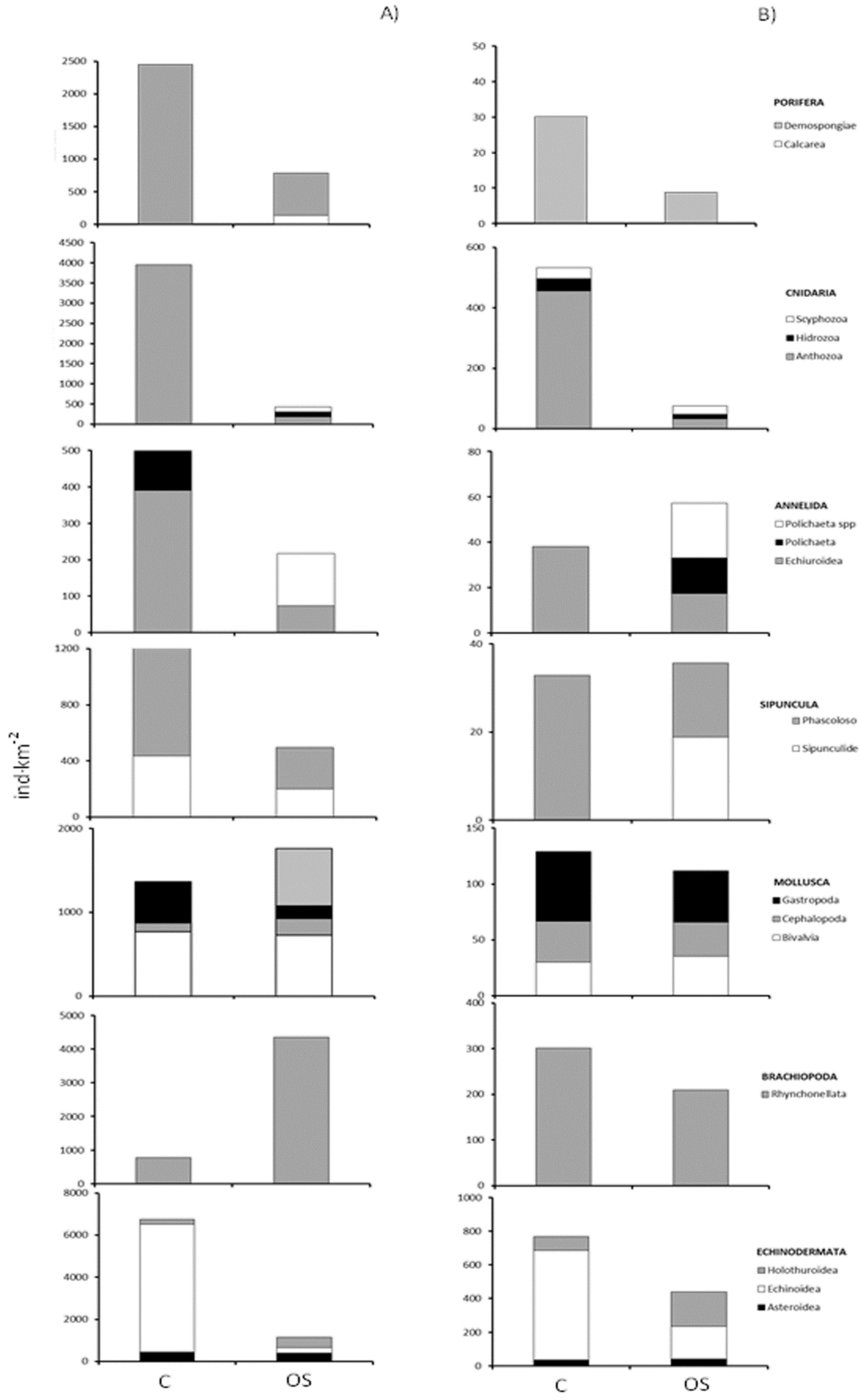


Figure 11. Density by Class for A) Agassiz trawl and B) OTMS in canyon (C) and open slope (OS) areas.

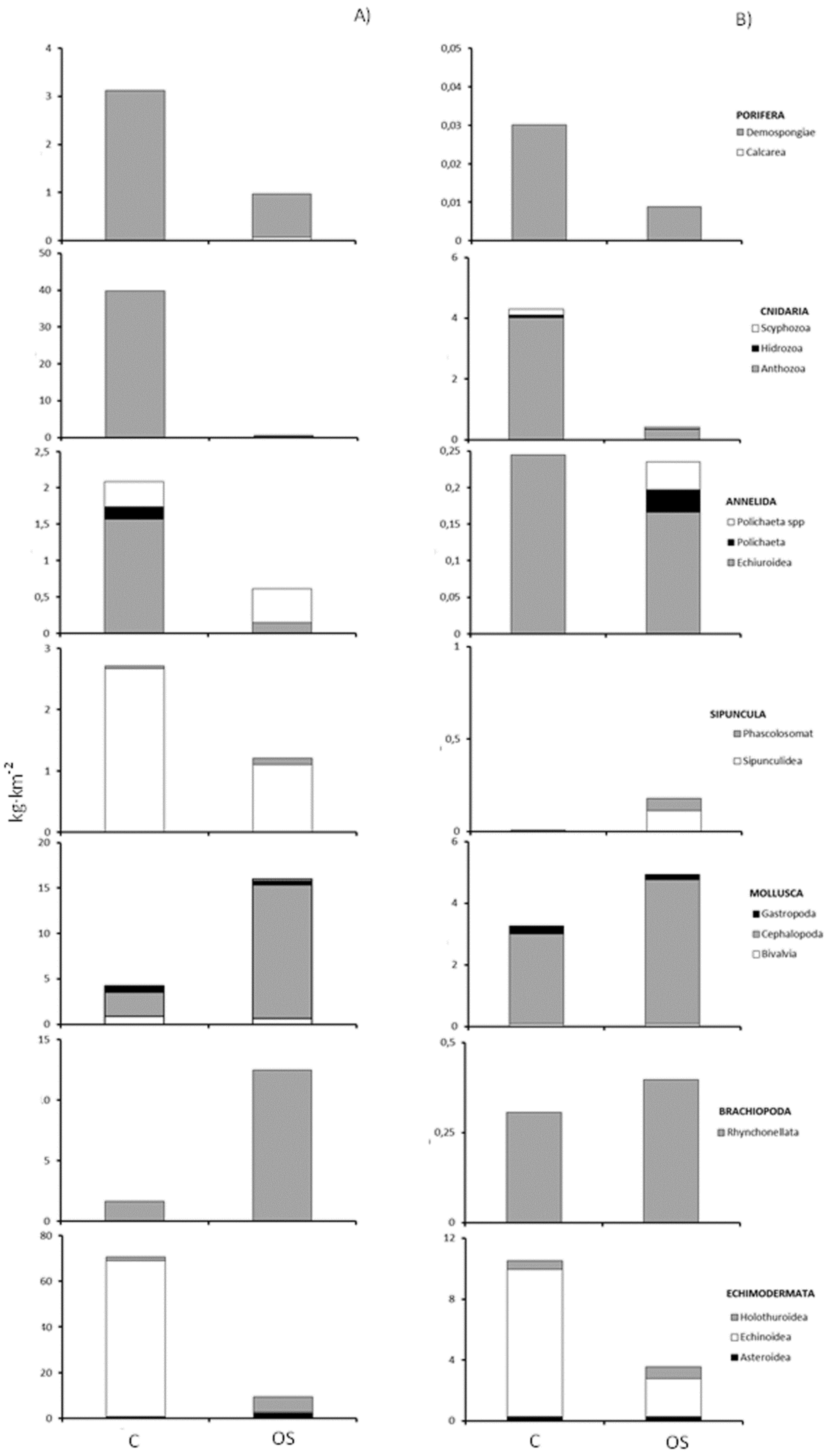


Figure 12. Biomass by Class for A) Agassiz trawl and B) OTMS in canyon (C) and open slope (OS) areas.

it more than three times. Some examples were the cold water corals *Lophelia pertusa* (Linnaeus, 1758) and *M. oculata*, the gastropoda *Pagodula echinata* (Kiener, 1840), and the irregular echinoid *G. elegans.*

Species accumulation plots for open slope and submarine canyons (Fig. 13) shows that neither habitat has been sampled fully (as you add samples, more species are found), but the open slope is better sampled than the canyons.

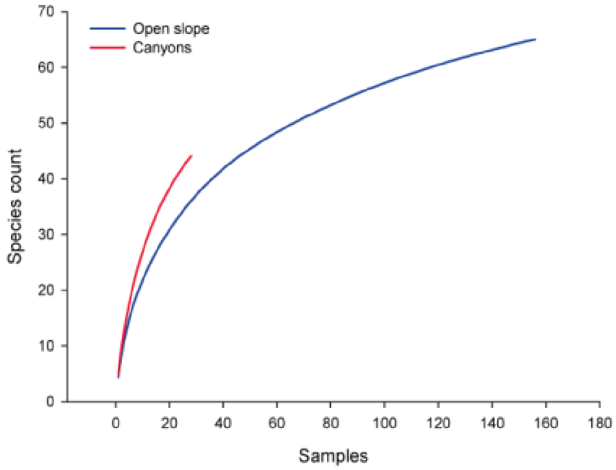


Figure 13. Species accumulation plots for open slope (blue) and submarine canyons (red) in the western Mediterranean.

Discussion

In this work, new faunistic and population characteristic records were provided for the deep-sea north-western Mediterranean Sea, highlighting the presence of 3 species considered as “rare”. Taking into account that the presence of these species was not punctual in the catches, that result point out their potential abundant presence in certain zones of the Mediterranean continental margin (Wheeler 2007; Fontaine et al. 2012). The lack of their previous report could be due to different factors, for example the weak sampling effort carried out in several deep-sea zones or alternatively, to the lack of taxonomic expertise in classifying correctly the specimens (Godfray 2002; Wheeler 2007).

In the same scenario, we can place our new findings about the deeper bathymetric expansion of previously reported species in our area and in the whole Mediterranean basin. For these reason we considered as a primary target the description of a detailed list of species in order to study faunistic communities.

A clear difference was observed in faunistic distributions and composition related to the employed trawl gear. That phenomenon has been already reported (Tecchio et al. 2011b), but to our best knowledge, no author provided such type of evidences for non-crustacean invertebrates in the north-western Mediterranean. Most published data in fact refer to otter benthic trawls (e.g. OTMS, Sardà et al., 1998) and no information is available for Agassiz sampling in the north-western Mediterranean Sea. We observed that densities and biomasses compiled in both gears were totally different, not only at faunistic composition level but also in the reported order of magnitude of density and biomass. OTMS sampling, generally captured higher number of benthopelagic species (i.e. Class Cephalopoda and Scyphozoa), while Agassiz presented a majority of strictly benthic species. Patterns of distribution between densities and biomasses were more accurately pictured with Agassiz trawl that collected more equally all benthonic phyla, with sampled species showing similar patterns of density and biomass. All these observations indicate that the sampling using the Agassiz trawling allows achieving a more trustful view of benthic non-crustacean invertebrate communities (Billett et al. 2010).

Present study highlighted the occurrence of higher densities of non-crustacean invertebrate below 1500 mdepth, mainly observed on Agassiz samples (i.e. a maximum peak of density and biomass at 1500 m; see Figure 2). This densities pattern were evidenced in studies analyzing all fractions of the megafauna such as fishes and crustaceans (Tecchio et al. 2011a; Tecchio et al. 2013) or other non-crustacean invertebrates (Ramirez-Llodra et al. 2008; Cartes et al. 2009). This discrepancy could be due to the differences in sampling methods. The depths of low density of non-crustacean benthic invertebrates coincide with depths were fishing activity is reported. As has been proved, (Martín et al. 2008; Clark and Rowden 2009) abundances and biomasses reported in fished areas were usually lower than non-fished areas. Our results suggest that this same pattern could be affecting the north-western Mediterranean Sea.

The most abundant species of non-crustacean invertebrates were reported at the deepest sampling areas of this study (from 1500 m down to 2250 m depth). A decreasing trend with depth, coinciding with a decrease of biodiversity indexes has been reported. This biodiversity diminution with depth has been suggested before in the same area of the north-western Mediterranean Sea (Tecchio et al. 2013). This was in accordance with an increasing number of patching and aggregative species with depth, explaining higher densities and lower number of species. This occurred with several species of the Phyla Echinodermata as *Pseudostichopus occultatus* and *Brissopsis lyrifera*. The high numbers of *G. vitreus* at 1750 m and 2250 m depth, coincide with an elevated quantity of “clinker” (burnt residue of charcoal from steam ships), that provides an ideal solid substrate for this species (Ramirez-Llodra et al. 2013). All the other Phyla presented maximum densities and biomasses at shallower depth (from 400 m to 1500 m depth). This phenomenon was more accentuated with samples caught with OTMS, where an elevated presence of Echinodermata and Brachiopoda in one single sampling depth was found. Patterns of biomass with depth for Agassiz trawl samples were in accordance with densities, but not for OTMS samples. For these samples, the depth pattern was deeply influenced by the depth distribution of Class Cephalopoda.

Bathymetrical pattern of density and biomass were highly influenced by canyon samples. Maximum values of density and biomass were reported inside canyon areas at 1200 m and 1500 m depth, mostly composed by echinoderms and cold water corals. Faunistic composition was also different inside and outside canyons, as commonly found in the Atlantic and Pacific Ocean and for some Mediterranean areas (Gili et al. 1999; Billett 2006; Duffy et al. 2013). This could be explained by the geomorphological and physic characteristics of canyon zones (Arzola et al. 2008; Masson et al. 2011).

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First records, rediscovery and compilation of deep-sea echinoderms in the middle and lower continental slope in the Mediterranean Sea

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Resumen

El presente estudio recopila toda la información disponible sobre equinodermos batiales del talud medio e inferior del Mar Mediterráneo con el fin de proporcionar una fuente unificada de información para la taxonomía de este grupo. La información bibliográfica recogida fue implementada con nuevos datos provenientes de 223 pescas de arrastre de 11 campañas oceanográficas realizadas en el Noroeste Mediterráneo entre 800 m y 2845 m de profundidad. Los nombres taxonómicos válidos, rangos batimétricos y distribuciones geográficas se recogieron para todas la especies. Los nuevos datos describen, por primera vez, la presencia del equinoideo Atlántico *Gracilechinus elegans* (Düben and Koren, 1844) en el Mar Mediterráneo. Asimismo señalan la presencia de las holoturias endémicas *Hedingia mediterranea* (Bartolini Baldelli, 1914) muestreada con anterioridad únicamente en 1914 en el Mar Tirreno y *Penilpidia ludwigi* (von Marenzeller, 1893), señalada únicamente en tres ocasiones, dos en el Mar Egeo y una en el Mar Balear. Adicionalmente se ampliaron los máximos de distribución batimétrica para cuatro especies: el asteroideo *Ceramaster grenadensis* (Perrier, 1881) hasta 2845 m; el equinoideo *Brissopsis lyrifera* (Forbes, 1841) hasta 2250 m; y las holoturias *Hedingia mediterranea* y *Holothuria (Panningothuria) forskali* Delle Chiaje, 1823, hasta 1500 m y 850 m, respectivamente.

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First records, rediscovery and compilation of deep-sea echinoderms in the middle and lower continental slope of the Mediterranean Sea

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Summary: This study provides a compilation of all available information on deep-sea echinoderms from the middle and lower slopes of the Mediterranean Sea, with the aim of providing a unified source of information on the taxonomy of this group. Previous records of species are updated with new data obtained from 223 trawl hauls conducted in 11 cruises within the northwestern Mediterranean Sea between 800 m and 2845 m depth. Valid names, bathymetric ranges and geographic distributions are given for all species. The new data report, for the first time, the presence of the Atlantic echinoid *Gracilechinus elegans* (Düben and Koren, 1844) in the Mediterranean Sea. We also report the presence of the endemic holothurians *Hedingia mediterranea* (Bartolini Baldelli, 1914), dredged only once previously in 1914 in the Tyrrhenian Sea, and *Penilpidia ludwigi* (von Marenzeller, 1893), known previously only from three samples, two in the Aegean Sea and one in the Balearic Sea. Additionally, the deeper limits of the bathymetric distribution of four species have been expanded: the asteroid *Ceramaster grenadensis* (Perrier, 1881) to 2845 m; the echinoid *Brissopsis lyrifera* (Forbes, 1841) to 2250 m; and the holothurians *Hedingia mediterranea* and *Holothuria* (*Panningothuria*) *forskali* Delle Chiaje, 1823, to 1500 m and 850 m, respectively.

Keywords: deep-sea echinoderms; Mediterranean Sea; *Gracilechinus elegans*; submarine canyons; taxonomy; bathymetric range.

Primera cita, redescubrimiento y recopilación de los equinodermos de profundidad en el talud continental medio e inferior del Mediterráneo

Resumen: Este estudio presenta una recopilación de toda la información disponible sobre los equinodermos de profundidad en el talud continental medio e inferior del mar Mediterráneo, con el fin de proporcionar una fuente de información unificada sobre la taxonomía de este grupo. Se han actualizado los registros anteriores mediante nuevos datos provenientes de 223 pescas de arrastre de 11 campañas oceanográficas realizadas en el noroeste Mediterráneo entre 800 y 2845 m de profundidad. Se ha actualizado el nombre de las especies, sus rangos batimétricos y sus distribuciones geográficas. Los nuevos datos presentan, por primera vez, la presencia del equinoideo Atlántico *Gracilechinus elegans* (Düben and Koren, 1844) en el mar Mediterráneo. También se cita la presencia en el noroeste Mediterráneo de dos especies de holoturias endémicas del Mediterráneo, *Hedingia mediterranea* (Bartolini Baldelli, 1914), muestreada una única vez en 1914 en el mar Tirreno, y *Penilpidia ludwigi* (von Marenzeller, 1893), muestreada tres veces, dos en el mar Egeo y una en el mar Balear. Además se expanden los límites de distribución batimétrica para cuatro especies: el asteroideo *Ceramaster grenadensis* (Perrier, 1881) hasta 2845 m; el equinoideo *Brissopsis lyrifera* (Forbes, 1841) hasta los 2250 m; y las holoturias *Hedingia mediterranea* y *Holothuria* (*Panningothuria*) *forskali* Delle Chiaje, 1823, hasta los 1500 m y 850 m respectivamente.

Palabras clave: equinodermos de profundidad; mar Mediterráneo; *Gracilechinus elegans*; cañones submarinos; taxonomía; rango batimétrico.

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INTRODUCTION

The deep Mediterranean Sea has a wide variety of geological and ecological settings. Their faunal composition and local biodiversity are largely unknown (Danovaro et al. 2010). The western Mediterranean deep basin is no exception. It has a complex assemblage of markedly different habitats (Sardà et al. 2004), including sedimentary slopes, submarine canyons and seamounts (Company et al. 2012). The specific geomorphological characteristics of these habitats (e.g. the elevation of seamounts, the walls and axes of the submarine canyons and the inclination of the continental slopes) and associated abiotic processes (e.g. variation in oceanographic currents, hard vs. soft substratum and food availability) result in large-scale heterogeneity of the continental margin seafloor (Carpine 1970, Emig 1997, D'Onghia et al. 2003). This high habitat heterogeneity plays a major role in the establishment and maintenance of diverse faunal communities (Levin et al. 2010), which, to date, are still largely unexplored in the deep Mediterranean Sea (Bienhold et al. 2013, Mecho et al. 2014).

The shallow Mediterranean marine fauna inhabiting the shelf and upper slope areas have been studied since ancient times. Consequently, they are relatively well known at many levels (taxonomic, ecological, and biological) (Riedl 1986, Bolam et al. 2002, Danovaro and Pusceddu 2007, Coll et al. 2010). Nevertheless, because of the difficulties in sampling the deep sea, the bathyal and abyssal fauna of the Mediterranean Sea remains poorly studied (Pérès and Picard 1956a, Fredj 1974, Galil and Goren 1995, Danovaro et al. 2010, Tecchio et al. 2011a,b).

The description of the benthic fauna occurring deeper than 800 m in the Mediterranean started in the 19th century. Cruises carried out by the RN *Washington* (1881-1882) and SMS *Pola* (1890-1898) provided the first extensive descriptions of bathyal and abyssal Mediterranean fauna (Marenzeller 1893, Bartolini Baldelli 1914), including many new species of non-crustacean invertebrates. From the late 1920s to the 1960s the number of deep-sea Mediterranean research cruises decreased, resulting in limited new information (Pérès and Picard 1956a,b, Pérès 1958). Since the late 1970s, improvements in sampling methods and equipment have allowed a second period of deep-sea scientific exploration and investigation below 1000 m depth, conducted by ships such as the *Bambu, Mango*, and *Ruth Ann* in Italian waters, the RV *Jean Charcot* in the Alboran Sea, and the RV *Garcia del Cid* in the Balearic Sea.

Specimens collected by these expeditions have stimulated a number of publications and new records of species (Carpine 1970, Parenzan 1970, Reyss 1971, Fredj 1974). However, most of this deep-sea literature focuses on the dominant groups such as fishes and crustaceans, the commercial use of Mediterranean marine resources, and the management of these resources (Sardà et al. 1994, 2004, Moranta et al. 1998, Company et al. 2004, Aguzzi et al. 2009, Bahamon et al. 2009). Thus, both fish and crustaceans are well known taxonomically in comparison with other megafaunal groups, such as ascidians, sponges, echinoderms, sipunculans and echiurans (Monniot and Monniot 1975, Alvà 1987a, Uriz and Rosell 1990, Villanueva 1992, Pancucci-Papadopoulou et al. 1999, Quetglas et al. 2000).

In this context, Mediterranean Echinodermata from middle and lower slopes have been poorly studied, particularly in comparison with the Atlantic Ocean, where echinoderms are important in terms of abundance, biomass and ecosystem function (Billett 1991). The large number of investigations conducted in the Atlantic Ocean have resulted in a good taxonomic knowledge of the echinoderms (Mortensen 1903, 1927, 1943, Koehler 1921, 1927, Hérouard 1923, Hyman 1955, Sibuet 1979, Borrero Perez et al. 2003, among others). In contrast, there have only been a few studies on the taxonomy of Mediterranean deep-sea echinoderms (Marenzeller 1893, Bartolini Baldelli 1914, Tortonese 1954, 1965, Sibuet 1974, Alvà 1987b). Most reports provide only species lists; morphological descriptions are of secondary importance (Cherbonnier and Guille 1967, Alvà 1987a, 1991, Koukouras et al. 2007) or totally absent (Tortonese 1958, 1972, 1979, Pérez-Ruzafa and López-Ibor 1988, Rinelli 1998, Coll et al. 2010).

It is in this context of dispersed and relatively scarce information that we have undertaken a study of all bathyal echinoderms, including samples collected in the last five years in the northwestern Mediterranean in the framework of four different projects. New records of species and their bathymetric distributions have been added to provide a thorough review of existing data and an updated account of the taxonomy, geographical and bathymetrical distribution of bathyal echinoderms in the Mediterranean Sea.

MATERIALS AND METHODS

New echinoderm samples

Ten oceanographic cruises were conducted between October 2008 and April 2013 to sample the deep seafloor of the western Mediterranean Sea. The sampling areas included the Blanes Canyon and its adjacent open slope, the Palamós Canyon (also named La Fonera) and the Cap de Creus Canyon (Fig. 1). These cruises took place in the framework of three Spanish research projects (PROMETEO, DOSMARES, and PROMARES) sampling at depths between 850 and 2845 m. Additionally, a trans-Mediterranean cruise took place in the context of the European project BIOFUN (EuroDEEP Eurocores, European Science Foundation) in July 2009. This cruise sampled the western, central and eastern Mediterranean basins at 1200, 2000 and 3000 m depth. In addition, a 4000-m depth station was sampled in the central basin. However, because of the low number of echinoderms collected in the central and eastern basins (n=2), only the western Mediterranean samples were used in the present study (Fig. 1).

A total of 223 deployments were completed (Table 1), resulting in a total swept area of 10.3 km². Of these

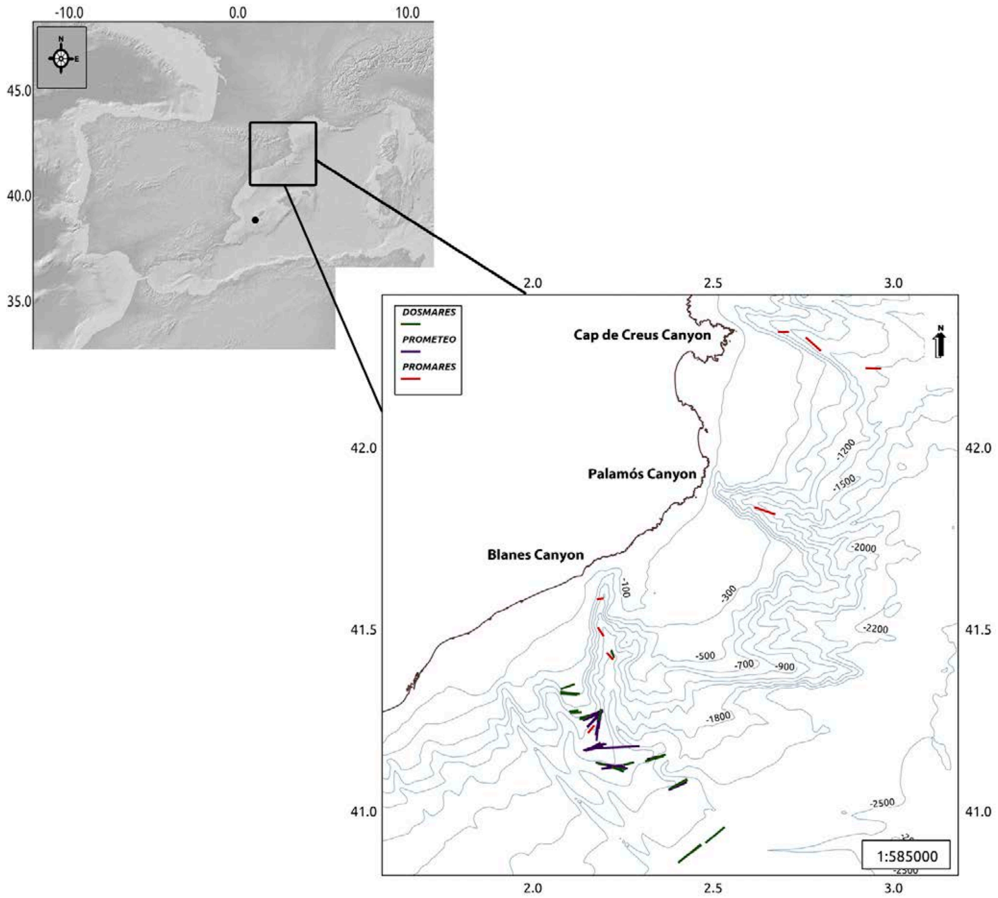

Fig. 1. – Study area. Areas sampled on the cruises PROMETEO, DOSMARES and PROMARES to the Blanes Canyon, Palamós Canyon, Cap de Creus Canyon and the adjacent open slope.

hauls, 119 samples were obtained by a single-warp otter-trawl Maireta system (OTMS, Sardà et al. 1998) with a net length of 25 m and a cod-end mesh size of 40 mm. A SCANMAR system was used to estimate the width of the net mouth. An average horizontal opening of 12.7±1.4 m was calculated. As the SCANMAR system can only operate down to 1200 m depth, the same value for the net mouth width was used also for deployments deeper than 1200 m. The height of the trawl mouth was estimated to be 1.4 m (Sardà et al. 1998). In addition, 49 hauls were conducted with an Agassiz dredge made of a square steel frame with a mouth width of 2.5 m and a mouth height of 1.2 m, and fitted with a 12-mm mesh net. Further, 55 samples were obtained with an epibenthic sledge, which consisted of a rectangular steel frame with three nets attached at different heights (10-50 cm, 55-95 cm and 100-140 cm above the bottom) with a mesh size of 300 μm (only one epibenthic sledge sample contained echinoderms).

Faunal samples were also obtained from 15 bottles in five different sediment traps deployed in the Blanes Canyon axis from November 2008 to February 2009, four of them at 1200 m and one at 1500 m depth. All were deployed at 22 m above the bottom.

Table 1. – Number of benthic trawls and dredges used in the present study by depth and geomorphological area. Canyon area (including La Fonera, Cap de Creus and Blanes canyons). A.C, Agassizz trawl sampled on Canyon area; OTMS.C, otter-trawl Maireta system sampled on Canyon area; ES.C, epibenthic sledge sampled on Canyon area; A.O.S, Agassizz trawl sampled on open slope; OTMS.O.S, otter-trawl Maireta system sampled on open slope; ES.O.S, epibenthic sledge sampled on open slope.

Depth		Canyon			Open slope		Total
	A.C	OTMS.C	ES.C	A.O.S	OTMS.O.S	ES.O.S	
850	1	1	0	0	0	0	2
900	4	1	2	7	18	7	39
1050	0	0	0	5	10	5	20
1200	2	0	1	9	21	10	43
1350	0	0	0	3	11	5	19
1500	6	8	3	5	18	11	51
1750	0	0	1	2	8	3	14
2000	0	0	1	2	12	3	18
2250	1	2	1	1	4	2	11
2850	0	0	0	1	5	0	6
Total	14	12	9	35	107	46	223

Table 2. – Echinoderms sampled in the present study from the deep Mediterranean Sea. * 19 specimens of *P. ludwigi* were collected in sediment trap samples in the Blanes Canyon.

Species	N. sampled Open slope	 Canyon	N total	Depth of occurrence (m)
ASTEROIDEA				
Ceramaster grenadensis (Perrier, 1881)	146	3	149	850-2845
Hymenodiscus coronata (G.O. Sars, 1872)	31	16	47	1500-2250
ECHINOIDEA				
Gracilechinus elegans (Danielssen and Koren, 1883)	0	7	7	1500
Brissopsis lyrifera (Forbes, 1841)	5	482	487	900-2250
HOLOTHUROIDEA				
Mesothuria (Allantis) intestinalis (Ascanius, 1805) Östergren, 1896	52	4	56	900-1750
Pseudostichopus occultatus von Marenzeller 1893	474	0	474	2000-2250
Holothuria (Panningothuria) forskali Delle Chiaje, 1823	0	1	1	850
Molpadia musculus Risso, 1826	25	0	25	900-1050
Hedingia mediterranea (Bartolini Baldelli, 1914) Tortonese, 1965	1	10	11	900-1500
Penilpidia ludwigi (von Marenzeller, 1893)	200	19*	219	900 -1500
Ypsilothuria bitentaculata (Ludwig, 1893)	27	0	27	900-1350
Total number of echinoderms collected	961	542	1503	850-2845

Finally, video-observations were made during the PROMARES cruise using the remotely operated vehicle (ROV) *Liropus 2000.* Thirty six video transects were conducted along the axes of the Blanes, Palamós and Cap de Creus canyons between depths of 300 and 1800 m.

A total of 1503 individuals belonging to 11 species were sampled (Table 2). Of these, 196 were asteroids, 494 echinoids and 813 holothurians. The classes Crinoidea and Ophiuroidea were absent from all samples.

Specimen identification

The echinoderms were sorted, weighed, counted and fixed with 40% formalin diluted with seawater and neutralized with borax on board ship. After 30 days, the samples were transferred to 70% alcohol in the laboratory for further examination. Some specimens were fixed in absolute ethanol on board to allow for molecular analyses (not included in this study). All specimens are stored in the Biological Reference Collection of the Institute of Marine Science, Barcelona (Spain).

In the laboratory, all specimens were classified to species level. For microscopic examination of holothurian spicules, small pieces of soft tissue (i.e. skin, tentacles and gonads) were dissolved in bleach solution and mounted on glass slides for identification. The taxonomic results were compared with previous taxonomic studies. The nomenclature was checked against the World Register of Marine Species (WoRMS). The identification of the echinoid *Gracilechinus elegans* (Düben and Koren, 1844) was based on taxonomic descriptions from the Atlantic Ocean (Mortensen 1903, 1927, 1943, Koehler 1927, Minin 2012). This species has not been cited previously in the Mediterranean Sea. Its geographic distribution was compared with data in the Atlantic Ocean and other echinoid records from the Mediterranean Sea.

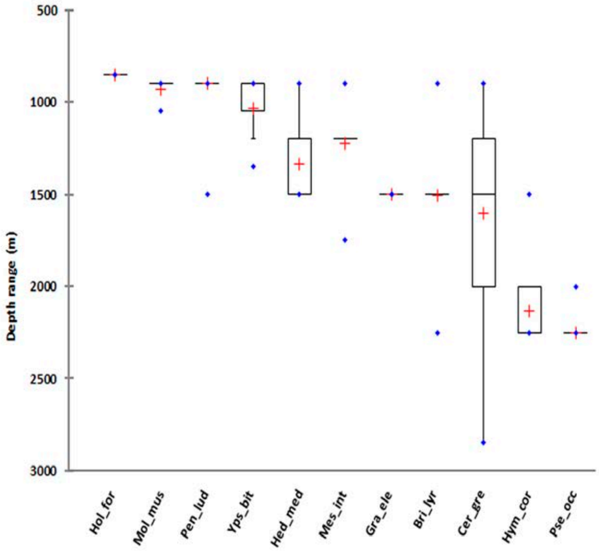

Fig. 2. – Bathymetric distribution and densities of echinoderms sampled in present study. The top and bottom of each box-plot represent 75% (upper quartile) and 25% (lower quartile) of all values, respectively. The horizontal line is the median. The ends of the whiskers represent the 10th and 90th percentiles. Cross marks represent means and blue spots maximum and minimum depth of occurrence. Species codes: Hol_for, *Holothuria* (*Panningothuria*) *forskali*; Mol_mus, *Molpadia musculus*; Pen_lud, *Penilpidia ludwigi*; Yps_bit, *Ypsilothuria bitentaculata*; Hed_med, *Hedingia mediterranea*; Mes_int, *Mesothuria* (*Allantis*) *intestinalis*; Gra_ele, *Gracilechinus elegans*; Bri_lyr, *Brissopsis lyrifera*; Cer_gre, *Ceramaster grenadensis*; Hym_cor, *Hymenodiscus coronata*; and Pse_occ, *Pseudostichopus occultatus.*

Synthesis of taxonomic information on deep-sea Mediterranean echinoderms

A comprehensive table was created of all the echinoderms present in the Mediterranean Sea and cited in the literature as having a maximum depth of occurrence below 800 m (see Table 3). This table was constructed based on Tortonese (1965) and Koukouras (2007). New data acquired during the PROMETEO, DOSMARES and PROMARES cruises was added (see above).

RESULTS

Class ASTERIODEA de Blainville, 1830

Two species of Asteroidea were collected in our study: *Ceramaster grenadensis* (Perrier, 1881) (n=149)

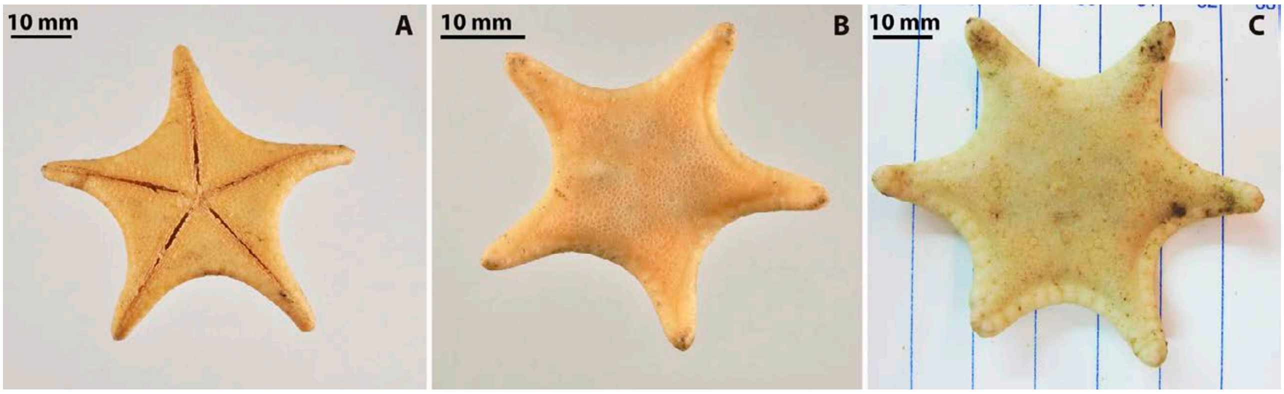

Fig. 3. – *Ceramaster grenadensis.* A, dorsal view; B, ventral view (Photo: A. Bozzano, ICM-CSIC); C, specimen with six arms.

and *Hymenodiscus coronata* (G.O. Sars, 1872) (n=47). Both are bathyal species. *Ceramaster grenadensis* sampled in the present study has a wide bathymetric range (850 to 2845 m, Fig. 2). The second species, *Hymenodiscus coronata* shows a narrower bathymetric range (1500 to 2250 m; Fig. 2).

Order VALVATIDA Perrier, 1884
Family GONIASTERIDAE Forbes, 1841
Genus *Ceramaster* Verrill, 1899
Ceramaster grenadensis (Perrier, 1881)
(Fig. 3)

Pentagonaster deplasi Perrier, 1885: 34.
Pentagonaster gosselini Perrier, 1885: 35.
Pentagonaster haesitans Perrier, 1885: 36.
Ceramaster grenadensis Halpern, 1970: 213-218, Figs. 8-9.

Material: 149 specimens collected during the PROMETEO 01-02-03-04-05, BIOFUN, PROMARES and DOSMARES 01-02-03-04 cruises. Depth of occurrence: from 850 to 2845 m. Zones: western Mediterranean Sea open slope, Blanes Canyon, Cap de Creus Canyon (Table 2).

Description: Shape pentagonal to stellate, very variable (Fig. 3A, B). Body flattened dorso-ventrally. Oral and aboral surface composed by more or less tabulate hexagonal plates covered by little granules. Marginal plates thick and massive, from 18 to 22; sampling methods could remove them. R=6 to 45 mm. r=3 to 25 mm. R/r=1.54 to 2.53. Colour variable, from cream, pale-yellow to pale pink. Polygonal madreporite, well defined, larger than surrounding plates. Adambulacral plate with 4 to 6 furrow spines, outside these a series of usually four club-shaped spines and outer spines similar to internal ones. Pedicellariae valvate, scarce on aboral side, larger and more numerous on oral side near ambulacral furrow. One of the specimens collected in the present study had six arms (Fig. 3C)

Distribution: Atlantic Ocean and Mediterranean Sea (Clark and Downey 1992).

New depth range: 200-2845 m (present study). The previous reported maximum depth of distribution for this species was 2500 m in the Atlantic Ocean (Clark and Downey 1992). The previous Mediterranean Sea bathymetric range was 600-2400 m (Tortonese 1972).

Remarks: Similarities were observed between the genus *Litonotaster* described by Halpern (1969, 1970). However, owing to 1) the absence of the characteristic flat and thin abactinial plates of the genus *Litonotaster*, and 2) the presence of tabulate abactinial plates covered by granules, the marginal plate disposition, and in agreement with available literature, we consider our specimens to be *Ceramaster grenadensis*. *Litonotaster* has not been reported in the Mediterranean Sea. Great intraspecific morphological variations have been signalled for *Ceramaster grenadensis* in the Mediterranean (Halpern 1970, Tortonese 1972, Sibuet 1974, Alvà 1987a). It is likely that a revision of the genus *Ceramaster* is needed.

Order BRISINGIDA Fisher, 1928
Family BRISINGIDAE G.O. Sars, 1875
Genus *Hymenodiscus* Perrier, 1884
Hymenodiscus coronata (G.O. Sars, 1872)
(Fig. 4)

Brisinga coronata Sars, 1873: 102
Brissingella coronata Tortonese, 1965: 194-196, Fig. 93.

Material: 47 specimens collected during cruises PROMETEO 05, BIOFUN and DOSMARES 01-02. Depth of occurrence: from 1500 to 2250 m. Zones: western Mediterranean Sea open slope and Blanes Canyon (Table 2).

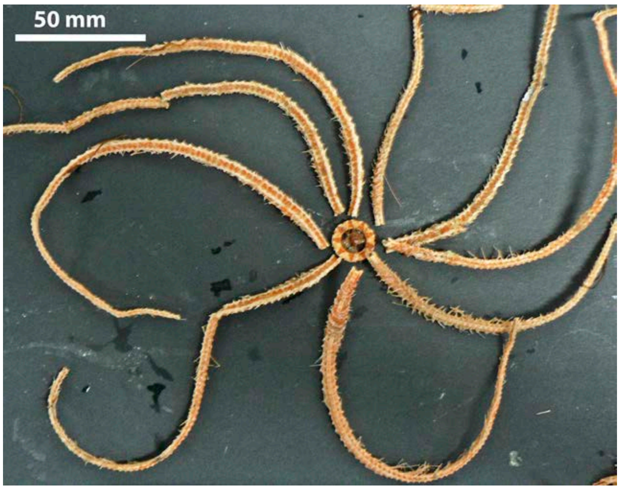

Fig. 4. – *Hymenodiscus coronata*.

Description: Diameter of disc 11 mm; from 9 to 13 long and slender arms. Colour orange to reddish. Very difficult to collect intact, usually the disc and the arms are broken and separate (Fig. 4). Madreporite large, channelled. Gonadal region slightly to highly inflated. Abactinal arm plates rod-like. Two to four tiny, acicular furrow spines and one to two moderately long subambulacral ones.

Distribution: North Atlantic and Mediterranean Sea (Alvà 1987a).

Depth range: 100-2904 m (Bartolini Baldelli 1914).

Remarks: Description taken from (Clark and Downey 1992).

Class ECHINOIDEA Leske, 1778

Only two sea urchin species were sampled: the regular echinoid *Gracilechinus elegans* (Düben and Koren, 1844) (n=7) and the irregular echinoid *Brissopsis lyrifera* (Forbes, 1841) (n=487). *Gracilechinus elegans*, known in the Atlantic, has been reported for the first time in the Mediterranean Sea in the present study. It was sampled in the Blanes Canyon at 1500 m depth (Fig. 2). Other specimens were observed and collected with the ROV during the PROMARES cruise (Mecho, pers. obs.) in the lower Palamós Canyon and Blanes Canyon areas (1500 m). *Brissopsis lyrifera* was found over a wide bathymetric range in the present study (from 900 to 2250 m; Fig. 2). It was abundant in some canyons between 900 and 1500 m (Table 2). In contrast, only five small specimens of *B. lyrifera* were collected on the open slope at depths between 1750 and 2250 m (Table 2).

Order CAMARODONTA Jackson, 1912
Family ECHINIDAE Gray, 1825
Genus *Gracilechinus* Fell and Pawson, in Moore, 1966
Gracilechinus elegans (Düben and Koren, 1844)
(Fig. 5)

Echinus elegans Düben and Koren, 1844: 272. Koehler, 1927: 51-53, pl. XII, Fig. 12 a-g; pl. XVII, Fig. 5

Material: 7 specimens from cruises PROMETEO 04, PROMARES and DOSMARES 04. Depth of occurrence: 1500 m. Zones: Blanes Canyon and Palamós Canyon (Table 2).

Description: Diameter test 38.5 to 48.3 mm; h=25.6 to 34.7mm. Test low, from conical and flattened above to slightly flattened on both sides, usually the height of the test is more than half the diameter (Fig. 5A). Colour whitish pink to pink, sometimes a few green (Fig. 5B, C). Long primary spines usually flat at the end. One primary tubercle present on each plate, forming a very regular series from oral to aboral side; usually secondary ones form a short longitudinal series from the middle to the oral side. A small tubercle is present between the pores and the primary tubercle, but not between the pores and the end of the plate. Some miliary tubercles are present, giving a rough appearance to the test. Three pairs of pores very clear and disposed in a sharp angle. The boundary between the areas was more straight than sinuous. Periproct (Fig. 5D) covered by large irregular plates, one of them with a spine. The plates surrounding the anus are irregularly club-shaped and smaller than the other plates. Ocular plates not in contact with the periproct. No spines on the buccal plates, where pedicellariae were present and abundant. Tridentate pedicellariae have the valves flat, narrow and mesh-worked, with the edge sinuate (500 to 650 µm long). In some cases small individuals had flatter valves than larger individuals (Fig. 5E). These valves have a narrow area near the base (Fig. 5F). Globiferous pedicellariae (500 to 550 µm) usually have 1 or 2 lateral teeth on either side of the blade and a more or less round to rectangular shape (Fig. 5G-I). Ophicephalus pedicellariae, broad, sinuate and with small teeth in the edge, and an intricate mesh-work.

Distribution: North Atlantic (OBIS). First record in the Mediterranean Sea.

Depth range: 50-1710 m. (Mortensen 1943, Minin 2012). Only reported at 1500 m depth in the Mediterranean Sea (present study).

Remarks: Mortensen (1903) reported this species from the Mediterranean, but he later discarded this identification (Mortensen 1943). Alvà (1987b) described another species, *Gracilechinus alexandri*, in the Mediterranean Sea. Both *G. elegans* and *G. alexandri* have many similar characteristics, making their true identification difficult (Mortensen 1903, Ramírez-Llodra and Tyler 2006, Minin 2012). Furthermore, juvenile *G. alexandri* have characteristics that might be confused with *G. elegans*. It is possible that the specimen of *G. alexandri* reported by Alvà (1987b) was a juvenile and was a misidentification of *G. elegans*. The specimen is no longer available for comparison. In our specimens, the presence of one or two teeth on the globiferous pedicellariae, their narrow base and their mesh-work are similar to those described in the literature (Mortensen 1903, Minin 2012). The tubercular pattern, the periproct, the shape of the ocular and genital plates and their disposition allowed us to classify these specimens as *G. elegans*. Mortensen (in 1903, p. 144, pl. XX, Fig. 9) found a small form for *G. elegans* with tridentate pedicellariae that had more flattened and truncate blades without mesh-work. This characteristic and the overlapping range in the number of teeth in the globiferous pedicellariae (1 to 4 in *G. elegans* and 2 to 5 in *G. alexandri*) could lead to a misidentification if only one individual was available, as appears to be the case in Alvà (1987b).

Order SPATANGOIDA Agassiz, 1840a
Family BRISSIDAE Gray, 1855
Genus *Brissopsis* Agassiz, 1847
Brissopsis lyrifera (Forbes, 1841)
(Fig. 6)

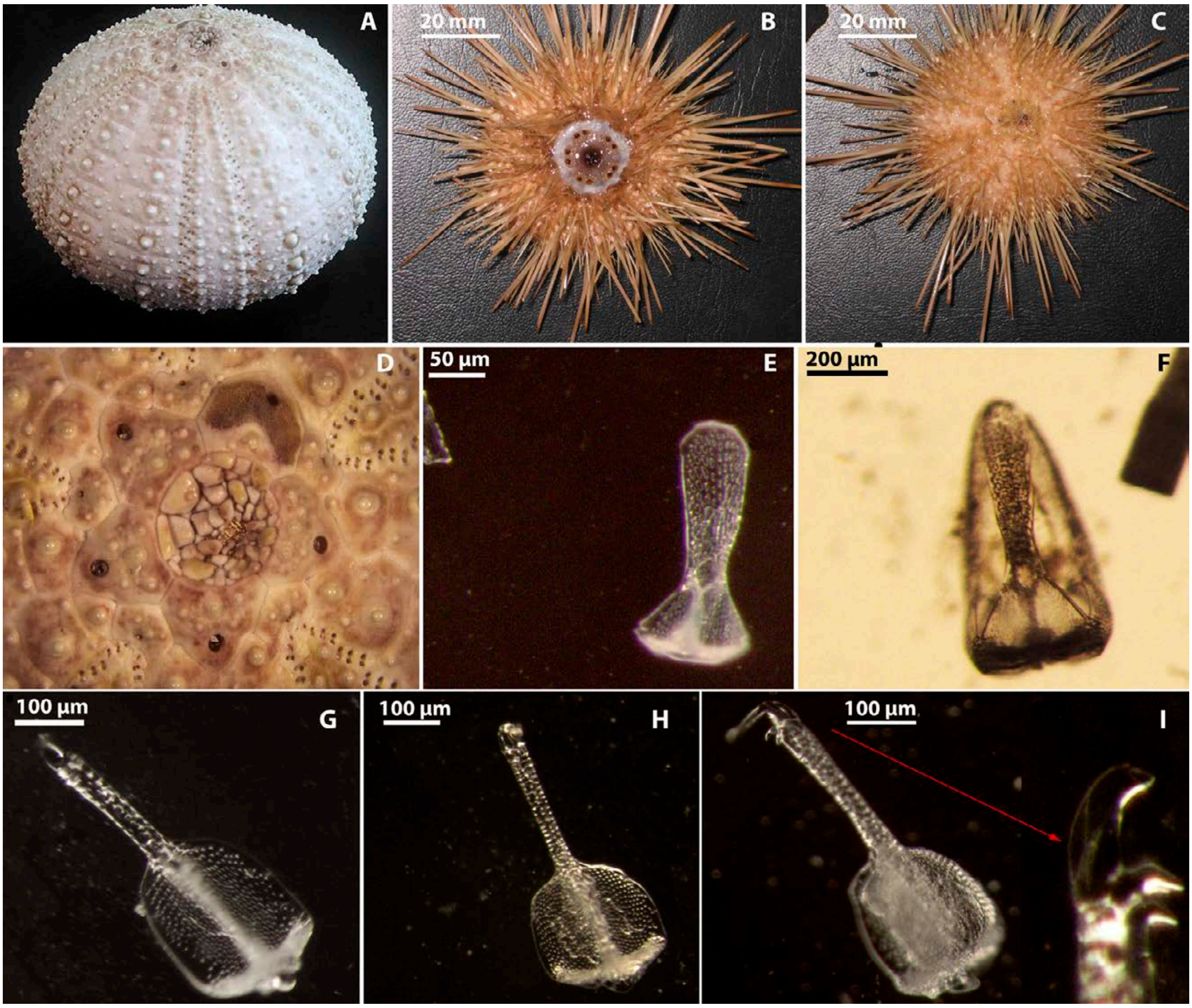

Fig. 5. – *Gracilechinus elegans*. A, test; B, oral view; C, aboral view; D, periproct structure; E, F, tridentate pedicellariae; G, H, globiferous pedicellariae; I, globiferous pedicellariae, detail of teeth.

Brissus lyrifeer Forbes, 1841: 187
Brissopsis lyrifera Tortonese 1965: 372-374

Material: 487 specimens from cruises PROMETEO 02-04-05, PROMARES and DOSMARES 01-03. Depth of occurrence: 900 to 2250 m. Zones: western Mediterranean Sea open slope, Blanes Canyon, Cap de Creus Canyon and Palamós Canyon (Table 2).

Description: Body oval, arched, sloping anteriorly. Colour from yellow to red-brown with a narrow band of ciliated dark spines which rings all five ambulacra petals on the upper surface (Fig. 6A, B). Anterior ambulacral zone slightly depressed. Periproct terminal, near aboral zone. Posterior petals shorter than the anterior ones, diverging and well separated. Globiferous pedicellariae short, ending in two long teeth. Tridentate pedicellariae of various forms, with three more or less leaf-shaped blades. Rostrate pedicellariae blade slender.

Distribution: Atlantic and Mediterranean Sea (OBIS).

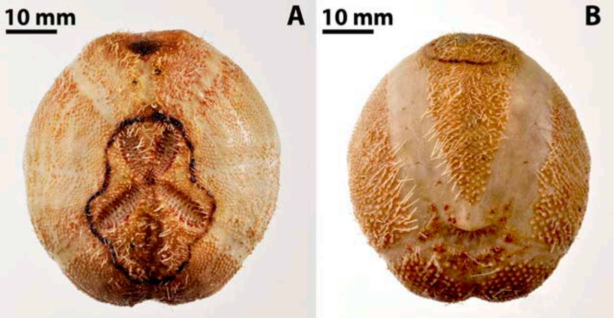

Fig. 6. – *Brissopsis lyrifera* A, oral view; B, ventral view (Photo from A. Bozzano).

New depth range: 200-2845 m (present study). The previous reported maximum depth of distribution for this species was 1650 m in the Atlantic Ocean (OBIS). Previous Mediterranean maximum depth was 1500 m (Tortonese 1965).

Remarks: Differences from *Brissopsis atlantica mediterranea* (Mortensen 1913) are evident in the posterior petals: diverging and well separated in *B. lyrifera* and confluent on the base, as opposed to nearly parallel in *B. atlantica mediterranea* (Lacour and Néraudeau 2000).

Class HOLOTHUROIDEA de Blainville, 1834

The Holothuroidea was the most abundant echinoderm class sampled in this study, with a total of 813 specimens and 7 species (Table 2). Three species belonging to the order Aspidochirotida were collected: *Mesothuria* (*Allantis*) *intestinalis,* (Ascanius, 1805) Östergren, 1896 (n=56), *Pseudostichopus occultatus,* Marenzeller 1893 (n=474) and *Holothuria* (*Panningothuria*) *forskali,* Delle Chiaje, 1823 (n=1).

In the present study, *Mesothuria intestinalis* had a bathymetric range between 900 and 1750 m depth (Fig. 2). In contrast *Pseudostichopus occultatus* had a very narrow depth range (2000 to 2250 m; Fig. 2). This species was sampled only in open slope areas. Although one individual was collected at 2250 m depth in the Blanes Canyon, we consider this as a residual sample, based on the high number of specimens collected in the previous catch, the bad condition of the specimen and the absence of this species in other trawls conducted at this depth in the canyon. This species was sampled in great numbers at 2250 m (maximum of 145 individuals) (Table 2). Only one individual of *H. forskali* was sampled (850 m in the Blanes Canyon).

The order Molpadiida was represented by two species: *Molpadia musculus,* Risso, 1826 (n=25) and *Hedingia mediterranea* (Bartolini Baldelli, 1914) Tortonese, 1965 (n=11). *Molpadia musculus* had a bathymetric range between 900 and 1050 m depth (Fig. 2) and was sampled only on the open slope. *Hedingia mediterranea* had a bathymetric range between 900 and 1500 m (Fig. 2) and was sampled mainly in canyon areas.

The order Elasipodida was represented by one species *Penilpidia ludwigi* (von Marenzeller, 1893) (n=219). The bathymetric distribution of this species ranged from 900 to 1500 m. Most of the individuals (n=200; Table 2) were sampled by the epibenthic sledge at a single open slope site in the western Mediterranean Sea at 900 m depth. A few individuals (n=19) were reported from sediment trap samples located in the Blanes Canyon at 1200 and 1500 m depth (Fig. 2).

The order Dactylochirotida was represented by a single species: *Ypsilothuria bitentaculata* (Ludwig, 1893) (n=27). This species was distributed in the present study between 900 and 1350 m depth (Fig. 2) and was sampled only in an open slope area (Table 2).

Order ASPIDOCHIROTIDA
Family Synallactidae Ludwig, 1894
Genus *Mesothuria* Ludwig, 1894
Subgenus *Allantis* Heding, 1942
Mesothuria* (*Allantis*) *intestinalis (Ascanius, 1805) Östergren, 1896
(Fig. 7)

Holothuria intestinalis Ascanius 1805: 5, pl. 45
Mesothuria intestinalis Gebruk 2012: 291-391, Fig.1-9C, D

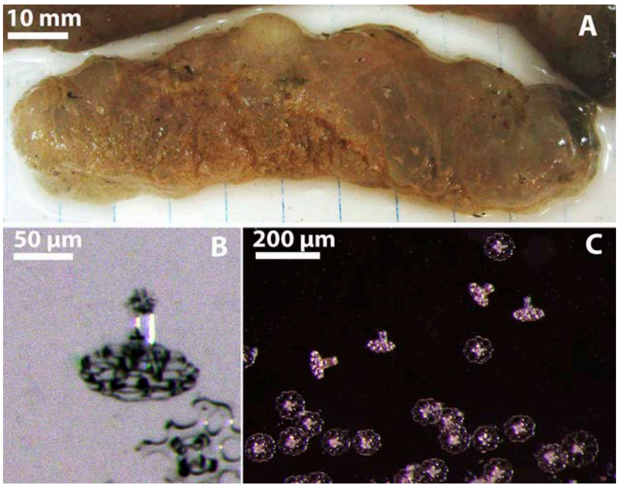

Fig. 7. – *Mesothuria* (*Allantis*) *intestinalis* characteristic. A, general view; B, ossicle crown with several thorns; C, ossicle plates with four rods and central spire.

Material: 56 specimens were collected during cruises PROMETEO 02-03-04-05, BIOFUN and PROMARES. Depth of occurrence: 900 to 1750 m. Zones: western Mediterranean Sea open slope, Blanes Canyon and Cap de Creus Canyon (Table 2).

Description: Large species, up to 30 cm long (Koehler 1927). Body nearly cylindrical with both ends flattened (Fig. 7A). Mouth subventral surrounded by 20 peltate tentacles. Scattered small tube feet all over the body, more abundant near the anterior and posterior ends. Dermis usually covered by shells, skin very fragile and thin in fresh specimens. On preservation, the dermis becomes thicker and more wrinkled. Characteristic ossicles are round tables (±100 µm), more or less regular with small peripheral holes around a central hole, and with central spire built by four rods, ending in a crown of several thorns (Perrier 1898) (Fig. 7B, C). Hermaphroditic species (Hyman 1955), gonads constituted by one branched tuft attached to left side of the dorsal mesentery, with some tubules male and some female, not found ripe at the same time (Mortensen 1927). Two respiratory trees, gelatinous and transparent. The species produces a substance which gels in formaldehyde and alcohol when preserved. Specimens usually eviscerate during capture.

Distribution: Mediterranean Sea, North Atlantic and West Indian seas (Gebruk et al. 2012).

Depth range: 18-2000 m (Gebruk et al. 2012). Mediterranean depth range 20 to 1927 m (Cartes et al. 2009).

Remarks: The presence of a second *Mesothuria* species of the genus in the Mediterranean Sea, *Mesothuria verrilli* (Théel, 1886), was discarded by Gebruk et al. (2012).

Genus *Pseudostichopus* Ludwig, 1894
Pseudostichopus occultatus Marenzeller 1893
(Fig. 8)

Pseudostichopus occultatus Marenzeller, 1893a: 15-17, pl. 4, Fig. 9. O'Loughlin, 2005: 173-174.

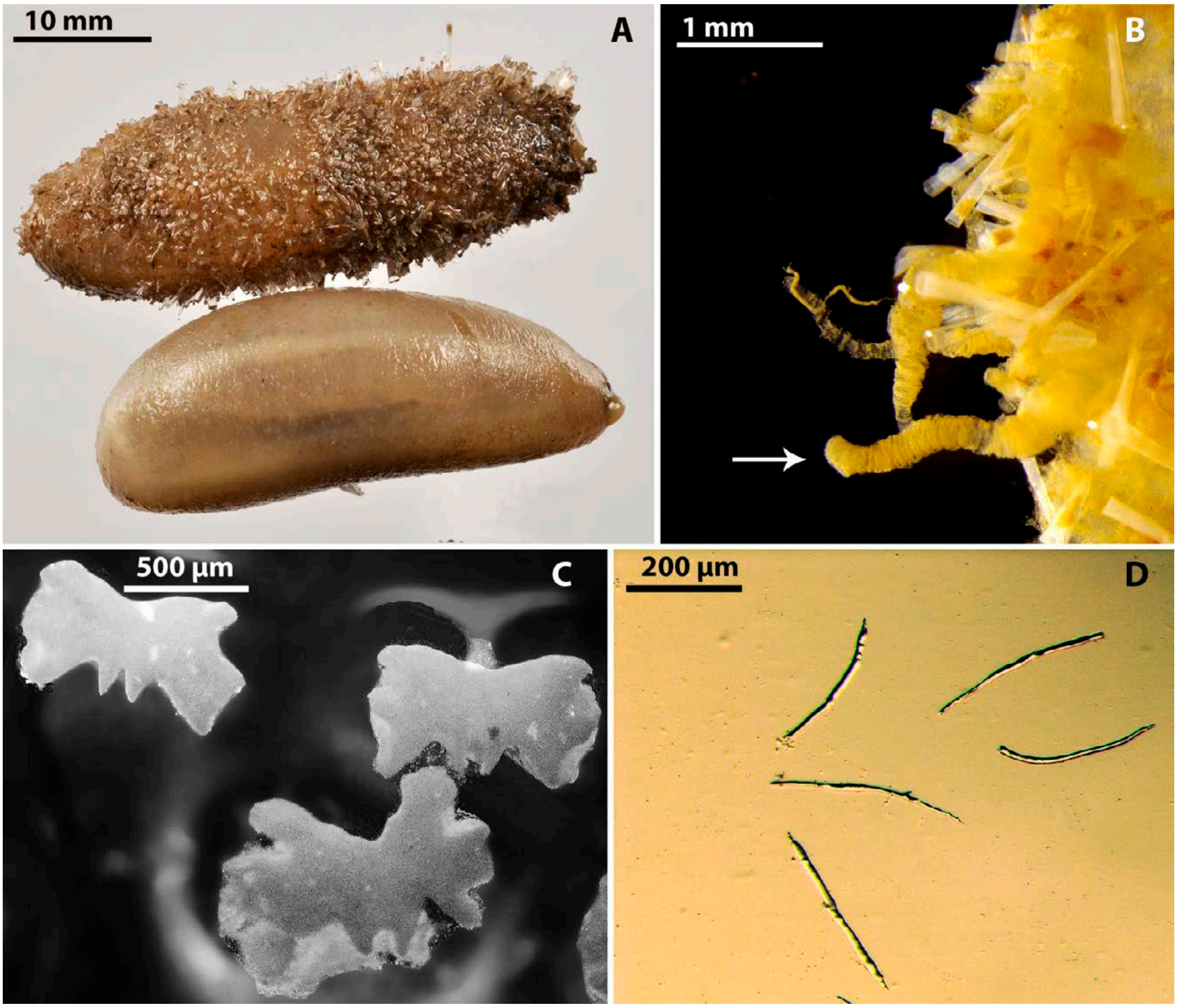

Fig. 8. – *Pseudostichopus occultatus* characteristics. A, general view, with and without pteropod cover (Photo from A. Bozzano); B, tube feet detail and encrusted pteropods; C, detached pieces of the calcareous ring; D, irregular spiny ossicles from respiratory trees and tentacles.

Material: 474 specimens collected from cruises DOSMARES 01-02-04. Depth of occurrence: 2000 and 2250 m. Zone: western Mediterranean Sea open slope (Table 2).

Description: Specimens smaller than 40 mm long; usually with pteropods and sand encrusted in the skin giving an external vitreous structure, colour dusty brown (Fig. 8A). Body dorsally convex, flat ventrally. The specimens sampled in this study do not have the pygal furrow which is generally characteristic of the group; some authors also note the absence of a pygal furrow in some specimens. Mouth subventral surrounded by 16-20 orange peltate tentacles, anus terminal. When the encrusted material is discarded the dermis is thin. The dorsolateral tube feet are sometimes difficult to see (Fig. 8B). Muscular bands cylindrical and subdivided, visible by transparency. Calcareous ring solid, radial plates with two central and lateral projections providing a ribbon-like shape to each plate (Fig. 8C). Two respiratory trees long and slim clustering along a central strap. Usually dredged in great numbers. Ossicles present in tentacles, tube feet, respiratory trees and near anus; absent in skin and gonads. Spiny rods (150 to 350 µm) (Fig. 8D) and scarce irregular, mesh-like perforate plates. Gonads in one tuft, with long unbranched tubules arising separately along gonoduct; one dissected specimen had little tufts full of eggs free in the coelom.

Distribution: Mediterranean Sea, North Atlantic (O'Loughlin and Ahearn 2005)

Depth range: 360-4400 m (Koehler 1927). Mediterranean depth range 415 to 3624 m (Bartolini Baldelli 1914).

Remarks: O'Loughlin (2002) reconsidered the genus *Pseudostichopus* and classified *P. occultatus* as *Meseres occultatus.* Later, (O'Loughlin and Ahearn 2005) returned this species to the genus *Pseudostichopus*. The colour of tentacles and internal structures shows great variability between individuals and is not suitable as a diagnostic character.

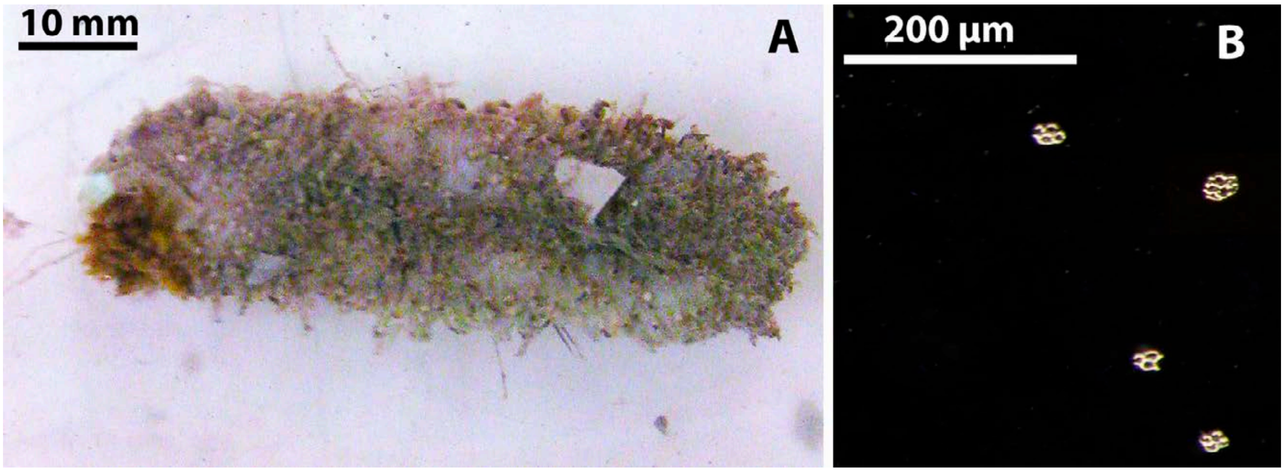

Fig. 9. – *Holothuria* (*Panningothuria*) *forskali* characteristics. A, general view; B, ossicles.

Family HOLOTHURIIDAE Ludwig, 1894
Genus *Holothuria* Linnaeus, 1767
Subgenus *Panningothuria* Rowe, 1969
Holothuria* (*Panningothuria*) *forskali Delle Chiaje, 1823
(Fig. 9)

Holothuria forskahli Delle Chiaje, 1824: 77-116, pl. 6-8. Tortonese, 1965: 64, Fig. 23

Material: 1 specimen from cruise PROMETEO 05. Depth of occurrence: 850 m. Zone: Blanes Canyon (Table 2).

Description: 60 mm long. Cylindrical body flattened ventrally (Fig. 9A). Numerous tube feet in three or four rows. Conical papillae on its dorsal surface. Subventral mouth with about 20 stumpy, branched tentacles. Calcareous deposits scarce, as small discs in skin (Fig. 9B) and branched and curved rods in tube feet and tentacles. Colour, usually black with white spots, sometimes brown with a yellow ventral side. Cuverian tubules are present.

Distribution: Mediterranean Sea and northeast Atlantic Ocean (Pérez Ruzafa et al. 1987).

New depth range: 20-850 m depth (present study). The previous maximum depth reported for this species in the Atlantic Ocean was 348 m (Pérez Ruzafa et al. 1987). The previous Mediterranean Sea maximum depth was 193 m (Pérez Ruzafa et al. 1987).

Remarks: The one small specimen collected had a pale grey-pinkish colour. Some authors (Koehler 1921, 1927, Tortonese 1965) described deeper specimens of *H. forskali* as pale in colour and smaller in body length compared with shallower individuals. O'Loughlin and Paulay (2007) describe a related species to *H. forskali*, living at greater depths (800 m) in Australian waters.

Order MOLPADIIDA
Family MOLPADIIDAE Müller, 1850
Genus *Molpadia* (Cuvier, 1817) Risso, 1826
Molpadia musculus Risso, 1826
(Fig. 10)

Molpadia musculus Risso, 1826: 293. Pawson, 2001: 317-318, Fig. 2A-B.

Material: 25 specimens collected during cruises PROMETEO 01-02-03-04-05, PROMARES and DOSMARES 04. Depth of occurrence: 900 and 1050 m. Zones: only present on western Mediterranean Sea open slope (Table 2).

Description: Up to 50 mm long. Sausage-shaped, with a small tail (Fig. 10A). Terminal, mouth surrounded by 15 pink digitate tentacles with two small prolongations (Fig. 10B). Skin rough and thick, coloured from grey to dark purple due to phosphatic deposits (Fig. 10A, B). Ossicle tables have few perforations and a small solid spine (500 to 700 µm). Rosette and racquet-shape plates and anchors present (Fig. 10C). Fusiform rods (±1000 µm) always present in tail, usually also on body wall (Fig. 10D). Calcareous ring with posterior bifurcate projections on radial plates. Two long and slender respiratory trees. Ossicles and body shape could vary, but fusiform rods of the tail are diagnostic. Colour varies with the age and growth of the animal. In the early stages they are grey-white and, when grown to the adult size, the colour turns darker from the accumulation of phosphatic deposits.

Distribution: Cosmopolitan (Pawson et al. 2001).

Depth range: 35-5205 m (Pawson et al. 2001). Mediterranean Sea depth range 50 to 2500 m (Parenzan 1970).

Remarks: In the Mediterranean Sea, the maximum depth of distribution for this species was 1050 m (Tortonese 1965, Sibuet 1974, Cartes et al. 2009, Ramírez-Llodra et al. 2010, present study). However, Parezan (1970, pp. 10 and 33) sampled ten *M. musculus* between 2300 and 2500 m, with the RV *Ruth Ann* in 1969 while dredging the Ionian Sea (central Mediterranean Sea).

Family CAUDINIDAE Heding, 1931
Genus *Hedingia* Deichmann, 1938
Hedingia mediterranea (Bartolini Baldelli, 1914) Tortonese, 1965
(Fig. 11)

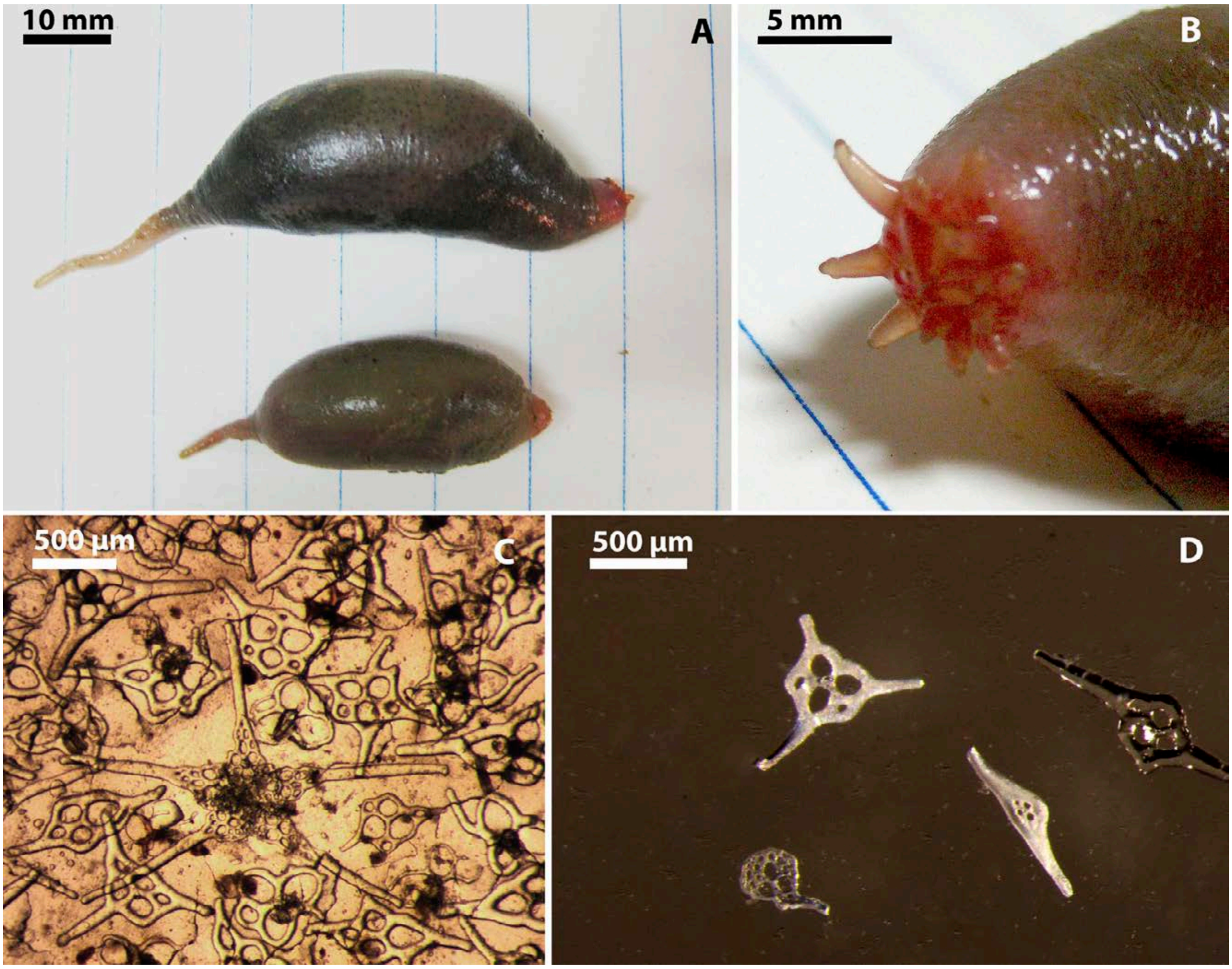

Fig. 10. – *Molpadia musculus* characteristics. A, general view; B, detail of the tentacles; C, rosettes and racquet-shaped ossicles with phosphatic deposits; D, fusiform rod ossicles from tail.

Trochostoma mediterraneum Bartolini Baldelli, 1914: 105-107, pl. 6 Figs. 9-10.
Hedingia mediterranea Tortonese 1965: 100-101, Fig. 43.

Material: 11 specimens collected from cruises PROMETEO 02-05 and PROMARES. Depth of occurrence: 900 to 1500 m. Zones: western Mediterranean Sea open slope and Blanes Canyon (Table 2).

Description: Fresh specimens pale violet or white, acquiring a yellowish white colouring when conserved (Fig. 11A, B). Body divided into two regions, an elongated body and a long caudal appendage (more than half the length of the body). Body oval, without podia. Rough skin due to calcareous plates. Anterior region wrinkled and cylindrical, with a terminal mouth. Skin without phosphatic deposits. Fifteen tentacles without digitations. Anus situated at the end of the caudal appendage. Five subdivided muscular bands visible by transparency. Ossicles very similar to *H. albicans*; tables (from 150 to nearly 250 µm) present all over the skin with very irregular holes and a central spine with three spiny columns (Fig. 11C -E). Smooth plates on anal papillae (Fig. 11F, G). Two respiratory trees (right and left), low-ramified and attached along the mesentery. Gonads long and unbranched tubules extending to the posterior end of the body, disposed in two tuffs attached to the mesentery on the upper part and free for the rest of their length in the coelom (Fig. 11H). Calcareous ring with five radial pieces, each with two posterior bifurcated projections and five interradial pieces (Fig. 11I, J). Tentacular ampullae long and digitate.

Distribution: Endemic from Mediterranean Sea, reported once on Tyrrhenian Sea. First citation in the western Mediterranean Sea.

Depth range: 800-1500 m (present study). The previous Mediterranean Sea depth range was 800 to 1000 m (Bartolini Baldelli 1914).

Remarks: Only one specimen has been reported previously in the Mediterranean Sea, dredged by RN *Washington* (1881-1882) in the Tyrrhenian Sea at 800-1000 m depth and described as *Trochostoma mediterraneum* by Bartolini Baldelli (1914). Later, Koehler (1927) classified the specimen as *Trochostoma articum*. Tortonese (1965) classified it definitively as *Hedingia mediterranea*. Pawson (2001) considered the specimen to be *Hedingia albicans* (Théel, 1886) Deichmann, 1938, and cited it in the Mediterranean. Molecular data are required for *Hedingia* species in order to resolve their taxonomic status.

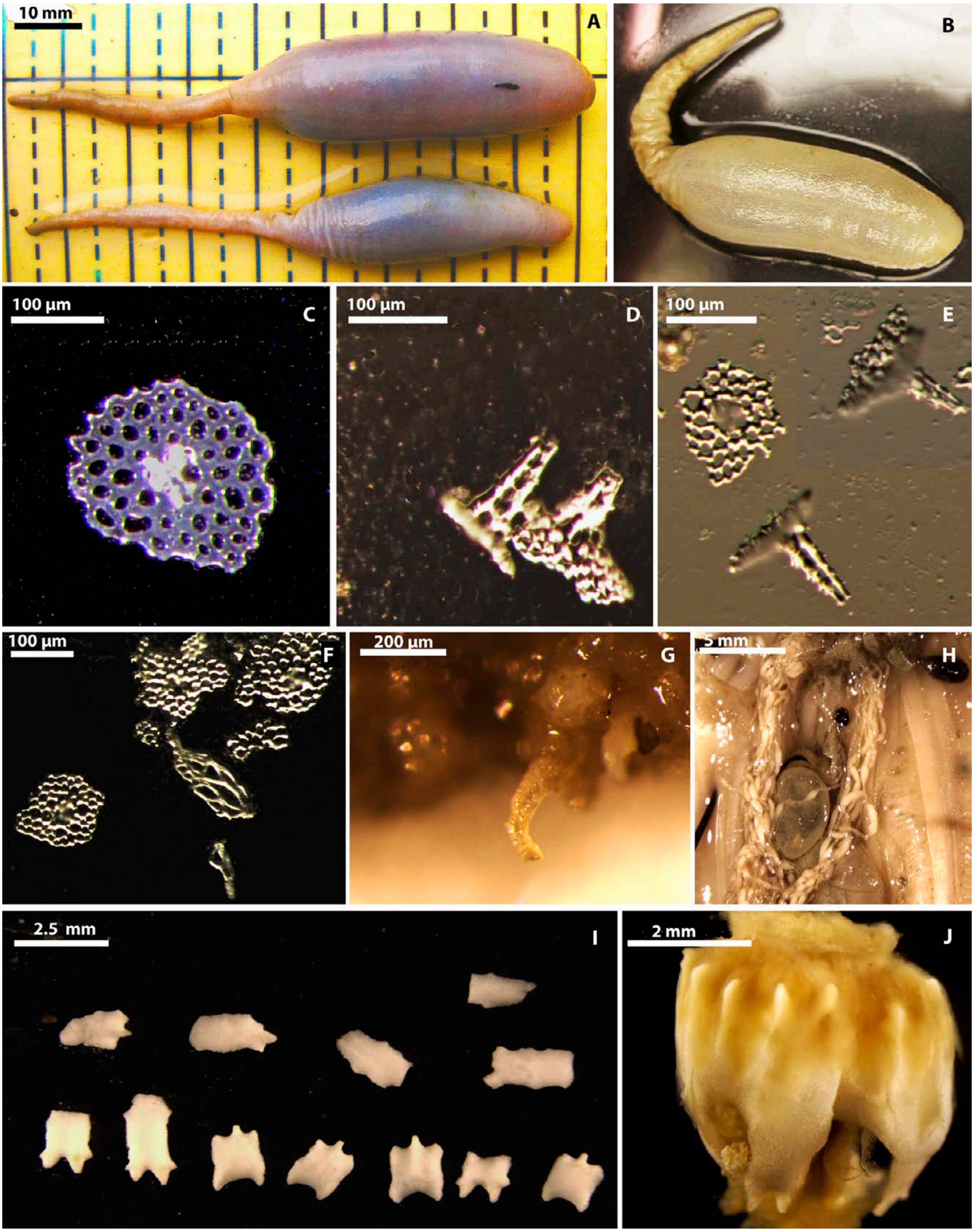

Fig. 11. – *Hedingia mediterranea* characteristics. A, B, external colour diversity; C, D, skin ossicles; E, detail of ossicles central spine; F, anal calcareous plates; G, anal papillae; H, gonadal tuffs and Polian vesicle; I, J, calcareous ring and detached pieces of calcareous ring.

Order ELASIPODIDA Théel, 1882
Family ELPIDIIDAE Théel, 1879
Genus *Penilpidia* Gebruk, 1988
Penilpidia ludwigi (von Marenzeller, 1893)

(Fig. 12A-G)

Kolga ludwigi Marenzeller, 1893: 20-23, pl. III Fig. 7, pl. IV Fig. 8.
Penilpidia ludwigi Gebruk, 2013: 1030-1032, Fig. 1.

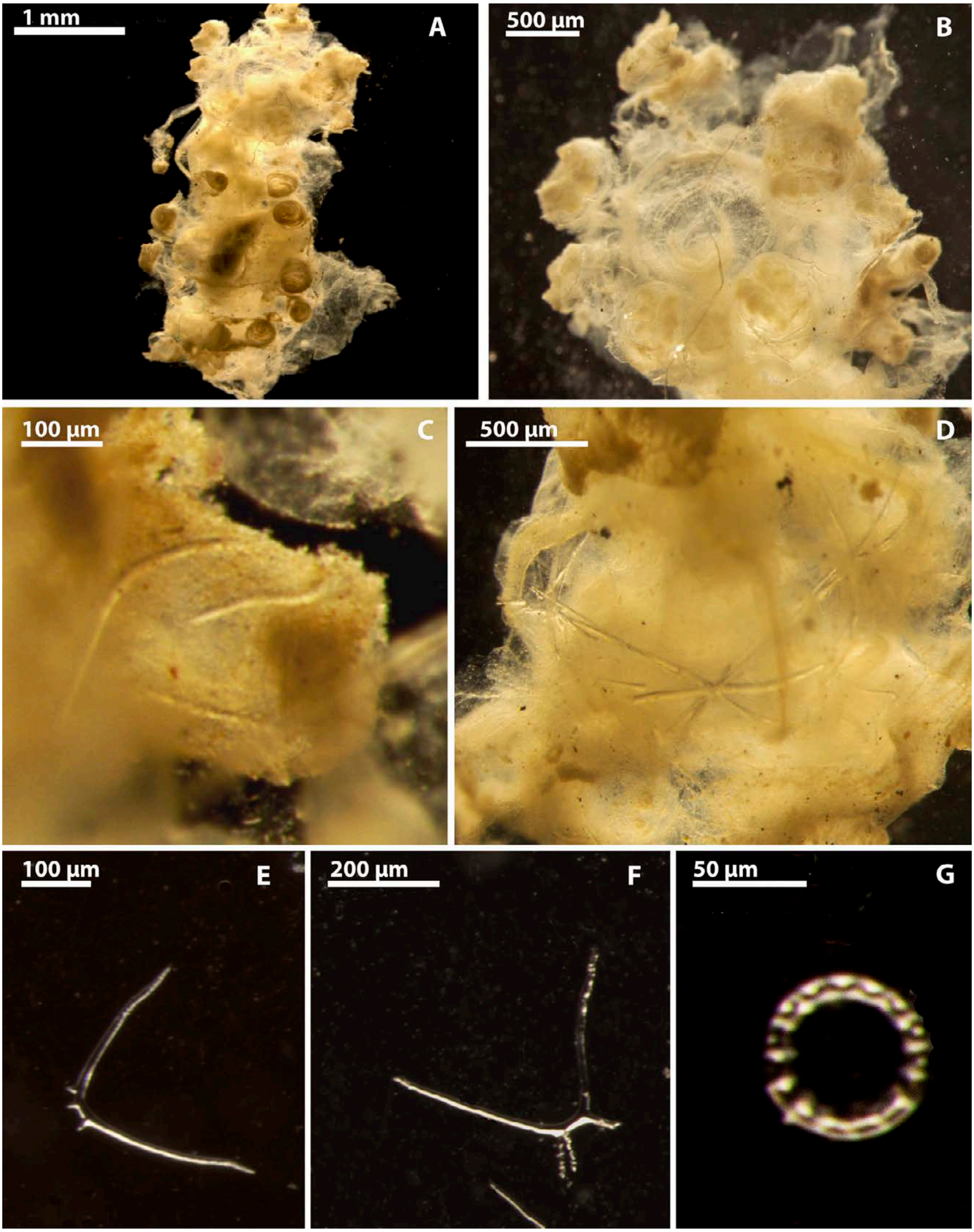

Fig. 12. – *Penilpidia ludwigi* characteristic. A, general view; B, oral region detail; C, tentacle ossicles; D, interlinked pieces of the calcareous ring; E, F, pieces of the calcareous ring G, wheel from skin.

Material: 219 specimens from cruise PROMETEO 01 and sediment traps of PROMETEO project. Depth of occurrence: from 900 to 1500 m. Zone: western Mediterranean Sea open slope and Blanes Canyon (Table 2).

Description: Small species of 5-20 mm in length. Fragile animals with skin usually broken. Digestive tract visible by transparency (Fig. 12A). Body elongated ovoid, with ventral side flattened. Six pairs of tube feet on the posterior half of the flattened ventral sole. Three pairs of papillae are present on the dorsal side, two pairs on the anterior part of the body and one pair on the posterior part. Ten tentacles surrounding the mouth (Fig. 12B), each divided into six to eight marginal lobes. Tentacles spicules curved rods with spines (130-300 μm) at their ends and in the middle on the external side of the curve (Fig. 12C). Calcareous ring with five interlinked pieces, usually visible by transparency. Each piece has four pair of arms radiating from the centre (Fig. 12D). Arched rods with one or two spines and four spiny leg ossicles (Fig. 12E, F). Papillae spicules smooth rods (Fig. 12G). Marenzeller (1893) reports males and females, describing gonads as one tuft slender and ramified for males and short and less ramified for females.

Distribution: Endemic to the Mediterranean Sea (Pagés et al. 2007, Gebruk et al. 2013).

Depth range: 755-4766 m (Fiege and Liao 1996).

Remarks: *Penilpidia ludwigi* has been reported twice in the eastern Mediterranean Sea basin (Marenzeller 1893, Fiege and Liao 1996) at depths of 755 to 4766 m. Its presence was reported in the northwestern Mediterranean Sea from sediment traps at 22 m above the bottom at depths between 1200 and 1700 m in the Palamós Canyon (Pagés et al. 2007). Although a specimen has been reported from a depth of only 48 m on the southwestern coast of Portugal (Cunha de Jesus and Cancela da Fonseca 1999), there is some doubt about this identification owing to depth (very shallow) and substrate (i.e. rocky area), as well as the poor condition of the specimen. Gebruk et al. (2008, 2013) described a related species in the North Atlantic and included a re-description of the genus and its species.

Order DACTYLOCHIROTIDA
Pawson and Fell (1965)
Family YPSILOTHURIIDAE Heding, 1942
Genus *Ypsilothuria* E. Perrier, 1886
Ypsilothuria bitentaculata (Ludwig, 1893)
(Fig. 13)

Sphaerothuria bitentaculata Ludwig, 1893:184. 1894: 141 pl. 12-14.
Ypsilothuria bitentaculata attenuata Alvà, 1991: 459-460.

Material: 27 specimens collected during cruises PROMETEO 01 to 05, PROMARES and DOSMARES 01. Depth of occurrence: 900 m to 1350 m. Zone: western Mediterranean Sea open slope (Table 2).

Description: Typically U-shaped (Fig. 13A). Two opposite siphons, oral and anal. Body wall thorny due to the presence of intricate scales, also visible with naked eye. Eight digitiform tentacles, of very unequal size, one on each side, being larger than the others. Calcareous plates visible with naked eye (Fig. 13B).

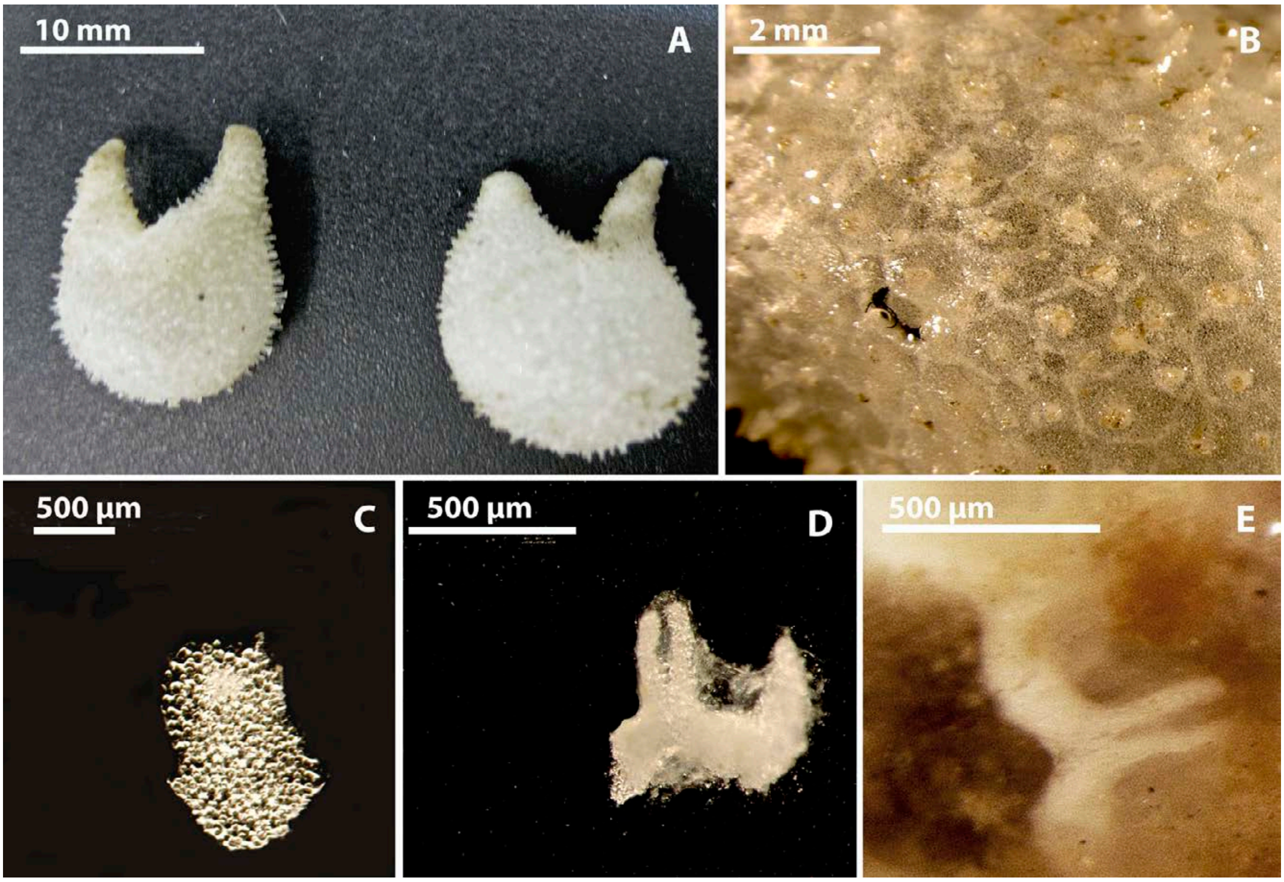

Fig. 13. – *Ypsilothuria bitentaculata* characteristics. A, general view; B, plates from skin; C, calcareous plate detail with central spine; D, E, calcareous ring detail of bifurcated projections.

Plates subcircular. Strong short spire placed near the edge of the plate (Fig. 13C). The plates are perforated by many small holes giving an irregular shape. Calcareous deposits in tentacles. Calcareous ring with eight plates. Lateral interradial plates with anterior bifurcated projections (Fig. 13D, E). The projections are often asymmetric.

Distribution: Cosmopolitan (Cherbonnier and Féral 1978).

Depth range: 225-4440 m (Cherbonnier and Féral 1978). Mediterranean Sea depth range 900 to 1560 m (Alvà 1991).

Remarks: Differs from *Y. talismani* in the bifurcated projections of the calcareous ring and the size of the plates (Gage et al. 1985, Alvà 1991).

Taxonomic information on deep-sea Mediterranean echinoderms

Fifty species of echinoderms present in the Mediterranean Sea and cited in the literature as presenting maximum depth of occurrence below 800 m were grouped in a table (Table 3). After carefully analysing all published data, we observed that from the initial 50 species shown in Table 3 only 29 were signalled at depths below 800 m depth in the Mediterranean Sea. Geographically, five of the studied species in Table 3 were endemic to the Mediterranean. Three were cosmopolitan and one had a broad Indo-Pacific and Mediterranean distribution (while all the other species had an Atlanto-Mediterranean distribution). Of the 50 species, 11 were sampled in our study. One of them was a first record for the Mediterranean. Four of the sampled species increased their maximum depth of distribution, and one increased the maximum depth of distribution in the Mediterranean Sea.

DISCUSSION

General remarks

This study provides a thorough review of all citations and distribution information of deep-sea echinoderms in the Mediterranean Sea. The literature review showed that for some species only very limited biological/ecological data were available, and in many cases only species lists were provided (Tortonese 1979, Pérez-Ruzafa and López-Ibor 1988). This paper provides new information of specimens collected in the last few years, including new records and extensions of geographic and bathymetric distributions. Our new data include information from areas with complex topography such as canyons, which previously have been sampled inadequately. We have collected together information of echinoderms living deeper than 800 m.

Our results report, for the first time, the presence of the echinoid *Gracilechinus elegans* (Düben and Koren, 1844) in the Mediterranean Sea. In addition, there are new records of two species considered previously as “rare” in the Mediterranean Sea. At present, there is no consensus regarding what determines a “rare species” (Cunningham and Lindenmayer 2005). In our study, taking into account all published information, we considered “rare” those species that have been reported less than five times in the whole basin. Based on this, two “rare” holothurians endemic to the Mediterranean Sea, *Hedingia mediterranea* (Bartolini Baldelli, 1914) Tortonese, 1965 and *Penilpidia ludwigi* (von Marenzeller, 1893), were identified. Additionally, we note greater bathymetric ranges for four species. The depth range of the asteroid *Ceramaster grenadensis* (Perrier, 1881), previously dredged in the Mediterranean Sea down to 2400 m (Carpine 1970, Tortonese 1979, Alvà 1987a), was extended to 2845 m. The echinoid *Brissopsis lyrifera* (Forbes, 1841), previously dredged around 1500 m (Sibuet 1974, Tortonese 1979, Cartes et al. 2009), was extended to 2250 m. Parezan (1970) reported the presence of *B. lyrifera* at 2500 m depth in the Ionian Sea. However, the specimen reported by Parezan (1970) was the test of a dead animal. Consequently later studies have not reported the presence of *B. lyrifera* at depths greater than 1500 m. The holothurian *Hedingia mediterranea* had been dredged previously only around 1000 m (Bartolini Baldelli 1914). Our data extend its bathymetric distribution range to 1500 m. Finally, the depth range of *Holothuria* (*Panningothuria*) *forskali* Delle Chiaje, 1823, which had been dredged previously down to 348 m in the Atlantic Ocean and around 193 m in the Mediterranean Sea (Pérez Ruzafa et al. 1987), is extended to 850 m in the Mediterranean Sea.

Below, we discuss the results by class. At the beginning of each section, if appropriate, we discuss first any new records and those of rare species. We then compare our results with the published literature, as detailed in Table 3.

Class Asteroidea

Our results for the class Asteroidea were based on two typical bathyal species, *Hymenodiscus coronata* (G.O. Sars, 1872) and *Ceramaster grenadensis* (Perrier, 1881). The depth range of *C. grenadensis* has been expanded to 2845 m. Where their depth ranges overlapped (1500 to 2250 m) the two species co-occurred, perhaps facilitated by their contrasting diets: *H. coronata* is a suspension feeder and *C. grenadensis* a secondary consumer (Carlier et al. 2009).

Other deep-sea asteroids reported previously from the Mediterranean at depths greater than 800 m (Table 3), such as *Astropecten irregularis irregularis* (Pennant, 1777), *Luidia sarsi sarsi* Düben and Koren, in Düben, 1845, *Odontaster mediterraneus* (Marenzeller, 1893), *Henricia cylindrella* (Sladen, 1883) and *Plutonaster bifrons* (W. Thompson, 1873), were not sampled in the recent work. *Plutonaster bifrons* was reported by Tortonese (1979) at 2715 m. However, this depth distribution was not supported by the specific data or citations in Tortonese’s publication. Thus, we consider the *Plutonaster bifrons* sample of the “Pola” (Marenzeller 1893) to be the deepest known record of

Table 3. – Echinoderms cited as present in the deep Mediterranean Sea. Atl. Depth, Maximum depth of distribution in the Atlantic Ocean; Med. Depth, Maximum depth of distribution in the Mediterranean Sea; Pre. Stu, Maximum depth sampled in the present study; Distribution, Atl-Med, Atlanto-Mediterranean distribution.

	Atl. Depth	Literature	Med. Depth	Literature	Pre. Stu.	Distribution
Crinoidea						
Leptometra celtica (Barrett and McAndrew, 1858)	1279 m	Mortensen 1927	538 m	Sibuet 1974	x	Atl-Med
Leptometra phalangium (J. Müller, 1841)	x	x	1300 m	Tortonese 1979	x	Mediterranean
Neocomatella europaea AH Clark, 1913	1700 m	Sibuet 1974	337 m	Sibuet 1974	x	Atl-Med
Asteroidea						
Astropecten irregularis pentacanthus (Delle Chiaje, 1827)	x	x	932 m	Tortonese 1958	x	Mediterranean
Astropecten irregularis irregularis (Pennant, 1777)	1000 m	Clark and Downey 1992	900 m	Koukouras et al. 2007	x	North Atl-Med
Ceramaster grenadensis grenadensis (Perrier, 1881)	2500 m	Clark and Downey 1992	**2845 m**	**Present study**	2845 m	Atl-Med
Chaetaster longipes (Retzius, 1805)	1140 m	Clark and Downey 1992	100 m	Tortonese 1958	x	Atl-Med
Henricia cylindrella (Sladen, 1883)	1383 m	Clark and Downey 1992	960 m	Sibuet 1974	x	Atl-Med
Hymenodiscus coronata (Sars G.O., 1872)	2600 m	Clark and Downey 1992	2904 m	Bartolini Baldelli 1914	2250 m	North Atl-Med
Luidia sarsi sarsi Düben and Koren, in Düben, 1845	1300 m	Clark and Downey 1992	1292 m	Marenzeller 1893	x	Atl-Med
Marginaster capreensis (Gasco, 1876)	x	x	600 m	Tortonese 1965	x	Mediterranean
Nymphaster arenatus (Perrier, 1881)	3000 m	Clark and Downey 1992	????	Pérez Ruzafa and López-Ibor 1988	x	Atl-Med
Odontaster mediterraneus (Marenzeller, 1893)	1804 m	Koehler 1909	1196 m	Tortonese 1965	x	Atl-Med
Plutonaster bifrons (W. Thompson, 1873)	2442 m	Cherbonnier and Sibuet 1972	2525 m	Marenzeller 1893	x	Atl-Med
Tethyaster subinermis (Philippi, 1837)	1425 m	Koehler 1895	320 m	Koukouras et al. 2007	x	Atl-Med
Ophiuroidea						
Amphilepis norvegica (Ljungman, 1865)	2900 m	Mortensen 1927	533 m	Tortonese 1965	x	North Atl-Med
Amphiura chiajei Forbes, 1843	1200 m	Mortensen 1927	766 m	Tortonese 1965	x	Atl-Med
Amphiura filiformis (O. F. Müller, 1776)	1200 m	Mortensen 1927	760 m	Marenzeller 1893	x	Atl-Med
Ophiacantha setosa (Retzius, 1805)	1480 m	Koehler 1921	300 m	Tortonese 1965	x	Atl-Med
Ophiactis balli (W. Thompson, 1840)	1765	Mortensen 1927	557 m	Sibuet 1974	x	Atl-Med
Ophiothrix fragilis (Abildgaard, in O. F. Müller, 1789)	1244 m	Mortensen 1933	450 m	Sibuet 1974	x	Atl-Med
Ophiotreta valenciennesi (Lyman, 1879)	1440 m	Paterson 1985	819 m	Misfud et al. 2009	x	Cosmopolitan
Ophiura albida Forbes, 1839	850 m	Mortensen 1927	500 m	Misfud et al. 2009	x	Atl-Med
Ophiura (Dictenophiura) carnea Lütken, 1858 ex M. Sars	1260 m	Mortensen 1927	1196 m	Tortonese 1979	x	Atl-Med
Echinoidea						
Asterechinus elegans Mortensen, 1942	1500	Samadi et al. 2010	1700 m	Bienhold et al. 2013	x	Indo-Pacific/Med
Brissopsis atlantica var. mediterranea Mortensen, 1913	3200 m	Tortonese 1965	679 m	Mastrotaro et al. 2010	x	Atl-Med
Brissopsis lyrifera (Forbes, 1841)	1650 m	OBIS	**2250 m**	**Present study**	**2250 m**	Atl-Med
Cidaris cidaris (Linnaeus, 1758)	1800 m	Tyler and Gage 1984	1777 m	Alvà 1987a	x	Atl-Med
Echinocyamus pusillus (O. F. Müller, 1776)	1250 m	Mortensen 1927	436 m	Misfud et al. 2009	x	Atl-Med
Echinus melo Olivi, 1792	1100 m	Minin et al. 2012	679 m	Mastrotaro et al. 2010	x	Atl-Med
Gracilechinus acutus Lamarck, 1816	1280 m	Minin et al. 2012	1880m	Cartes et al. 2009	x	Atl-Med
Gracilechinus elegans (Düben and Koren, 1844)	1750 m	Mortensen 1943/ Minin 2012	**1500 m**	**Present study**	**1500 m**	Atl-Med
Hemiaster expergitus Lovén, 1874	3120 m	Tortonese 1972	1249 m	Koukouras et al. 2007	x	Atl-Med
Neolampas rostellata A. Agassiz, 1869	1260 m	Tortonese 1958	400 m	Bartolini Baldelli 1914	x	Atl-Med
Spatangus purpureus O.F. Müller, 1776	969 m	Koehler 1927	932 m	Tortonese 1958	x	Atl-Med
Stylocidaris affinis (Philippi, 1845)	779 m	Mortensen 1903	1000 m	Fredj 1974	x	Atl-Med
Holothuroidea						
Hedingia mediterranea (Bartolini Baldelli, 1914) Tortonese, 1965	x	x	**1500 m**	**Present study**	**1500 m**	Mediterranean
Holothuria (Panningothuria) forskali Delle Chiaje, 1823	348 m	Pérez- Ruzafa et al. 1987	**850 m**	**Present study**	**850 m**	Atl-Med
Leptosynapta inhaerens (O.F. Müller, 1776)	Uncertain	WorMs	1200 m	Ramírez Llodra et al. 2008	x	Atl-Med
Mesothuria intestinalis (Ascanius, 1805) Östergren, 1896	2000 m	Gebruk et al. 2012	1927 m	Cartes et al. 2009	1750 m	Atl-Med
Mesothuria verrilli (Théel, 1886)	2600 m	Gebruket al. 2012	x	x	x	Atlantic
Molpadia musculus Risso, 1826	5205 m	Pawson et al. 2001	2500 m	Parezan 1970	1050 m	Atl-Med
Oestergrenia digitata (Montagu, 1815) var. *profundicola* (Kemp, 1905)	268 m	Mortensen 1927	914 m	Tortonese 1958	x	Atl-Med
Panningia hyndmanni (W. Thompson, 1840)	1150 m	Mortensen 1927/ Harvey 1988	150 m	Fredj 1974	x	Atl-Med
Parastichopus regalis (Cuvier, 1817)	747	OBIS	834 m	Marenzeller 1893	x	Atl-Med
Penilpidia ludwigi (Marenzeller, 1893)	x	x	4766 m	Fiege and Liao 1996	1500 m	Mediterranean
Pseudostichopus occultatus Marenzeller, 1893	4400 m	Herouard 1902	3624 m	Bartolini Baldelli 1914	2250 m	Cosmopolitan
Pseudothyone raphanus (Düben and Koren, 1846)	1150 m	Harvey et al. 1988	110 m	Cherbonnier and Guille 1967	x	Atl-Med
Thyone gadeana R. Perrier, 1902	970 m	WoRMS	300 m	Fredj 1974	x	Atl.-Med
Ypsilothuria bitentaculata (Ludwig, 1893)	4440 m	Cherbonnier and Féral 1978	1580 m	Cartes et al. 2009	1350 m	Cosmopolitan

P. bifrons (2525 m), in agreement with other authors (Alvà 1987a, Koukouras et al. 2007). Two other asteroid species, *Marginaster capreensis* (Gasco, 1876) and *Astropecten irregularis pentacanthus* (Delle Chiaje, 1827), have been considered to be Atlanto-Mediterranean species. Both species were reviewed by Clark and Downey (1992), who considered them to be endemic to the Mediterranean Sea. *Astropecten irregularis pentacanthus* (Delle Chiaje, 1827) was cited by Tortonese (1958, 1965) at 932 m depth from the "Pola" cruise. Two other species with a maximum depth of distribution at 1000-1500 m in the Atlantic Ocean, *Chaetaster longipes* (Retzius, 1805) and *Tethyaster subinermis* (Philippi, 1837), occurred considerably shallower (100 and 320 m, respectively) in the Mediterranean Sea. Finally, *Nymphaster arenatus* (Perrier, 1881), with a maximum depth at 3000 m in the Atlantic Ocean, has been cited from the Mediterranean Sea by Pérez-Ruzafa and López-Ibor (1988) and Koukouras (2007), but no depth data were given.

Class Echinoidea

This study reports for the first time the presence of *Gracilechinus elegans* (Düben and Koren, 1844) in the Mediterranean Sea. While Mortensen (1903) reported this species from the Mediterranean, he discarded the record in a later publication (Mortensen 1943). The lack of observations of *G. elegans* in the Mediterranean Sea could be caused by misidentification of congeneric species. For instance, adults of *G. elegans* are similar to juveniles of *G. alexandri* (see *G. elegans* description above). The only specimen of *G. alexandri* reported from the Mediterranean Sea (Alva 1987b) was not available for comparison. Another species that could lead to misidentification in the Mediterranean Sea is *Gracilechinus acutus* var. *norvegicus* (Düben and Koren, 1844). The possibility of hybridization between species should be taken into account. Hybridization has been described for other species of the same genus in the Atlantic (Shearer et al. 1911). Hybrids themselves may be responsible for some failures in identification. Molecular studies of Mediterranean Sea and Atlantic Ocean specimens may be able to determine the species more clearly in the future, including hybridization and phylogenetic differences.

Brissopsis lyrifera was present in canyon muddy sediments below 900 m, as suggested originally by Carpine (1970). Large and dense aggregations of dead and live *Brissopsis* were observed by ROV in canyons. The gregarious behaviour of this species has been reported in previous studies (Laubier and Emig 1993, Ramírez-Llodra et al. 2008). Many echinoid tracks were visible on the sediment, suggesting a "herd" in movement, similar to what has been observed for other bathyal echinoids (Salazar 1970, Gage and Tyler 1991). Although the number of collected specimens was too low to conduct population structure analyses, we observed that smaller specimens appeared to occur at greater depths. This contrasts with the results of Ferrand et al. (1988), who proposed the recruitment of smaller individuals at shallower depths. Our results are in agreement with Harvey et al. (1988), who suggested a possible 'dwarfism' for this species at greater depths. *Brissopsis lyrifera* is usually reported from the upper slope (250-400 m depth) on the Mediterranean continental margin (Tortonese 1965, Carpine 1970, Ferrand et al. 1988, Koukouras et al. 2007, Ramírez-Llodra et al. 2008, Cartes et al. 2009). The abundance of this species has decreased greatly in recent years on the upper and middle continental slopes at depths down to 1000 m (Mecho, pers. obs.), which may be related to intensive commercial trawling activity down to depths of 900 m (Ramírez-Llodra et al. 2010, Puig et al. 2012). Local fishermen have noted a large decrease in *B. lyrifera* in their by-catch in the last decade.

No specimens of the closely related species *Brissopsis atlantica* var. *mediterranea* Mortensen, 1913 were found.

Eight other species of echinoids have been reported from the Mediterranean Sea at depths below 800 m (Table 3). Two of these species, *Stylocidaris affinis* (Philippi, 1845) and *Cidaris cidaris* (Linnaeus, 1758), are common in the deep sea and have been sampled frequently below 800 m in the Mediterranean Sea (Alvà 1987a, Cartes et al. 2009). However, these two species were absent from our samples. Other species that occur mainly at shallower depths, such as *Spatangus purpureus* O.F. Müller, 1776 and *Gracilechinus acutus* Lamarck, 1816, were also not sampled in the recent cruises, even though they have been reported previously at depths greater than 800 m.

Two deep "rare echinoid species" are reported in the literature from the Mediterranean Sea: *Hemiaster expergitus* Lovén, 1874, sampled only three times (Cherbonnier 1958, Tortonese 1972, Koukouras et al. 2007) and *Asterechinus elegans* Mortensen, 1942, an Indo-Pacific species recently found in the eastern Mediterranean in association with sunken wood (Bienhold et al. 2013). These two species were not sampled in the present study. Three other species, *Echinocyamus pusillus* (O. F. Müller, 1776), *Echinus melo* Olivi, 1792, and *Neolampas rostellata* A. Agassiz, 1869, have maximum depths of distribution at 1100 m in the Atlantic Ocean. Their maximum depths of distributions are shallower (not exceeding 700 m depth) in the Mediterranean Sea.

Class Holothuroidea

The holothurian *Hedingia mediterranea* was first described by Bartolini Baldelli (1914) in the Tyrrhenian Sea. Its presence has not been reported since in the Mediterranean. It is possible that specimens reported as *H. mediterranea* have been misclassified as sipunculids because of the similar body shape of the two groups. Some studies have cited *H. mediterranea* as endemic to the Mediterranean Sea (Koehler 1921, 1927, Tortonese 1963, 1965, Parenzan 1970, Fredj 1974, Koukouras et al. 2007, Matarrese 2010), but only by referring to the original record of the type specimen. Accordingly, we consider the individuals sampled in this study as a truly 'rediscovered' species and extending both its geographic range to the northwestern Mediterranean

Sea and its bathymetrical distribution. One sample collected in the Blanes Canyon at 1200 m included four individuals and another at 1500 m in the same area included five individuals, suggesting a greater presence of this species in canyons. Pawson et al. (2001) considered the Bartolini Baldelli specimen as *Hedingia albicans* (Théel, 1886) Deichmann, 1938. This species is known from several locations in the North Atlantic. However, no explanation was provided for the synonymy of *H. albicans* and *H. mediterranea*. The information available does not allow us to clarify whether the Mediterranean specimens (classified as *Hedingia mediterranea*) are the same species as or distinct from the Atlantic species (classified as *Hedingia albicans*). In the present study we continue to classify the species as *H. mediterranea* following Tortonese (1963, 65). A molecular comparison between species of *Hedingia* would help to resolve the taxonomic discrepancies.

The only species of Elpidiidae present in the Mediterranean Sea is *Penilpidia ludwigi*. This is also considered to be a "rare" species, because it has been reported only three times previously, twice from the eastern Mediterranean Sea (Marenzeller 1893, Fiege and Liao 1996) and once from the deep western Mediterranean Sea (Pagés et al. 2007). However, when it does occur it may be found in abundance. Pagés et al. (2007) collected 150 individuals. More than 200 individuals were collected in one epibenthic sledge sample, suggesting that the species may occur in dense aggregations (Fiege and Liao 1996, Pagés et al. 2007) similar to those reported for other Elpidiidae in the Atlantic Ocean (Billett and Hansen 1982, Billett et al. 2001, 2010, Gebruk et al. 2003, Ruhl and Smith 2004). The presence of *P. ludwigi* in the Blanes Canyon sediment traps adds new faunistic records for this area. Pagés et al. (2007) collected *P. ludwigi* in the Palamós Canyon also with sediment traps moored at 22 m above the bottom. Our sediment traps sampled greater numbers in autumn and winter, coinciding with a stormy period in the northwestern Mediterranean (Sanchez-Vidal et al. 2012). This may have resulted in greater resuspension of bottom sediments and associated small fauna, such as *P. ludwigi.* Another factor that can cause resuspension of sediments, and thus the collection of small holothurians in sediment traps, are deep currents (Gebruk et al. 2013). In addition, swimming behaviour has been described in other Elpidiidae (Ohta 1985, Pawson and Foell 1986, Miller and Pawson 1990) and has also been proposed for *P. ludwigi* (Pagés et al. 2007). Swimming cannot be discarded as an explanation of the presence of this species in sediment traps. Pagés (2007) suggested that aggregations of *P. ludwigi* might occur during periods coincident with phytoplankton spring blooms and the flux of new organic matter to the seafloor. Although our sediment traps sampled greater numbers of specimens in autumn (similarly to the epibenthic sledge sample) and winter, these seasonal peaks of abundance may also indicate periodic recruitment of opportunistic species, as reported for other small species of Elpidiidae (Billett and Hansen 1982, Ohta 1985, Billett 1991, Billett et al. 2001, 2010).

The class Holothuroidea was the most speciose and most abundant of all the groups collected in our samples, as in the North Atlantic deep sea (Billett 1991, Gage and Tyler 1991). The order Aspidochirotida had the greatest number of species. Unlike in other studies, we did not observe dense aggregations of *Mesothuria* (*Allantis*) *intestinalis* (Ascanius, 1805) Östergren, 1896, as reported by Cartes et al. (2009) from 1600 m in the same region. Another species of the same genus, *Mesothuria verrilli* (Théel, 1886), has been reported from the Mediterranean Sea (Koukouras et al. 2007), but the presence of this species in the Mediterranean Sea was reviewed and discarded by Gebruk et al. (2012). *Pseudostichopus occultatus* Marenzeller 1893, a cosmopolitan aspidochirotid species, showed a restricted geographic and bathymetric distribution in our samples, occurring only between 2000 and 2200 m on the open slope, but in very high abundances.

The presence of large aggregations of individuals near the canyon axis could be related to food inputs (Morgan and Neal 2012). Submarine canyons act as conduits of organic matter from the shelf to bathyal/abyssal depths (Company et al. 2012). The aggregations of *P. occultatus* may be due to the periodic changes in food availability originating from canyon refluxes, as proposed for *Mesothuria.* To the best of our knowledge, the presence of *Holothuria* (*Panningothuria*) *forskali* Delle Chiaje 1823 at mid-bathyal depths has not been reported previously. The deepest records were at 345 m off the Canary Islands (Pérez Ruzafa et al. 1987, Hernández et al. 2013). The specimen sampled in the present study came from the Blanes Canyon at 850 m depth.

Two species of the order Molpadiida were collected. *Molpadia musculus* Risso, 1826 was present only in open slope areas. *Hedingia mediterranea* occurred mainly in canyon areas. Both species are deposit feeders and live infaunally. *Molpadia musculus* was reported as a typical canyon species in the Atlantic Ocean (Amaro et al. 2009) and in other Mediterranean Sea areas (Ramírez-Llodra et al. 2008, Cartes et al. 2009). However, no specimens of *M. musculus* were found in our canyon samples. The high presence of *H. mediterranea* inside canyons suggests habitat specialization, but further sampling inside canyons is necessary to confirm this hypothesis.

The order Dactylochirotida was represented by a single species, *Ypsilothuria bitentaculata* (Ludwig, 1893). The presence of this species only at middle slope depths is commonly reported (Pawson 1965, Gage et al. 1985). This species was reported from the Mediterranean Sea only in the early 1990s (Alvà 1991). Subsequently, *Ypsilothuria bitentaculata* has been cited by other authors (Massin 1996, Cartes et al. 2009) and also as *Y. talismani* by Ramírez-Llodra et al. (2008). Little information is available for *Ypsilothuria* in the Mediterranean Sea. A detailed discussion on its taxonomy must await further sampling.

Of the holothurians species reported previously from the deep (occurrence below 800 m) Mediterranean Sea, only two species did not occur in our study (Table 3). First, *Leptosynapta inhaerens* (O.F. Müller, 1776) occurs at shallower depths of around 500 m. A record of this species by Ramírez-Llodra et al. (2008)

from 1200 m on the Catalan margin off Barcelona is uncertain and may have been misidentified (Company, pers. com). Second, *Oestergrenia digitata* (Montagu, 1815) var. *profundicola* (Kemp, 1905) has been reported at 900 m (Marenzeller 1893, Tortonese 1958). One species typical of shallower Mediterranean waters, *Parastichopus regalis* (Cuvier, 1817), has been cited at 834 m depth by Marenzeller (1893), but no other reports are known for these depths. Finally, there are three other species, *Panningia hyndmanni* (W. Thompson, 1840), *Pseudothyone raphanus* (Düben and Koren, 1846) and *Thyone gadeana* Perrier R., 1898, which have maximum depth ranges extending to around 1000 m in the Atlantic Ocean but occur no deeper than 300 m in the Mediterranean Sea.

Class Crinoidea

Crinoids were totally absent from our samples. Three species of crinoids have been cited from the bathyal Mediterranean seafloor (Table 3). Only one of them, the endemic crinoid *Leptometra phalangium* (J. Müller, 1841), has a maximum depth of distribution greater than 800 m. Stalked crinoids were not reported in the Mediterranean Sea (David et al. 2006).

There are some records of high abundances of *Leptometra phalangium* in upper slope areas (100 to 400 m depth) (Pérès and Picard 1956a, Mifsud et al. 2009), as observed for the same genus in other areas (Fonseca et al. 2013). The deepest record for this species is 1292 m (Marenzeller 1893). However, despite these deeper records, not a single crinoid was collected in any of our hauls or observed during the ROV dives. Their occurrence at predominantly shallower depths (Hellal 2012) may explain the absence of these crinoids in our samples.

Class Ophiuroidea

Ophiuroids were also totally absent from our samples. Nine species of ophiuroids have been cited previously from the Mediterranean Sea at depths between 300 and 1219 m (Table 3), with only two species, *Ophiura* (*Dictenophiura*) *carnea* Lütken, 1858 ex M. Sars, and *Ophiotreta valenciennesi* (Lyman, 1879), cited below 800 m (Tortonese 1979, Mifsud et al. 2009). All nine species have been reported from depths greater than 800 m in the Atlantic Ocean, but their maximum depth of distribution in the Mediterranean Sea is shallower. This may explain the lack of ophiuroids in our study.

Endemicity in echinoderms from the Mediterranean

There has been considerable debate as to whether the deep-sea fauna of the Mediterranean is truly endemic or is a sub-population of Atlantic species (Bouchet and Taviani 1992, Tyler 2003). The shallow Gibraltar Sill may be a significant barrier for the influx of larvae of echinoderms from the Atlantic and may act as an isolating mechanism once populations are established in the Mediterranean. The higher temperatures of deep water in the Mediterranean may mitigate the immigration of species from the deep Atlantic. However, an increased sampling effort and molecular analyses are required before this aspect is fully resolved. Our samples increase the availability of genetic data necessary for future comparative studies between populations.

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Reproductive biology of the deep-sea asteroid *Ceramaster grenadensis* (Perrier, 1881) from the north-western Mediterranean Sea

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Resumen

Ceramaster grenadensis (Perrier, 1881) es el asteroideo batial más abundante del Mediterráneo nord-occidental. Este asteroideo, presenta una amplia distribución geográfica estando presente tanto en el Mediterráneo como en el Océano Atlántico. A pesar de ello, la información referente a su biología y ciclo reproductivo es escasa, al igual que en otras especies del mismo género. En este contexto, el presente estudio describe por primera vez el ciclo reproductivo y los patrones de distribución de la especie el Mediterráneo nord-occidental profundo. Los 141 especímenes utilizados fueron recogidos estacionalmente durante 10 campañas oceanográficas realizadas entre Octubre del 2008 y Abril del 2013. Se realizaron 194 pescas de arrastre (141 realizadas mediante Redes de Arrastre Maireta y 53 con patín de arrastre Agassiz) a profundidades comprendidas entre los 900 y los 2800 m de profundidad. Se observó que la distribución poblacional de *C. grenadensis* presenta un patrón batimétrico en el cual los especímenes adultos de tamaño más pequeño y los juveniles se encuentran a mayor profundidad. El sex ratio es de 2:1 hembras por macho, y se mantiene constante tanto estacional como batimétricamente. Los análisis histológicos de las gónadas presentan una organización típica de especies de reproducción asincrónica, conteniendo oocitos previtelogénicos y vitelogénicos durante todo el año. Este tipo de oogenesis se suele presentar en especies de reproducción continua, sin embargo la disminución del Índice Pilórico (PCI) en verano y el aumento del Índice Gonadosomático (GI) en otoño sugiere en este caso una mayor capacidad de desove en otoño. Asimismo en ambos sexos se observa un incremento de los valores de GI y PCI con la profundidad.

Abstract

The bathyal asteroid *Ceramaster grenadensis* (Perrier, 1881) is one of the most abundant deep-sea asteroids in the north-western Mediterranean Sea and presents also a wide geographic distribution in the Atlantic Ocean. As for other species in this genus, little information is available on the biology and reproductive strategy of *C. grenadensis*. In this context, we describe for the first time the reproductive cycle of this species from bathyal depths in the north-western Mediterranean. Specimens (n = 141) were collected seasonally from 194 benthic trawls (141 Otter Trawls and 53 Agassiz sledge) conducted in 10 cruises from October 2008 to April 2013. Open slope and canyon systems were sampled at depths between 900 m and 2250 m. The population distribution of *C. grenadensis* showed a depth related structure, presenting the smaller adult specimens and juveniles at greater depths. Sex ratio was of 2:1 females per male, constant along seasons and depths. Histological analyses of the gonads showed an asynchronous ovarian organization, with previtellogenic and vitellogenic oocytes thorough the year. This oogenesis patterns suggest a continuous reproduction. However, the Pyloric Caeca Index (PCI) decreased in summer while the Gonad Index (GI) increased in autumn, suggesting a higher spawning capacity in autumn. On both sexes, an increasing GI and PCI trend was observed with increasing depth.

KEY-WORDS: *Ceramaster grenadensis*, deep sea, reproductive biology, oogenesis, Mediterranean Sea.

Introduction

Information on life history patterns of deep-sea fauna has been improved greatly in the past few years (Ramirez-Llodra, 2002a; Young, 2003). Nonetheless this information remains scarce for deep-sea Mediterranean megafauna (Company *et al*., 2003; Fernandez-Arcaya *et al*., 2012), including the echinoderms, which are still poorly studied in the Mediterranean Sea. In the Atlantic Ocean, the echinoderms are an important component of the bathyal and abyssal fauna in terms of density, biomass and ecosystem function (Billett, 1991; Billett *et al*., 2001; Ginger *et al*., 2001; Wigham *et al*., 2003), and their life history has been described for many species (Tyler, 1983; Gage *et al*,. 1986; Tyler *et al*,. 1992; Wigham *et al*., 2003; Galley *et al*., 2008; Benítez-Villalobos & Díaz-Martínez 2010; Ross *et al*., 2013). In the deep Mediterranean Sea, the most abundant groups are fish and decapod crustaceans (Company *et al*., 2004; Danovaro *et al*., 2010; Tecchio *et al*., 2011; Fernandez-Arcaya *et al*., 2013a), and consequently deep-sea echinoderms have been less studied (Alvà, 1987; Mecho *et al*., 2014) and their life history, remains mostly unknown. In the Atlantic Ocean, a wide diversity of reproductive strategies has been reported for deep-sea asteroids, from the most common quasi-continuous reproductive patterns to seasonal patterns (Tyler & Pain, 1982a; Tyler & Pain, 1982b). Also, there is a diversity in fecundity from high to low assets (Ramirez-Llodra *et al*., 2002b; Young, 2003), and brooding or broadcasting strategies have been described (Mercier & Hamel 2008).

The asteroid *Ceramaster grenadensis* (Perrier, 1881) belongs to the Family Goniasteridae and presents a wide geographic and bathymetric distribution. It is present both in the Atlantic Ocean and the Mediterranean Sea between 200 and 2845 m depth (Clark & Downey, 1992; Mecho *et al*., 2014). Its shallow bathymetric range is slightly deeper in the Mediterranean Sea, starting at 600 m, and its presence has been reported from the eastern, central and western Mediterranean basins (Koukouras *et al*., 2007; Carlier *et al*., 2009). This species is the most abundant asteroid below 850 m in the north-western Mediterranean Sea (Mecho *et al*., 2014). Although *C. grenadensis* represents an important component of the deep benthic ecosystem, information about its general biology remains scarce. The trophic behaviour of some species within this genus has been studied, with some species (i.e. *Ceramaster granularis* (Retzius, 1783)) described as active sponge predators (Gale *et al*., 2013) and others described as deposit feeders (i.e. *Ceramaster patagonicus* (Sladen, 1889)) (Anderson & Shimek, 1993). *Ceramaster grenadensis* is a secondary consumer that may feed on decayed organic material (Carlier *et al*., 2009). Life-history traits in relation to the reproductive biology are presently unknown. Thus, in this study we focused on the population distribution and the seasonal and bathymetric patterns of the reproductive biology of *C. grenadensis* along the middle and lower slope of the Catalan margin of the north-western Mediterranean Sea.

Materials and Methods

Study area and sampling methods

From October 2008 to April 2013, ten oceanographic cruises were conducted in the north-western Mediterranean Sea. The sampling areas included the submarine canyons of Blanes, Palamós (also named La Fonera) and Cap de Creus, as well as the adjacent slope to the Blanes canyon (Fig. 1). Because of the low number of individuals sampled

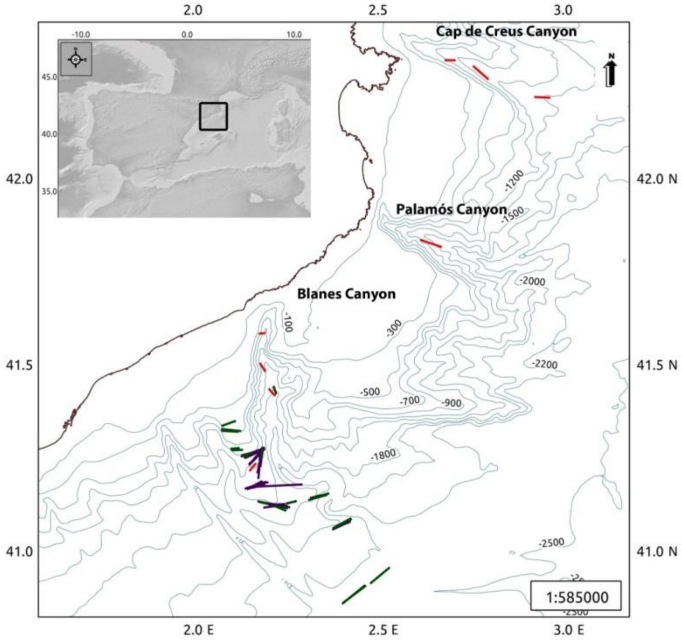

Figure 1. Study area representing the three sampled canyons and adjacent open slopes in the North-western Mediterranean Sea. Colour lines represent trawl stations. Red: PROMARES trawls; Blue: PROMETEO trawls; Green: DOSMARES trawls.

inside canyon areas (n = 3), no comparative analyses between habitats was conducted. These cruises took place in the framework of three Spanish research projects (PROMETEO, DOSMARES and PROMARES), sampling at depths comprised between 900 and 2250 m every 150 m.

A total of 141 hauls were conducted by a single warp Otter-trawl Maireta System (OTMS, Sardà *et al*., 1998) with a net length of 25 m fitted with a cod-end liner of 6 mm strength mesh size. A SCANMAR system was used to estimate the width of the mouth of the net. An average horizontal opening of 12.7 ± 1.4 m, n = 36 was recorded. As the SCANMAR system can only operate down to 1200 m depth, the same value for the mouth's width of the net was used also for hauls deeper than 1200 m. The height of the trawl mouth was estimated to be 1.4 m (Sardà *et al*., 1998). In addition, 53 hauls were conducted with an Agassiz dredge, made of a square steel frame with a mouth width and height of 2.5 and 1.2 m respectively, and a cod-end liner of 6 mm strength mesh size. The total sweep area fished was of 10.3 km^2.

Because of the low number of individuals available at each depth, and based on available literature on deep-sea communities depth structuring (Quetglas *et al*., 2000; Company *et al*., 2004), the individuals were grouped in four bathymetrical strata comprising the whole sampled depth range as follow: 900-1050 m, 1200-1350 m, 1500-1750 m and 2000-2250 m depth. Density and biomass were standardized to km^2 estimated from vessel speed, distance from initial and final trawl position and average of the mouth opening of the sampling gear.

Samples analysis

All specimens (n = 141, Table 1) were sorted on board. The major radius (R) from the anus to the tip of the D arm was measured for all specimens. The specimens were then fixed with 40% formalin diluted with seawater and neutralized with borax. To study the population size distribution, R size were grouped into 5 mm classes and percentage frequency analysed.

Table 1. Number of individual sampled during the oceanographic cruises. See trawl information in Figure 1 (DM: DOSMARES trawls; PR: PROMETEO trawls).

Cruise code	Season	Depth strata (m)				Total
		900-1050	1200-1350	1500-1750	2000-2250	
DM01	Winter	0	3	5	17	25
DM02	Spring	0	1	0	0	1
DM03	Autumn	2	1	0	0	3
DM04	Spring	2	7	5	9	23
PR01	Autumn	1	0	0	0	1
PR02	Winter	1	14	14	0	29
PR03	Spring	3	8	3	0	14
PR04	Summer	2	11	4	0	17
PR05	Autumn	3	3	9	12	27
PROMARES	Summer	0	0	1	0	1
Total		14	48	41	38	141

After 30 days, the samples were transferred to 70% alcohol in the laboratory and weighted to the nearest ± 0.01 g. The 141 specimen were dissected on the oral side, from which the five pairs of gonads were extracted and weighted to the nearest ± 0.001 g. The same procedure was conducted for the pyloric caeca. The gonad index (GI) and pyloric caecum index (PCI) were calculated:

$$\mathrm{GI} = \frac{Gonad\ Mass\ (\mathrm{g})}{Female\ Mass\ (\mathrm{g})} \times 100$$

$$\mathrm{PCI} = \frac{Pyloric\ caeca\ Mass\ (\mathrm{g})}{Female\ Mass\ (\mathrm{g})} \times 100$$

After being weighted, gonads were preserved in 70% alcohol prior to histological preparation. In a subsample of 125 individuals, gonads were dehydrated in graded alcohols, cleared in Histoclear and included in paraffin wax. The gonads processed were sectioned at 7 µm and stained with Haematoxylin and Eosin. These 125 individuals were used for sex identification. Of these, 71 were females, of which 42 were processed and the Feret diameter of 100 oocytes (whenever possible) sectioned through the nucleus was measured for each individual using the image analysis package SigmaScan Pro 5.

The Feret diameter gives the diameter of a disc with the equivalent area of the measured object. Oocyte sizes were grouped in 50-µm classes and oocyte-size frequency diagrams constructed by depth and season.

Statistical data treatment

All the data were tested for normality using the Kolmogorov-Smirnov non-parametric tests. Two Analyses of variance (ANOVA) were used to test population size differences between depths and between seasons. Meanwhile, the difference for density, biomass, GI and PCI between depths, and between seasons were tested by Mann-Whitney non-parametric tests. Chi-squared test was used to analyse sex ratios in relation to depth and season.

Results

Density and biomass

The bathymetric distribution of *C. grenadensis* ranged from 900 m to 2250 m depth, with mean density values not statistically different along depth strata (Fig. 2A) (Kruskal–Wallis test, $H_3 = 1.35$, $P > 0.05$). Although not significant, the maximum mean density was higher at 1500-1750 m depth. In contrast, a decrease in biomass with depth was observed (Fig. 2B).

The mean biomass distribution showed significant differences between the two shallower strata (900-1050 m and 1200-1350 m) (Mann-Whitney U test, $U = 68$, $N_1 = 12$, $N_2 = 24$, $P < 0.01$) and between the shallower and deepest strata (900-1050 m and 2000-2250 m) (Mann-Whitney U test, $U = 18$, $N_1 = 12$, $N_2 = 12$, $P < 0.01$).

Although the mean density and biomass was not significantly different between seasons (Kruskal–Wallis test, $H_3 = 0.31$, $P > 0.5$ for density; Kruskal–Wallis test, $H_3 = 1.47$, $P > 0.5$ for biomass), a general trend of lower values in summer was found (Fig. 3A, B).

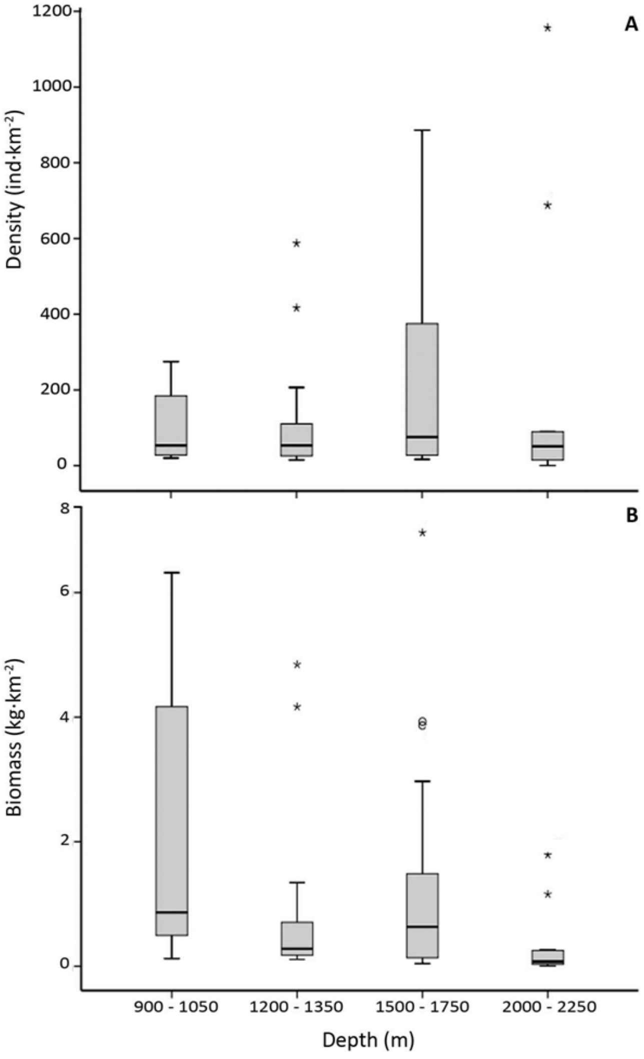

Figure 2. Density (ind·km^{-2}) (A) and biomass (kg·km^{-2}) (B) of *C. grenadensis* by depth strata. The top and bottom of each box-plot represent 75% (upper quartile) and 25% (lower quartile) of all values, respectively. The horizontal line is the median. The ends of the whiskers represent the 10th and 90th percentiles. Cross marks represent outliers

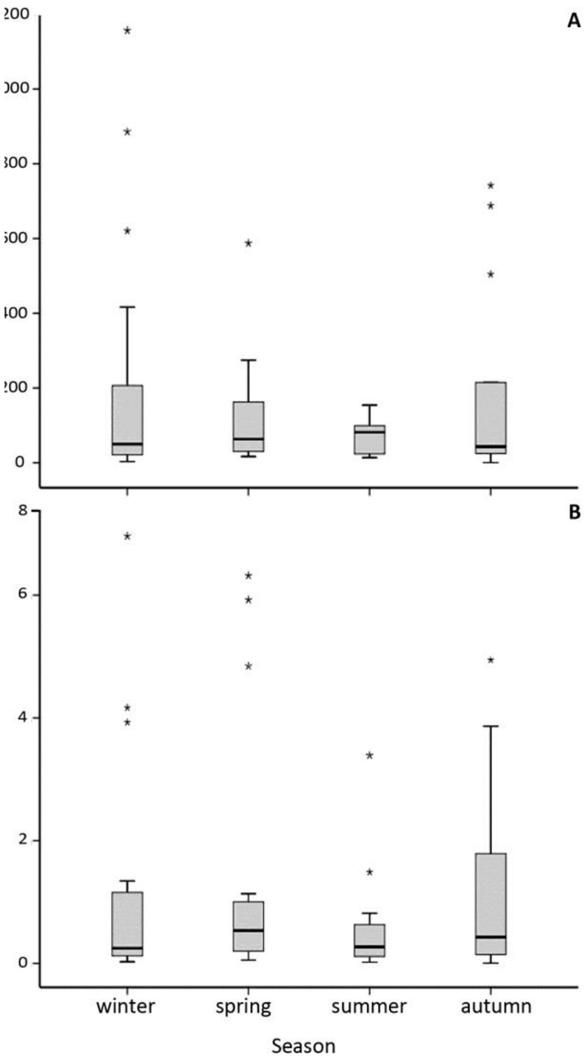

Figure 3. Density (ind·km^{-2}) (A) and biomass (kg·km^{-2}) (B) of *C. grenadensis* by season. The top and bottom of each box-plot represent 75% (upper quartile) and 25% (lower quartile) of all values, respectively. The horizontal line is the median. The ends of the whiskers represent the 10th and 90th percentiles. Cross marks represent outliers.

Population size distribution

The population structure of *C. grenadensis* describes a normal distribution (Kolmogorov-Smirnov, D = 120, df = 137, $P < 0.0001$, Lilliefors significance correction), with most specimens (70%) presenting size classes between R = 20 and 35 mm (Major Radium size) (Fig. 4A, B). The population size frequency distribution presented a depth related pattern, with size decreasing significantly with depth (ANOVA, $F_{(3,134)} = 91.79$, $P < 0.0001$), with mean sizes not significantly different between the middle strata (1200-1350 m and 1500-1750 m). A mean individual size of R = 38 mm was observed at 900-1050 m stratum decreasing to R = 15 mm mean individual size at 2000-2250 m stratum (Fig. 4A). The adults were distributed along all the bathymetric range while juvenile specimens were limited to the deepest areas below 1750 m depth. At the deepest stratum (2000-2250 m), the ratio of adults/juveniles was nearly 1:1.

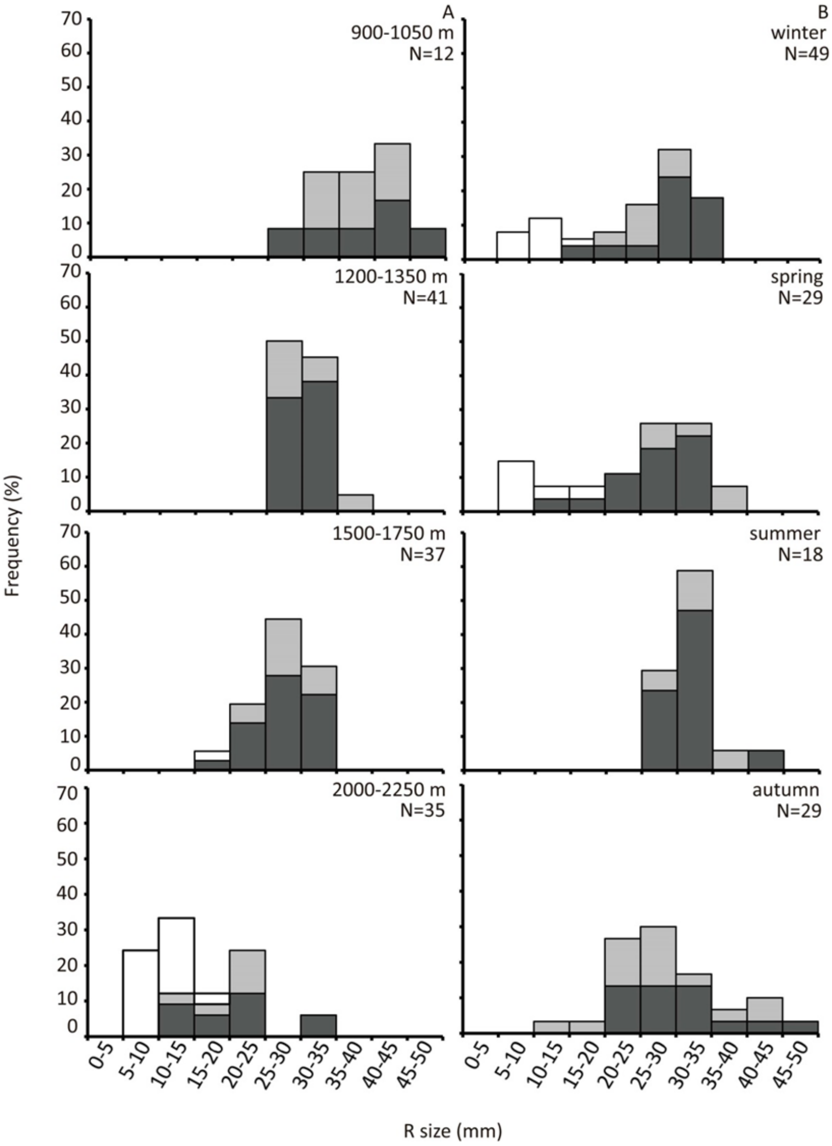

Figure 4. Individual size frequency distribution by depth (A) and season (B). Dark grey bars, females; light grey bars, males; white bars, juveniles.

Seasonally, the mean individual size decreased from summer (R = 31.9 mm) to spring when the smallest individuals were captured (R = 22.5 mm) (Fig. 4B). Significant differences between summer and all the other seasons were found (ANOVA, $F_{(3,123)} = 6.637$, $P < 0.0001$). The individual size frequency distribution by season showed the presence of juveniles during winter and spring, while in summer and autumn only adult individuals were captured The smallest individual was caught at 2250 m depth in spring, with a R =

6.34 mm while the largest specimen was collected at 1050 m depth in autumn with a R = 45.26 mm.

Sex ratio and size at first maturity

Over the 125 specimens sexed, 71 were female and 36 males, with 18 juveniles where sex could not be determined. Sex ratio was significantly biased towards females 2:1 (Chi-square test, $X^2_2 = 0.5$, $P > 0.05$). No significant differences were found in the sex ratio with depth or season (Chi-square test, $X^2_2 = 0.5$, $P > 0.05$). Minimum size at sexual maturity was R = 10.9 mm for females and R = 13.3 mm for males. All the 18 juvenile specimens were found deeper than 1750 m depth.

Gonad morphology

Ceramaster grenadensis presented macroscopically the typical gonad morphology of asteroids, with five pairs of gonads per individual, one pair in each interradius. Each pair of gonads was suspended in the coelom and was attached to the body wall by a short gonoduct opening aborally at the gonopore. Macroscopically, mature ovaries and testes presented the same morphology of tuffs of digitate tubules and could not be distinguished (Fig. 5A).

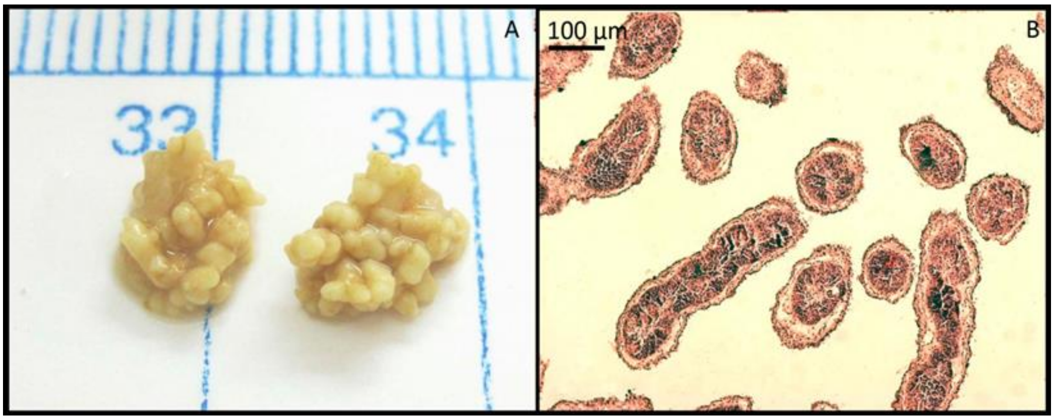

Figure 5. Macroscopic view of a *C. grenadensis* gonad (A). Histological sections of gonads of mature testis (B).

Males in maturity stages presented the spermatozoa accumulated as dense masses of gametes in the lumen of the testes (Fig. 5B). Juveniles were classified as indeterminate specimens and they were identified by the presence of immature follicles, with no distinguishable oocytes or spermatozoa (Fig. 6A).

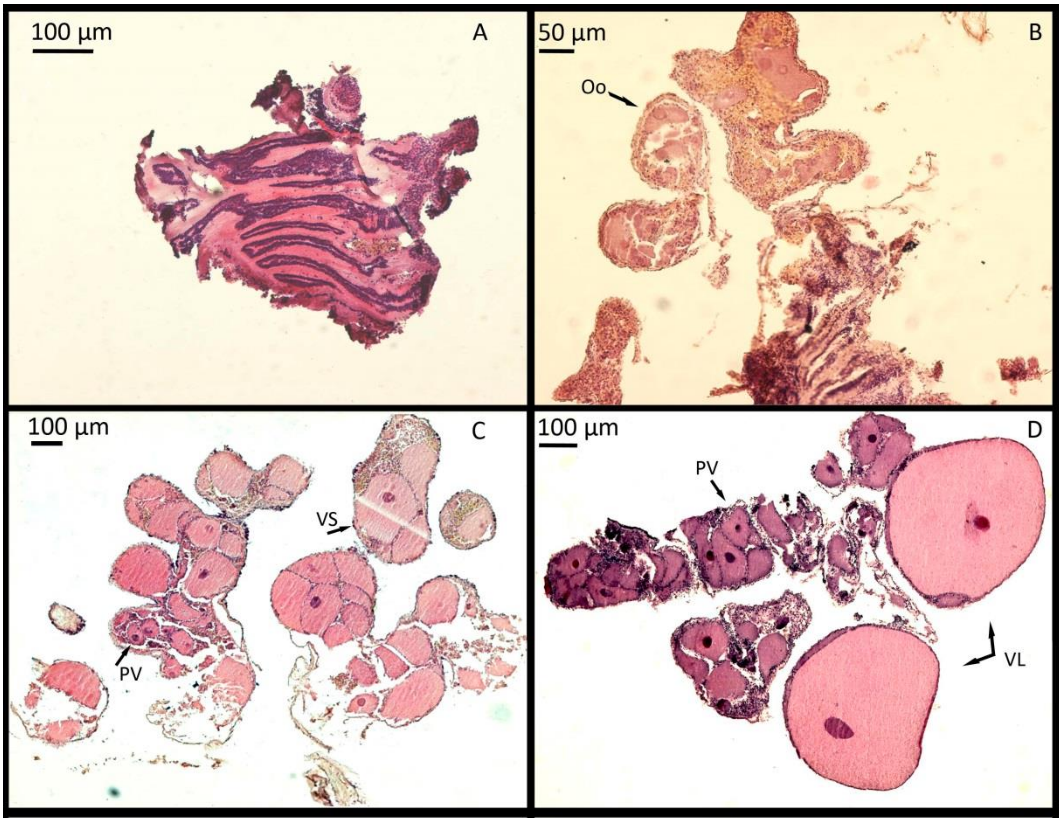

Figure 6. Histological sections of gonads. Juvenile specimen with indeterminate gonad (A); Inmature female with oogonia (Oo)(B); Section showing previtellogenic oocytes (PV) and small vitellogenic oocyte (VS) (C); Section showing large vitellogenic oocytes (VL) and, previtellogenic oocytes (PV) (D).

In immature females, only oogonia were present, observed as small cells (<100 µm) with large nucleus/ cytoplasm ratio (Fig. 6B). In females with developing ovaries, we observed all stages of oogenesis present at one time, including oogonia previtellogenic and vitellogenic oocytes (Fig. 6C). The previtellogenic oocytes (22.76 to 208.9 µm), presented a central nucleus with an eccentric nucleolus. These cells could be identified because their cytoplasm stains in dark purple with haematoxylin due to their basophilic composition. The vitellogenic oocytes were larger cells (between 136 µm and 691 µm) with a smaller nucleus/cytoplasm ratio resulting from the accumulation of vitellum in the cytoplasm. The acidophilic cytoplasm of vitellogenic oocytes stained pale pink with Eosin. In the vitellogenesis stage, the oocytes were usually small or medium in size (from ± 130 µm to ± 450 µm) (Fig. 6C). Meanwhile, in maturity stage, females presented previtellogenic (<200 µm) and large vitellogenic oocytes (from 450 µm to 691 µm) (Fig. 6D).

Oocyte-size frequency distribution

Females of *C. grenadensis* presented a broad range of oocyte stages, independently of season or depth (Fig. 7A, B), suggesting that oogenesis is asynchronous in this species.

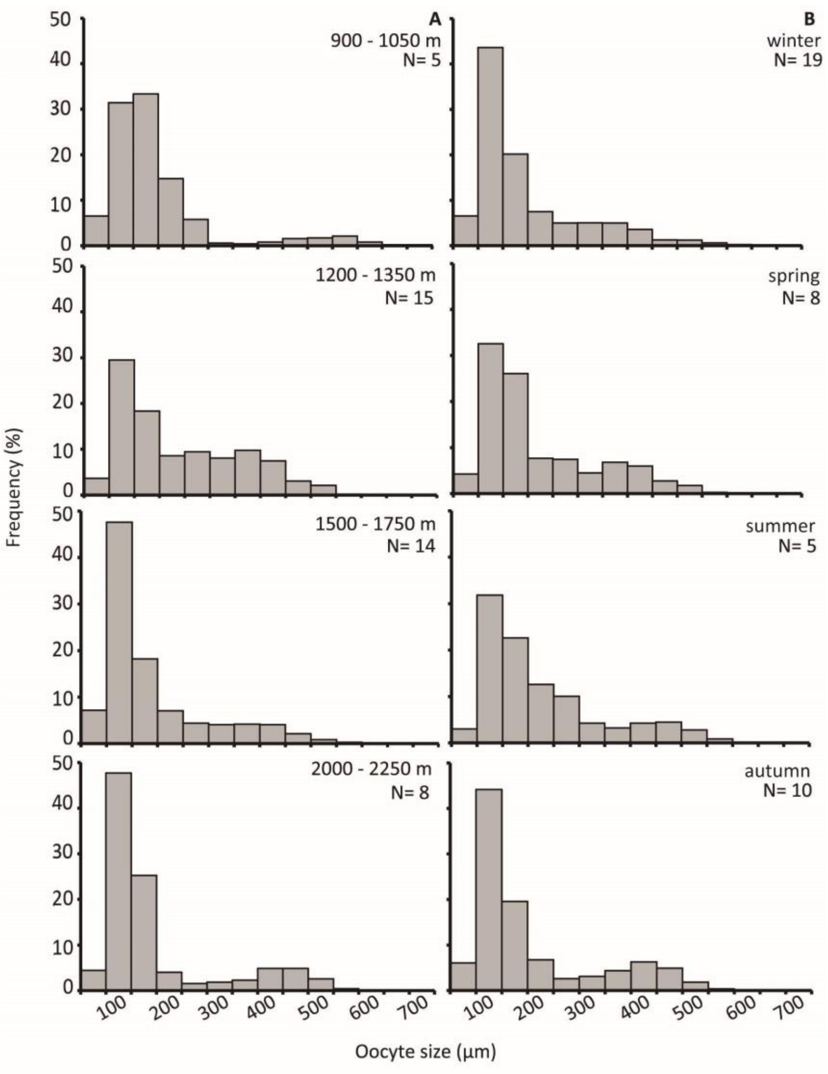

Figure 7. Bathymetric oocyte-size frequency distribution (A) and seasonal oocyte-size frequency distribution (B).

There was a wide peak of previtellogenic oocytes (<250 µm) representing from 73% to 77% of the total measured oocytes in all seasons and from 68% to 91% of the measured oocytes in all depth strata. Small vitellogenic oocytes (from 250-350 µm) represented 7% to 10% of the measured oocytes in all seasons and from 1% to 17% of the measured oocytes in all depth strata. Developing vitellogenic oocytes (350-500 µm) represent 6% to 13% in all seasons and from 4% to 12% of the measured oocytes at all depths. The largest oocytes (500–700 µm) represented less than 1% of the measured oocytes for all seasons and between 0.1% and 3% for all depth strata.

Gonad Index (GI) and Pyloric Caecum Index (PCI)

The bathymetric values for GI and PCI were not constant bathymetrically (Table 2). In females, GI presented higher values at 1200-1350 m and 2000-2250 m depth (Fig. 8A). Significant higher mean GI and PCI values for females were reported at the lowest depth stratum (2000–2250 m depth) (Fig. 8A) (Table 2).

Table 2. Mann-Whitney U values for females' GI and PCI values between depth strata. Significance of the U values (*U*) is indicated using: * *P < 0.1*; ** *P < 0.05*; *** *P < 0.001*. Distance values with no asterisk indicate non-significant values

GI	900-1050	1200-1350	1500-1750
1200-1350	45*		
1500-1750	51	225**	
2000-2250	14*	152	87
PCI	900-1050	1200-1350	1500-1750
1200-1350	64		
1500-1750	40*	298	
2000-2250	0***	10***	18***

In males, the same pattern of higher mean values at lower depths was observed (Fig. 8B). The mean GI was significantly higher at the deepest depth stratum (Table 3). Meanwhile, the mean PCI increased significantly at 1500-1750 m stratum (Table 3), with a maximum at 2000-2250 m (Fig. 8B) (Table 3)

Table 3. Mann-Whitney U values for males GI and PCI values between depth strata. Significance of the U values (*U*) is indicated using: * *P < 0.1*; ** *P < 0.05; *** *P < 0.001*. Distance values with no asterisk indicate non-significant values.

GI	900-1050	1200-1350	1500-1750
1200-1350	29		
1500-1750	31	59	
2000-2250	2**	2***	3**
PCI	900-1050	1200-1350	1500-1750
1200-1350	31		
1500-1750	13**	28	
2000-2250	10	15	29

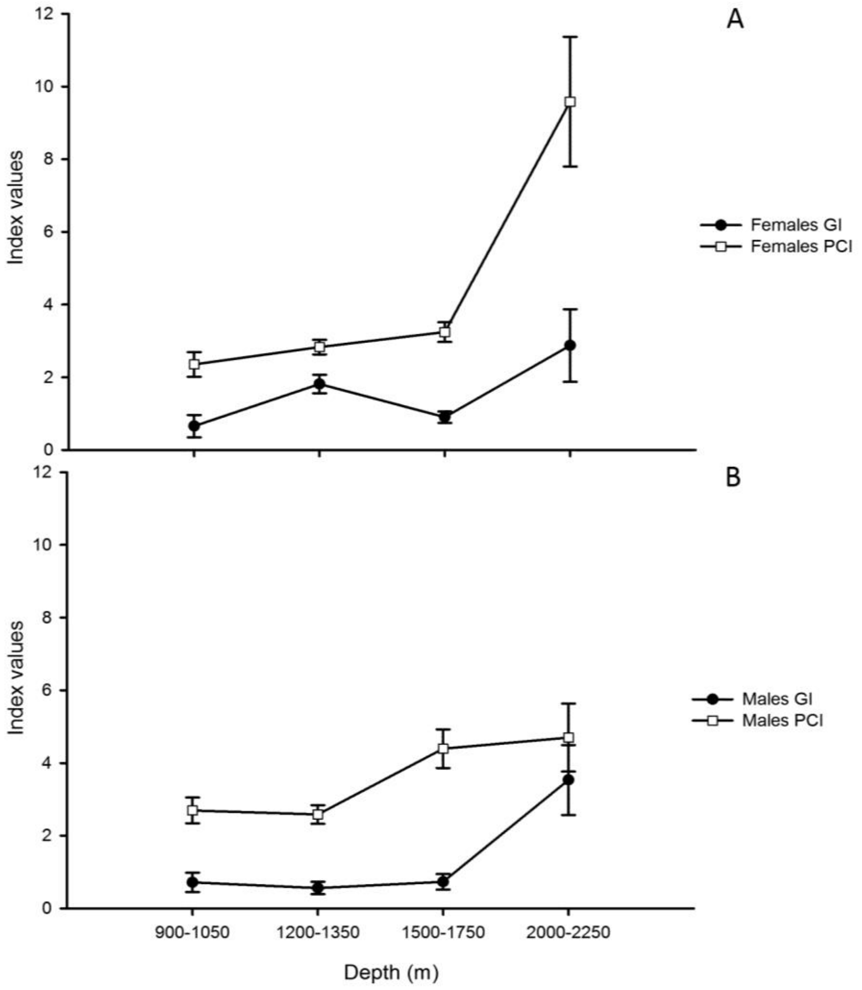

Figure 8. Bathymetrical changes in Gonad Index (GI) and Pyloric Caeca Index (PCI) (mean ± SE) in females (A) and males (B).

No significant differences were observed in the mean GI values of females throughout the year (Fig. 9A, Table 4). A decrease in mean PCI was observed in summer (Fig. 9A), but the differences were not statistically significant (Table 4).

In males, we observed a seasonal pattern, presenting a significant increase of mean GI in summer and autumn (Fig. 9B, Table 5), while there were no significant differences in mean PCI throughout the year (Fig. 9B, Table 5).

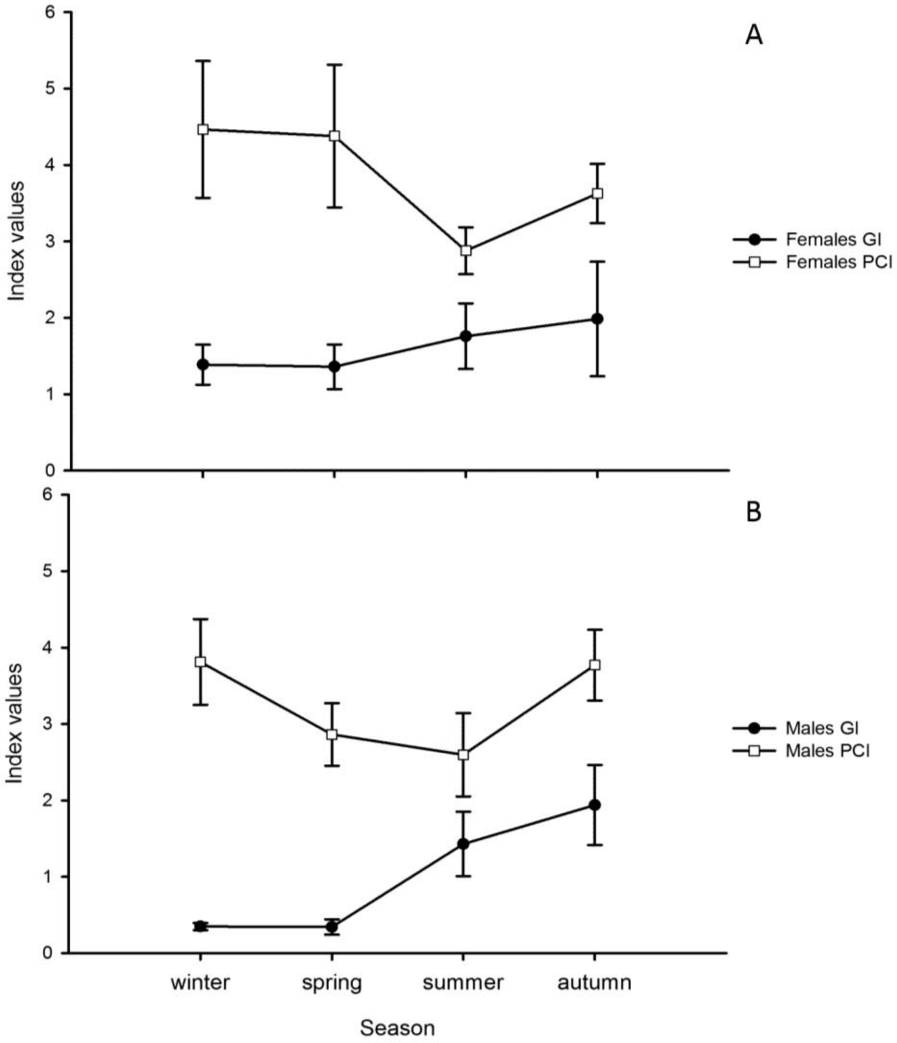

Figure 9. Seasonal changes in Gonad Index (GI) and Pyloric Caeca Index (PCI) (mean ± SE) in females (A) and males (B).

Table 4. Mann-Whitney U values for females GI and PCI values between seasons. Significance of the U values (*U*) is indicated using: $* P < 0.1$; $** P < 0.05$; $*** P < 0.001$. Distance values with no asterisk indicate non-significant values.

GI	winter	spring	summer
spring	207		
summer	154	89	
autumn	199	116	79
PCI	winter	spring	summer
spring	210		
summer	148	86	
autumn	199	114	76

Discussion

The density of the asteroid *C. grenadensis* was similar at the different depths sampled on the continental margin of the north-western Mediterranean Sea, with a slight, but not statistically significant, increase at 1500-1750 m. This pattern coincides with what has been reported for other echinoderms, such as the holothurian *Mesothuria (Allantis) intestinalis* (Ascanius, 1805) Östergren, 1896, found in large numbers at 1600 m depth in the Balearic basin (Cartes *et al.*, 2009), and invertebrates species (i.e *Aristeus antennatus* (Risso, 1816)) (Sardà *et al.*, 2003). Sporadic food inputs at these depths provided by particulate matter transport down nearby submarine canyons (Sanchez-Vidal *et al.*, 2008) have been suggested as one of the possible factors for the higher presence of invertebrates at these intermediate depths (Sardà *et al.*, 2003; Cartes *et al.*, 2009). The biomass pattern of *C. grenadensis* presented the inverse trend than density, with a decrease with depth and a significantly lower biomass found at 2000-2200 m compared to the other depth strata. This pattern coincides with the biomass patterns observed for the whole megafauna from the same area (Tecchio *et al.*, 2013). This decrease of megafaunal biomass with depth in the north-western Mediterranean has been related to the decrease of the megafauna individual size (Stefanescu *et al.*, 1993; Moranta *et al.*, 1998).

A decreasing trend of the individual size with depth was also observed for *C. grenadensis*. If we considered exclusively adult specimens, the observed bathymetric changes in size structure were in accordance with the deep-sea dwarfism theory (Harvey *et al.*, 1988), as found in the irregular echinoid *Brissopsis lyrifera* (Forbes, 1841) in the Atlantic Ocean. Mecho *et al.*, (2014) also found this pattern in individuals of *B. lyrifera*, with the smallest individuals found at the deepest distribution range (1750-2250 m depth) of this species in the Mediterranean Sea. This phenomenon has also been observed in *Molpadia musculus* Risso, 1826 (Mecho, unpubl. data). The smaller size observed in certain deep-sea species has been related to the low food availability in the deeper areas compared with more productive shelf and upper slope habitats (Rex *et al.*, 2006). Food limitation could be particularly important in the deep Mediterranean seafloor, because of the oligotrophic nature of the Mediterranean waters (Company *et al.*, 2003; Zúñiga *et al.*, 2009).

Juvenile specimens of *C. grenadensis* were only caught deeper than 1750 m depth. The lower bathymetric distribution of the juveniles compared to the adult population has been observed also for other asteroids, such as *Luidia sarsii sarsii* Duben & Koren in Duben 1845, and *Pontaster tenuispinus* (Düben & Koren, 1846), both in the Porcupine Seabight (Sumida *et al*., 2001; Howell *et al*., 2002). Additionally, a previous analysis of the same smaples reported juveniles of *C. grenadensis*, below the previously reported adult lowest depth of distribution in the Western Mediterranean Sea (South Balearic Sea), thus extending the maximum depth range of distribution for this species down to 2845 m (Mecho *et al*., 2014). The factors driving this depth-related recruitment pattern in several species of deep-sea asteroids is not fully understood and further information on larval distribution and dispersal in relation to environmental variables is necessary (Howell *et al*., 2002).

The gonads and pyloric caeca indices increased with depth, with maximum values observed at the deepest stratum (2000-2200 m). A higher GI value at the deeper species distribution range was also found in the Atlantic asteroid *Solaster endeca* (Linnaeus, 1771) (Ross *et al*., 2013). Ross *et al*., (2013) attribute these higher GI values to the larger oocyte size of the individuals distributed at their maximum depth of distribution. However, no bathymetric differences in oocyte size were found in the present study. We suggest that, in Mediterranean *C. grenadensis*, individuals dwelling at greater depths probably increase their reproductive effort by reducing the somatic investment (small sizes) and devoting a higher amount of energy to oocyte (i.e. higher GI values). The PCI values quantified for females *C. grenadensis* caught at 2000-2200 m depth were four times higher than values recorded at 900-1050 m. This high PCI recorded from individuals in the lower slope suggest that these females may be storing nutrients in the pyloric caeca in order to maintain a constant production of gametes in a food-limited environment (Benítez-Villalobos & Díaz-Martínez, 2010), allowing a constant transfer of energy from the pyloric caeca to the gonads (Mcclintock *et al*., 1995).

The gonad index values were higher in autumn, while lower pyloric caeca index values were reported in summer, coinciding with other studies (Mcclintock *et al*., 1995). Our results suggest a transfer of nutrients from the pyloric caeca to the gonads during periods of gonadal growth (summer–autumn). Higher reproductive activity in autumn have also been described for deep-sea fishes in the same study area (Fernandez-Arcaya *et al.*, 2013a; Fernandez-Arcaya *et al.*, 2013b). We observed that in males, PCI values started to decrease in spring and the GI started to increase at the following season (i.e. summer). In females PCI decreased in summer with a subsequent increase GI in autumn. Thus, males seem to have a maximum gonad development before females. Unless if this time-separation was usually shorter (i.e. minutes or hours) (Mercier & Hamel, 2008) here it could be an adaptation to high fertilisation success by ensuring that sperm is available when females start spawning.

In females, the distribution of oocytes remains constant thorough seasons and depths, with the ovary mainly occupied by previtellogenic and medium vitellogenic oocytes and a low percentage of vitellogenic oocytes in the largest size range. This constant presence of all kind of oocytes thorough the year was also reported in the Atlantic Ocean for *Styracaster elongatus* Koehler, 1907 (Benítez-Villalobos & Díaz-Martínez, 2010) and bathymetrically for *Henricia lisa* A. H. Clark 1949 (Mercier & Hamel, 2008), were the deepest specimens presented a constant presence of all kind of oocytes.

In deep-sea echinoderms, quasi-continuous reproductive patterns as well as seasonal patterns have been described (Ramirez-Llodra, 2002a; Mercier & Hamel, 2008; Baillon *et al.*, 2011). For deep-sea Mediterranean echinoderms, the reproductive biology has been only described for one species, the echinoid *Brissopsis lyrifera,* in the Gulf of Lion. This species has a well-defined seasonal pattern of reproduction, with maximum gonad maturation and spawning in autumn (Ferrand *et al.*, 1988), and presenting small eggs between 15 and 80 µm. The ovaries of *C. grenadensis* from the Mediterranean presented a wide range of oocyte sizes at all seasons and depths, with only a few mature gametes at any single time. This oogenesis patterns is characteristic of semi-continuous reproduction (Young, 2003). Thus, *C. grenadensis* follows a similar reproductive pattern than what has been described for many deep-sea echinoderms (Mcclintock *et al*. 1995; Ramirez-Llodra *et al*., 2002b; Galley *et al*., 2008), which has been related to the low food availability at great depths (Mcclintock *et al*., 1995). The deep Mediterranean seafloor is particularly food-limited because of the overlying relatively oligotrophic waters (Company *et al*., 2003; Tyler, 2003; Zúñiga *et al*., 2009). We suggest that this environmental food limitation together with the capacity of storing and redistributing nutrients from the pyloric caecum (high PCI values all year round) play key roles in shaping the semi-continuous reproductive pattern observed in *C. grenadensis*.

The diversity of eggs sizes in echinoderms, estimated from the largest oocyte size in the ovary, results in different nutrient input in the spawned egg and thus differences in larval development (Eckelbarger & Watling, 1995). In *C. grenadensis*, the eggs size are relatively large (650–700 µm), similarly to other studied species of deep sea asteroids in Atlantic waters, such as *Styracaster elongatus* Koehler, 1907 and *Hyphalaster inermis* Sladen, 1883) (Ramirez-Llodra *et al*., 2002b; Young, 2003; Benítez-Villalobos & Díaz-Martínez, 2010). This large egg size should to provide enough energy to the embryo and larva for pelagic until settlement (Benítez-Villalobos & Díaz-Martínez, 2010). The lecithotrophic larvae do not need to feed in the water column and thus provide an advantage in food-limited environments such as the deep Mediterranean Sea (Eckelbarger & Watling, 1995; Ramirez-Llodra, 2002a).

In summary, the population structure and reproductive biology of *C. grenadensis* has been described for the first time. The results suggest that adult size and somatic growth in *C. grenadensis* is lower in the individuals distributed at the deeper range of the species, where food quantity and quality are lower. Nonetheless, the reproductive output was higher in the individuals distributed at deeper depths, suggesting a higher investment in reproduction in detriment of somatic growth. Seasonally, highest values of GI were observed in autumn, suggesting a higher spawning capacity in this season. The gametogenesis of *C. grenadensis* form the bathyal Mediterranean Sea is similar to that of other deep-sea echinoderms, and showed a quasi-continuous production of sperm and large oocytes, which has been reported as a common reproductive pattern for deep-sea echinoderms.

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Visual faunistic exploration of geomorphological human-impacted deep-sea habitats of the north-western Mediterranean Sea

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Resumen

Las observaciones realizadas *in situ* del Mediterráneo profundo y su fauna, siguen siendo escasas a día de hoy en comparación con otros océanos y mares. En este estudio, se describe por primera vez la composición y distribución de la megafauna demersal proveniente de tres hábitats diferentes del Mediterráneo noroccidental como son cañones, montes submarinos y deslizamientos de tierra submarinos. Esto fue posible mediante el uso de un Remote Operated Vehicle (ROV) a profundidades comprendidas entre los 60 y los 800 m de profundidad. En las 30 horas de video obtenidas (equivalente a 14.5 km de suelo marino muestreado), se describieron un total de 4519 observaciones faunísticas. Estas observaciones se clasificaron taxonómicamente hasta el nivel más bajo posible. Posteriormente, estos datos fueron analizados en relación a los factores ambientales como son topografía, tipo de substrato y profundidad. Igualmente se cuantificó el impacto antropogénico presente en las diversas zonas. Este impacto se agrupó en cuatro categorías: redes de pesca, palangres, marcas de arrastre de pesca y otros tipos de broza (botellas de plástico, cristal, zapatos, cajas, etc...). Los resultados obtenidos indicaron la presencia de hábitats considerablemente diferentes en los cuales la broza y las marcas de arrastre son los efectos antropogénicos más observados. Los diferentes hábitats muestreados presentan similitudes en cuanto a composición faunística en relación al tipo de substrato, profundidad y topografía. Esto permitiría predecir la composición faunística presente en otras áreas desconocidas de características similares.

Abstract

In situ observations of the deep Mediterranean Sea and its associated fauna are presently scarce in comparison to those conducted in other oceans. This study reports, for the first time, the composition and distribution of demersal megafauna from different north-western Mediterranean submarine habitats such as canyons, sea hills and landslides between 60-800 m depth, by Remote Operated Vehicle (ROV). From a total of 30 hours of video recording (equivalent to 14.5 km of surveyed seafloor), 4519 faunistic observations were made, being items identified to the lowest possible taxonomic level and analysed in relation to environmental factors (i.e. topography, substrate type, and depth). In addition, anthropogenic impact was quantified by grouping observations in four categories: fishing nets, longlines, trawl marks and other litter. Our results indicate the presence of a complex collection of markedly different habitats in which litter and trawl marks were the most observed signs of anthropogenic impact. The different targeted environments showed similarities in faunal composition according to substrate, depth, and topography. This aspect justifies a seascape approach in further ecosystem studies within north-western Mediterranean deep-sea areas. Canyons, sea hills and landslides can be classified as seascape units, since sharing similar compositions and distributions of taxonomic groups. This would allow faunistic predictions in other presently unexplored but similar areas in the western basin.

KEY-WORDS: north-western Mediterranean; faunal composition; ROV; behaviour; anthropogenic impact; seascape; submarine canyon; sea hills; submarine landslide, trawl marks, litter

Introduction

The identification of deep-sea environments to be considered as "essential habitats" for the conservation and management of benthic biodiversity is currently a major focus of European Community research programs [1]. In this context, the study of cold seeps, mud volcanoes, seamounts, and canyons as "hot spots" for local biodiversity is of strategic relevance, when aiming to the ecosystem integrated management and conservation of demersal sources [2,3]. In this scenario, the deep Mediterranean Sea hosts a complex collection of geological and ecological relevant environments for which faunal composition and local biodiversity are largely unknown [4]. *In situ* video observations of Mediterranean deep-sea fauna are presently much more reduced in comparison to those conducted to date in other geographical areas [5–9]. The western Mediterranean basin is not an exception. Its middle slope encompasses markedly different geomorphologic structures, occurring over short geographic distances of few kilometres [4,10]. This asset may potentially result in a highly variable faunal composition, but only few data have been gathered up to date [3,11], because of the difficulty in performing sampling with classic methodologies such as bottom trawling in most of these sites.

The presence of submarine canyons is one of the most distinctive features of the north-western Mediterranean continental margin [10,12–14]. Canyons are large incisions in the continental shelf and slope that often result in abrupt depressions just a few miles off the coastline. They act as conducts of concentration and downward transport of sediment and organic particles [12–17] as well as drivers for local current regimes [18,19]. Their biodiversity and species distributions have been object of study in the past two decades mostly by indirect sampling methods like trawl hauling [14,20–24]. Sea hills are also present in the north-western Mediterranean area [25], defined as topographic structures that rise up to 500 m above the surrounding seafloor, with their morphology largely shaped by their lithological nature. These topographic seafloor features can alter the local hydrological regime and sedimentation. In general, they expose hard substratum that makes them ideal spots for sessile filter-feeder fauna [26–29]. Nevertheless, only recently have submarine hills received some scientific attention in the north-western Mediterranean area [30]. Finally, a third type of structure is represented by muddy continental shelves and slopes, the latter affected in certain areas by submarine landslides [31]. These habitats have been studied typically in relation to species distribution and biodiversity, mostly when commercially trawl fisheries also occur [21,32–35].

All these complex geomorphologic structures from the deep Mediterranean Sea are presently threatened by a diversified variety of anthropogenic impacts, spanning from the accumulation of litter [36–39], to fisheries activity, including lost or discarded gears (i.e. nets and longlines), as well as trawling itself that produces consistent habitat damage [40–42]. Also, the ecotoxicology of dispersed chemical compounds at plastic decomposition acts on the metabolism of species and on the dynamism of resulting trophic webs [43–45]. For all these reasons, the quantification of anthropogenic impacts on deep-sea ecosystems is presently a source of concern for both the science community and policy makers [46].

The use of ROV video-imaging surveys for the description of species compositions, ethology, and anthropic impacts has increased worldwide in recent years [2,11,36,46,47]. In this context, the objective of the present study is to describe and compare, by means of ROV imagery, the faunistic distribution in different deep-sea geomorphologic zones of ecological relevance within the north-western Mediterranean area. In order to do so, we selected habitats for which there was very limited or no direct (i.e. ROV observations) or indirect (i.e. trawling) data about the inhabiting megabenthic communities. In addition, we quantified the anthropogenic impact within each area. As an important by-product of this exploration, we also described a number of relevant ethological aspects for some species.

Materials and Methods

Data collection

The ROV *Max Rover II* of the Hellenic Centre of Marine Research (HCMR) was used to conduct visual observations along seven seafloor transects (referred from now as “Dives”) during the research cruise EUROLEON, which took place in October 2007, on board *BIO Hespérides*. The ROV survey imaged a total of 14.5 km, equivalent to a total of 30 hours of video of the seafloor (Table 1).

Table 1. Depth range (m) and km of surveyed seafloor of the seven dives conducted during the exploratory surveys in different geomorphological deep-sea zones of the Balearic Sea.

Dive number	Dive location	Geomorphology area	Substrate type	Bathymetric range (m)	Surveyed km
1	Blanes Canyon	Submarine canyon	Mud+Sand	70-450	2.5
2	Blanes Canyon	Submarine canyon	Mud+Sand	60-450	0.7
3	Gulf of Valencia	Sea hill	Mud+Rock	450-800	3.7
4	Eivissa Channel	Sea hill	Sand+Rock	280-500	0.6
5	Eivissa Channel	Sea hill	Sand+Rock	196-250	2.2
6	Eivissa Channel	Landslide	Mud+Rock+CoR	575-600	2.0
7	Eivissa Channel	Landslide	Mud	650-700	2.8

Typologies of observed substrate are indicated as follow: CoR, coral rubble; Mud; Sand and Rock.

The ROV was equipped with two wide angle colour CCD cameras, offering a frontal and a lateral view, plus a third one with a macro-zoom. Lighting asset was composed by 2 x 100 W HID lights and 4 x 150 W Quartz lights. ROV speed and seabed height during filming operations were approximately 2.3 knots and 2 m, respectively.

Three areas of the north-western Mediterranean were video-surveyed (Fig. 1): the Blanes Canyon, the Gulf of Valencia, and the Eivissa Channel. A total of seven dives (Table 1) were performed in the three areas, providing data from 3 distinct geomorphological zones (Fig. 1): dives 1 and 2 in the Blanes canyon head (depth range 60-450 m) [48]; dive 3 in an unreported sea hill in the Gulf of Valencia (depth range 450-800 m); and dives from 4 to 7 in the Eivissa Channel, two of them (dives 4 and 5) along a small flat-topped sea hill (depth range 280-500 m and 196-250 m), and the other two (dives 6 and 7) close to the escarpments of two submarine landslides (Jersi and Ana landslides) [49–51]. These mass wasting events occurred about 60 ka [50], over a depth range of 575-600 m and 650-690 m, respectively.

Data processing and analysis

Video considered for the faunistic analyses was obtained from the frontal CCD camera. All faunistic characterizations were performed in a *time-lapse* mode (i.e. at 50% of real speed), using the software application Intervideo WinDVD 9.0 (Windows).

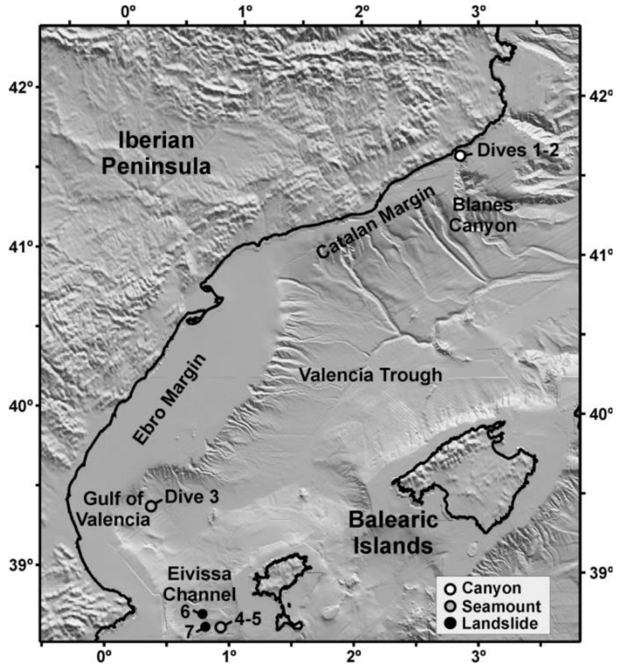

Figure 1. Study area. Blanes canyon head in the Balearic Sea, sea hills in the Gulf of Valencia and Eivissa Channel and landslides in the Eivissa Channel.

All observed organisms were classified to the lowest possible taxonomical level. For a more precise taxonomic determination, digital frames were extracted after video partitioning. Classification was accomplished by the use of currently used taxonomical guides [52–54].

Data on faunal composition, substrate type (i.e. mud, rock, sand, and coral rubble), ethology, and anthropogenic impact were annotated and coupled to dive time. For that purpose, we firstly grouped observed organisms in six taxonomical groups. Five of them corresponded to the four more frequently observed Phyla (Porifera, Cnidaria, Echinodermata, and Chordata) and Subphyla (i.e. Crustacea). We also considered a sixth miscellaneous group (named as “Other”), made by those Phyla reported at a too low frequency to be considered as individual units for the faunistic analysis (i.e. Brachiopoda, Annelida, Ctenophora and Echiura).

ROV navigation tracks were processed and recalculated to eliminate outliers produced by navigation errors and lost pings, and, finally, filtered to smooth the track. Quality control has been performed by matching the time variable with depth readings of the ROV related to the video record and the bathymetric depth of the multibeam maps based on the ROV track navigation.

All observations were loaded in a database according to their timing of occurrence in the video footage and then elated to the exact geographic location, through the coupling with ROV navigation data. Observations were grouped over a distance of 100 m along the ROV navigation track, allowing for comparisons in different sections. We reported the number of taxonomic groups by distance (i.e. each 100 m) in relation to substrate type, depth, and geomorphological structure.

The same procedure was done for anthropogenic impact. For a better visualization of faunistic spatial trends, the numbers of individuals per each taxonomic group were plotted by 100 m and represented along each dive transect. Finally, behavioural observations were reported and classified when occurring in videos more than twice, as suggested by other studies [55].

Statistical methods

To identify significant connections between taxonomical groups, abundances, and habitat parameters, levels of similarity among taxa were ordered in a two-dimensional plane through count-based distance matrices with the non-metric multidimensional scaling (NMDS). MetaMDS function in `vegan’ library [56] in R (R Project for Statistical Computing, http://www.r-project.org/) was used to perform the taxa ordination. Then, ordered taxa were regressed against the depth (continuous variable), using Generalised Additive Models (GAMs).

The function gam in `mgcv’ library [57] in R was used to fit the regressions. GAMs allowed identifying linear and non-linear connections between taxa ordination and depths.

Finally, to identify connections between ordered taxa and substrate type, and habitats (factor variables), factor fitting permutation tests were applied using the function ‘envfit’ in the ‘vegan’ package in R. The method calculated centroids for the factor variables and fitted them onto taxa ordination.

Results

General remarks

We reported a total of 4519 faunistic observations (Table 2) from three different geomorphologic features (i.e. canyon, sea hill, and landslide) (Fig. 1). The fauna belonging to the Phyla Chordata, Cnidaria, and the Subphylum Crustacea was the most abundant (Fig. 2), respectively representing 25%, 23% and 20% of the total observations. The Phylum Porifera was less abundant (12%), while Echinodermata represented only the 6%. Finally, animals within the category “Other” (i.e. Brachiopoda, Annelida, Ctenophora, and Echiura) together summed up 14%.

Table 2. Number of observations for each taxonomical group observed at each geomorphological zone.

Groups	Canyon	Sea hill	Landslide	Total obs.
Porifera	5	550	5	560
Cnidaria	252	551	244	1047
Crustacea	204	227	472	903
Echinodermata	49	87	141	277
Chordata	162	363	577	1102
Other	74	496	60	630
Total obs.	746	2274	1499	4519
%	16	51	33	100

There were no significant differences in taxa composition and abundance between habitats (Table 3, Case 1). The substrate type significantly explained taxa composition in all the habitats together and within habitats, except for the case of the landslide where species are not significantly related to that parameter (Table 3, Case 4).

Finally, depth significantly explained taxa composition in all the habitats together and within habitats, particularly in the sea hill, where depth accounted for about a half (47.4%, Table 3) of the taxa structure.

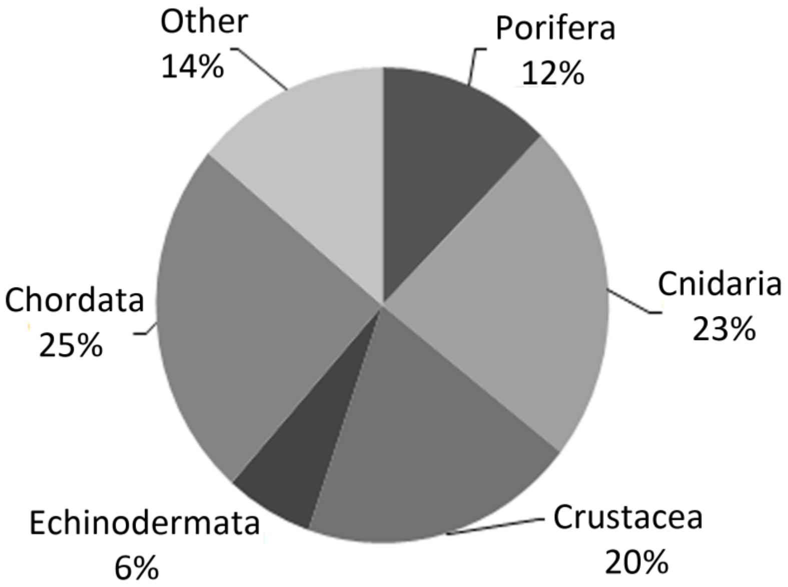

Figure 2. Percentage of total faunistic observations

Table 3. Summary of statistical validations for the connections between taxa ordination and environmental variables.

Response variable	Environmental variable	Statistical Method	Significance (p)	Variability explained (%)
1. Taxa in all the habitats	Habitat	FF	* 0.10729	2.5
	Sediment	FF	0.00033	11.7
	Depth	GAM	< 0.00001	17.9
2. Taxa in the canyon	Sediment	FF	0.003	16.4
	Depth	GAM	< 0.00001	17.8
3. Taxa in the hill	Sediment	FF	0.001	19.0
	Depth	GAM	< 0.00001	47.4
4. Taxa in the landslide	Sediment	FF	* 0.939	1.2
	Depth	GAM	< 0.00001	37.1

**Non-significant result. For the factor fitting (FF) method,* p-*values based on 1000 permutations. For GAMs,* p-*values of the smooth term are provided*

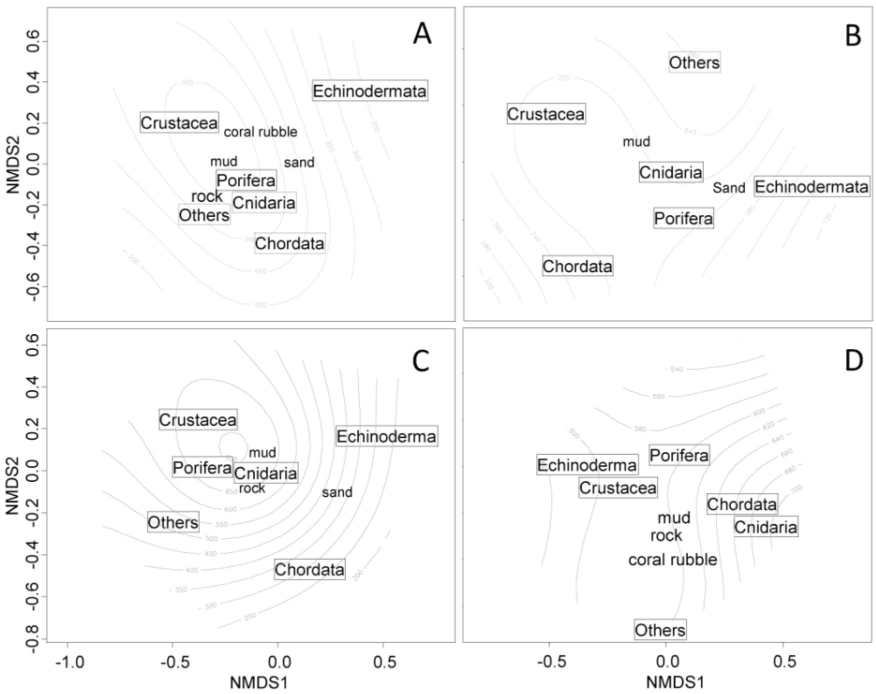

Figure 3. Spatial ordination of taxa composition and abundances related to depth (m) (grey curves), and sediment types, for A) all the habitats together; B) for the canyon only; C) for the hill only and D) for the landslide only.

When we considered all the taxonomical groups in all the habitats together (Fig 3A), we observed that the Phylum Echinodermata prefers shallower depths whereas Porifera, Cnidaria, and Crustacea prefer deeper zones. We also show that Echinodermata is the more dissimilar taxa (because of its abundance) compared to the others taxa (it appears far from the others in the spatial ordination plot). Finally, individuals of the Crustacea seem to be found closer to coral rubble, whereas the Porifera prefer mud and sand. The “Other” were closely related with rocky substrate.

Canyon head

A total of 746 faunistic observations were made in the western flank of the Blanes canyon head (Table 2, Fig. 1), where two transects covered a total distance of 3.2 km of seafloor. Two kinds of substrates were observed in the Blanes canyon head: a muddy area on the deepest part, and a sandy area with strong tanathocenosis on the shallower part of both dives. The two dives were similar in setting with the exception of the southern dive (no. 2), which crossed an area with a steeper slope in its deepest section. The Phylum Cnidaria was the most abundant, with a 34% of the total observations (Fig. 4A). This group had also the highest number of taxonomic groups (n= 14), as well as the highest number of individuals per group (i.e. the Anthozoan *Pennatula* spp. with

149 observations). The Subphylum Crustacea represented 27% of the total observations, most of them corresponding to the Infraorder Brachyura (i.e. crabs). This Phylum was followed by the Phyla Chordata (22%) and the Phylum Echinodermata (6%). For the latter, the Class Asteroidea and Holothuroidea were the most abundant. The group “Other” represented 10% of the total observations.

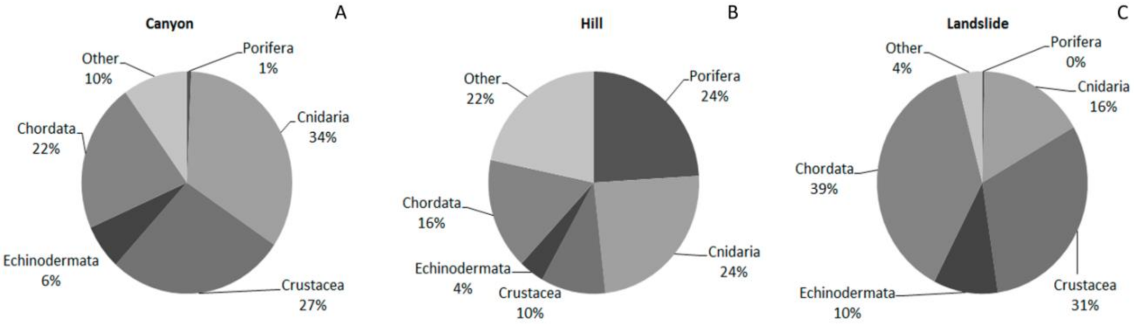

Figure 4. Percentage of faunal observations at each geomorphological zone studied. A) Canyon B) Sea hill C) Landslide

On the western flank of the Blanes canyon head, we could distinguish three different faunistic distributions (Fig. 5), related to depth and slope angle. The deepest part surveyed (450 - 250 m), showed a low number of observations and high diversity of Phyla. In general the area shows a two-step slope change at 250 - 300 m and 150 m depth. The deep areas with steep muddy slopes are dominated by crustaceans. From 150 to 60 m depth, the seafloor is relatively flat and it was dominated by the Phyla Cnidaria (Anthozoans, *Pennatula* spp.) and Echinodermata, mostly the Asteroidea *Anseropoda placenta* (Pennant, 1777) and the Holothuroidea *Parastichopus regalis* (Cuvier, 1817).

NMDS analysis in the canyon (Fig. 3B) revealed dissimilarity among taxonomical groups (these are not clustered in the plot). We also observed that Echinodermata remains shallower than the differences in depth are found in the habitat. Finally, Cnidaria and Crustacea were closely related with muddy substrate.

Sea hill

Two sea-hill transects were analysed (Fig. 1), one in the Gulf of Valencia and the other in the Eivissa Channel (Table 1). The first one presented a conical morphology in a depth interval from 450 to 800 m depth. The ROV transect comprised 10 hours of recorded images, resulting on 3.7 km (Fig. 6) of surveyed seafloor. The second sea hill in the Eivissa Channel was surveyed separately on its eastern flank and on its flat top, from 196 to 500 m depth, along 2.8 km (Fig. 7). Results will be described separately by sea hill and then total results of both sea hills.

The Gulf of Valencia sea hill (Fig. 6) raises from 800 m depth up to 450 m on the top. It is characterised by two kinds of substrates: a rocky area constituted by steep slopes and rocky substratum (from 450 to 600 m depth) and a large muddy plain surrounding the rocky area, from 600 to 800 m depth. A significant denser concentration of benthic fauna was observed on the shallowest rocky areas (Fig. 6A) in comparison to a drastically diminution of that fauna toward deepest muddy areas (Fig. 6B). The sea hill presented two well separated faunistic distributions related to these substrates and other taxa and they are related to sand, although no major depth. The rocky substratum was located on the flank of the seamount (Fig. 6A) and presented a fauna basically composed by benthic species in the Phyla Porifera (31% of the total observations within the rocky area), Cnidaria (25%, benthic species as corals, anemones and gorgonians) and “Other” (28%, mainly Brachiopoda). The second substratum, the muddy plain surrounding the rocky area (Fig. 6B), was dominated by Crustacea (33% of the total observations), Chordata (mainly fishes, 32%), and Cnidaria (mostly deep-sea anemones of the genus *Cerianthus*, 22%). In the case of the muddy plain, the distribution of the benthic communities was patchy along the dive and it was related to subtle changes of slope and substrate (Fig. 6B).

On the Eivissa Channel sea hill (Fig. 1), two areas were studied (Fig. 7): the upper slope (flank) and the flat top. The flank presented a south-eastern orientation in a depth interval between 280 - 500 m depth. The flank is subdivided in two areas with different substrates and slopes (Fig. 7A, Dive 4). At its bottom, we observed a flat area mainly composed of mud with boulders.

This area was dominated by motile fauna such as Crustacea (24% of the total observations of the flank) and Chordata (22%, mainly fishes), but included also sessile fauna (24%, as benthic cnidarians), on the cobbles. Moving upwards, the flank was occupied by rocky outcrops dominated by the benthic Phyla Porifera (14%), and “Other” (8%, in this case mainly Brachiopoda) (Fig. 7A).

The transect over the flat top of the Eivissa Channel sea hill (Fig. 7B, Dive 5) comprises only one substratum type, a bioclastic sand with sparse rocky outcrops. The top of the hill was dominated by motile fauna, including Chordata (fishes, 48%), Cnidarians (Class Scyphozoa, 26%) and Echinodermata (Class Holothuria, 14%). This transect covered the shallowest parts (196 – 250 m depth) of the surveyed area (Fig. 1) and it was dominated by shallow-water species of Cnidarians (*Pelagia noctiluca* (Forsskål, 1775)), Echinoderms (*Holothuria (Holothuria) tubulosa* Gmelin, 1791) and fishes. It is worth reporting the high number of fish schools observed over the rocky areas of the top of the sea hill.

A total of 2274 faunistic observations were reported from both sea hills over a distance of 6.5 km (Table 2). The most common observed groups were the Phyla Porifera and Cnidaria, representing each one 24% of the total entries (Fig. 4B). The group “Other” mostly consisted, of items within the Phylum Brachiopoda, representing 22% of all the observations. The Phylum Chordata (16%) and Subphylum Crustacea (10%) were less abundant in the sea-hill dives. The Phylum Echinodermata represented only a 4% of the total observation.

The NMDS analysis conducted in the sea hill (Fig 3C) showed that taxa in the hill are dissimilar among them (not clustering together in the plot) and that Echinodermata and Chordata were found in shallower depths with sand, whereas Porifera, Cnidaria and Crustacea prefer deeper areas with rocks and mud. The “Other” species were more abundant at middle depths.

Submarine landslide

Two submarine landslides (Jersi and Ana) were surveyed in the Eivissa Channel (see Fig. 1), resulting in 4.8 km of video recordings, from which 1499 taxonomic observations were made (Table 2). The depth range was similar in both studied sites, with Jersi landslide surveyed along the 575 m contour (Fig. 8A, Dive 6) and the Ana landslide between 650-700 m depth (Fig. 8B, Dive 7). The landslide scars consisted of more consolidated sediments and, in the Jersi landslide area, even rocky pebbles and coral rubble have been observed. The depositional areas of the landslides were composed of mud, similar in gross morphology to the undisturbed upslope area (i.e.

above the landslide scars). As for the sea-hill habitat, results are described firstly in separated mode for each landslide and then in general terms (comprising both landslides).

When we considered the landslides separately, we found Jersi landslide dominated by Crustaceans (60% of the total observations of this landslide), and Chordata (19%). None of the other groups exceed 8% in this landslide. We could observe an increase of Crustaceans on the scar area. Nonetheless, this landslide presented a mostly constant faunal composition along all its surveyed area (Fig. 8A).

The substratum along the Ana landslide was mostly mud (Fig 8B). Sediment along the scar area appeared as more consolidated. Ana landslide was dominated by Chordata (44% of the total observations of this landslide) followed by the Subphylum Crustaceans (24%), and the Phyla Cnidaria (19%) plus Echinodermata (10%). The Echinodermata were more abundant in scar than on the mud plain, and was basically constituted by the Class Ophiuroidea.

Comprising both landslides, the most representative groups were the Phylum Chordata (mostly fishes) and the Subphylum Crustacea, representing respectively the 39% and the 31% of total observations (Fig. 4C). The Cnidaria and Echinodermata were less abundant (respectively 16% and a 10%), and finally the group “Other” was only the 4% out of the total. The different faunal groups identified fit well with the topographic features recognised on the bathymetry (Fig. 8A, B).

Landslides scars, deposits and undisturbed seafloor had different phyla compositions and abundances. The most abundant fauna in the scars were the Crustacean. Landslide deposits were dominated by pelagic cnidarians (Order Coronatae) and Chordata (mainly fishes). Finally, the undisturbed seafloor upslope of the landslides was dominated by Crustaceans and Echinoderms (most of them belonging to the Class Ophiuroidea).

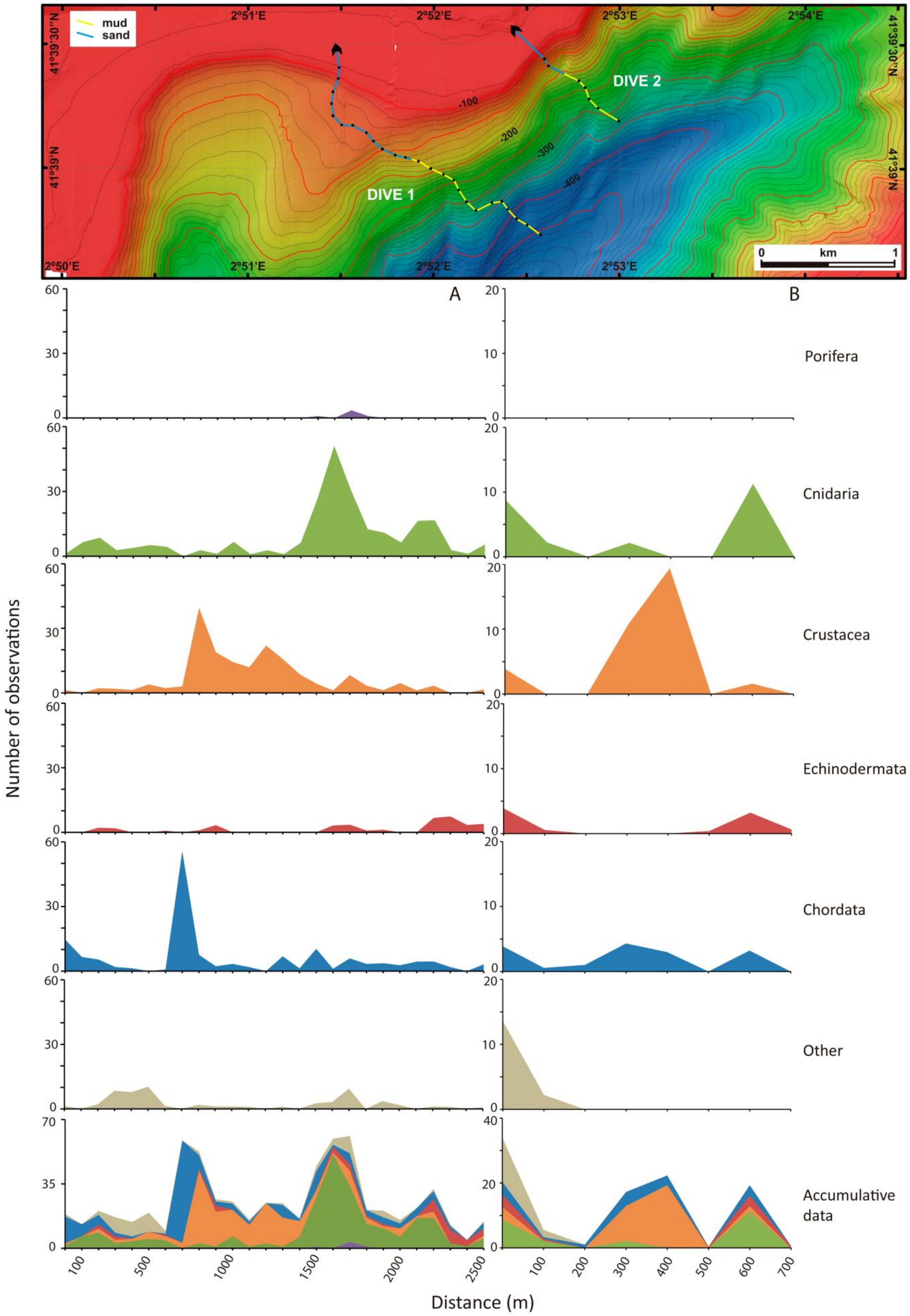

Figure 5. Blanes canyon head. Number of faunistic observations plotted by taxonomical group every 100 m. A) Dive 1; B) Dive 2

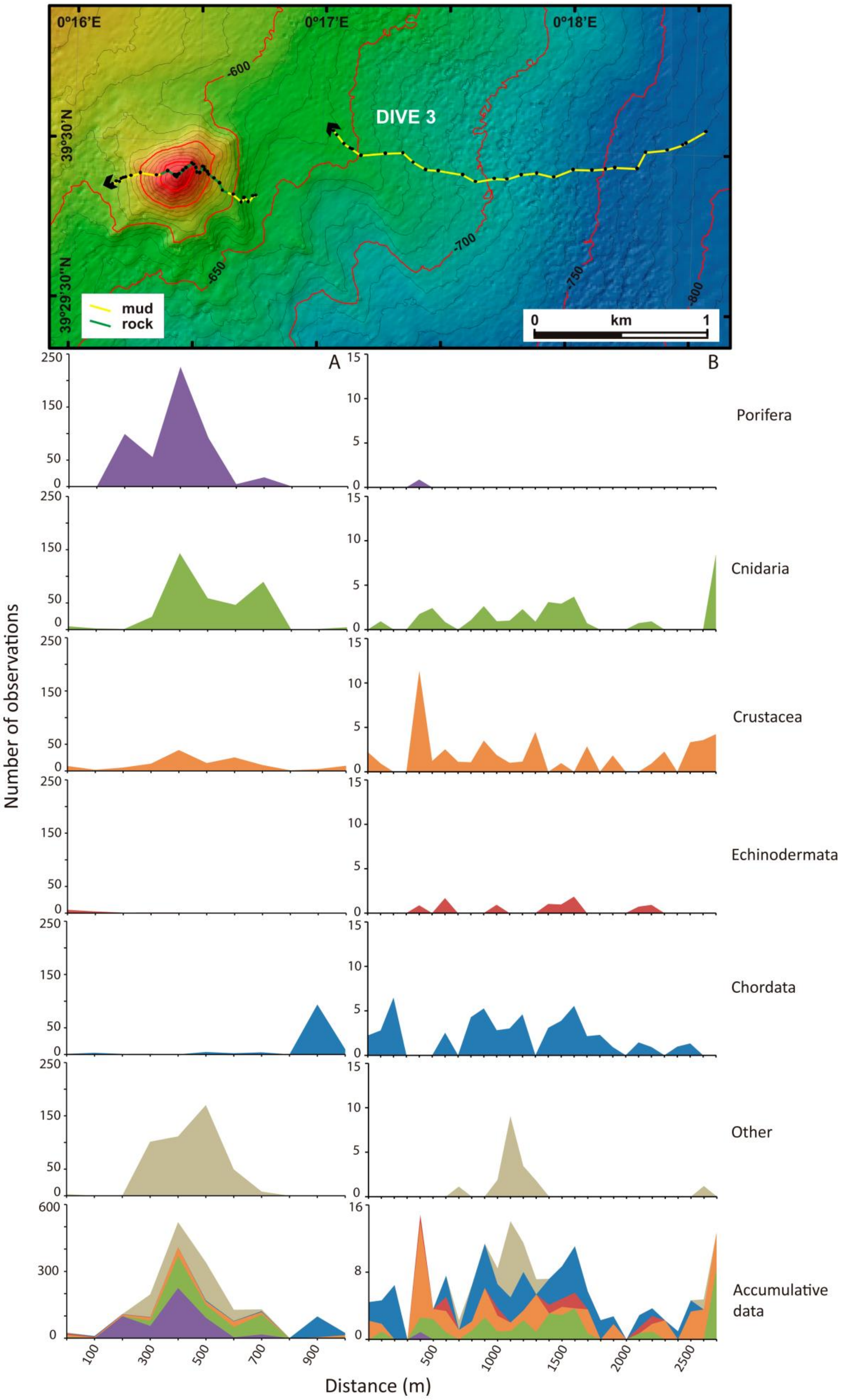

Figure 6. Gulf of Valencia sea hill. Number of faunistic observations plotted by taxonomical group every 100 m. A) Seamount rocky area; B) Surrounding muddy plain.

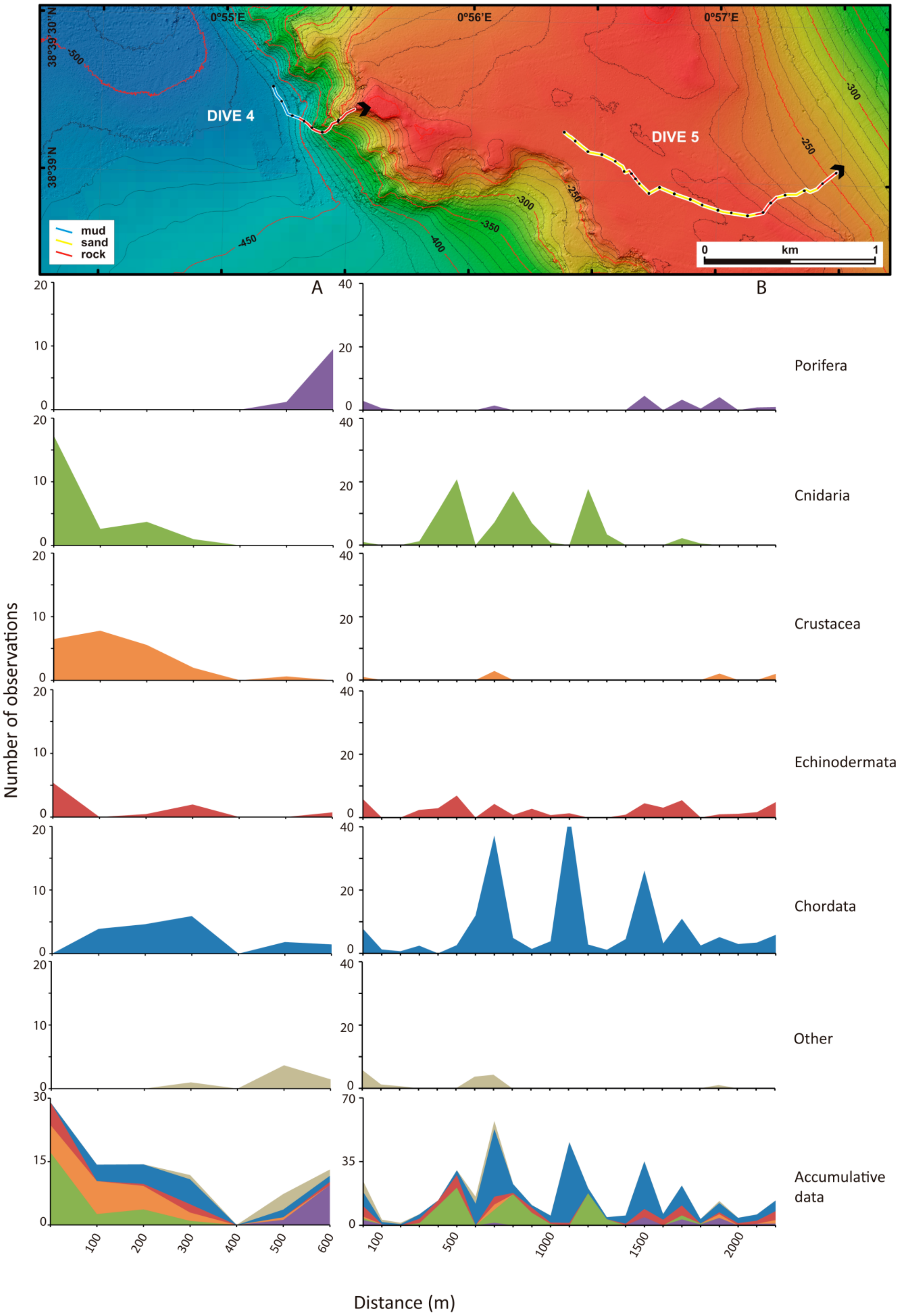

Figure 7. Eivissa Channel sea hill. Number of faunistic observations plotted by taxonomical group each 100 m. A) Dive 4, flank of the hill; B) Dive 5, flat top of the hill.

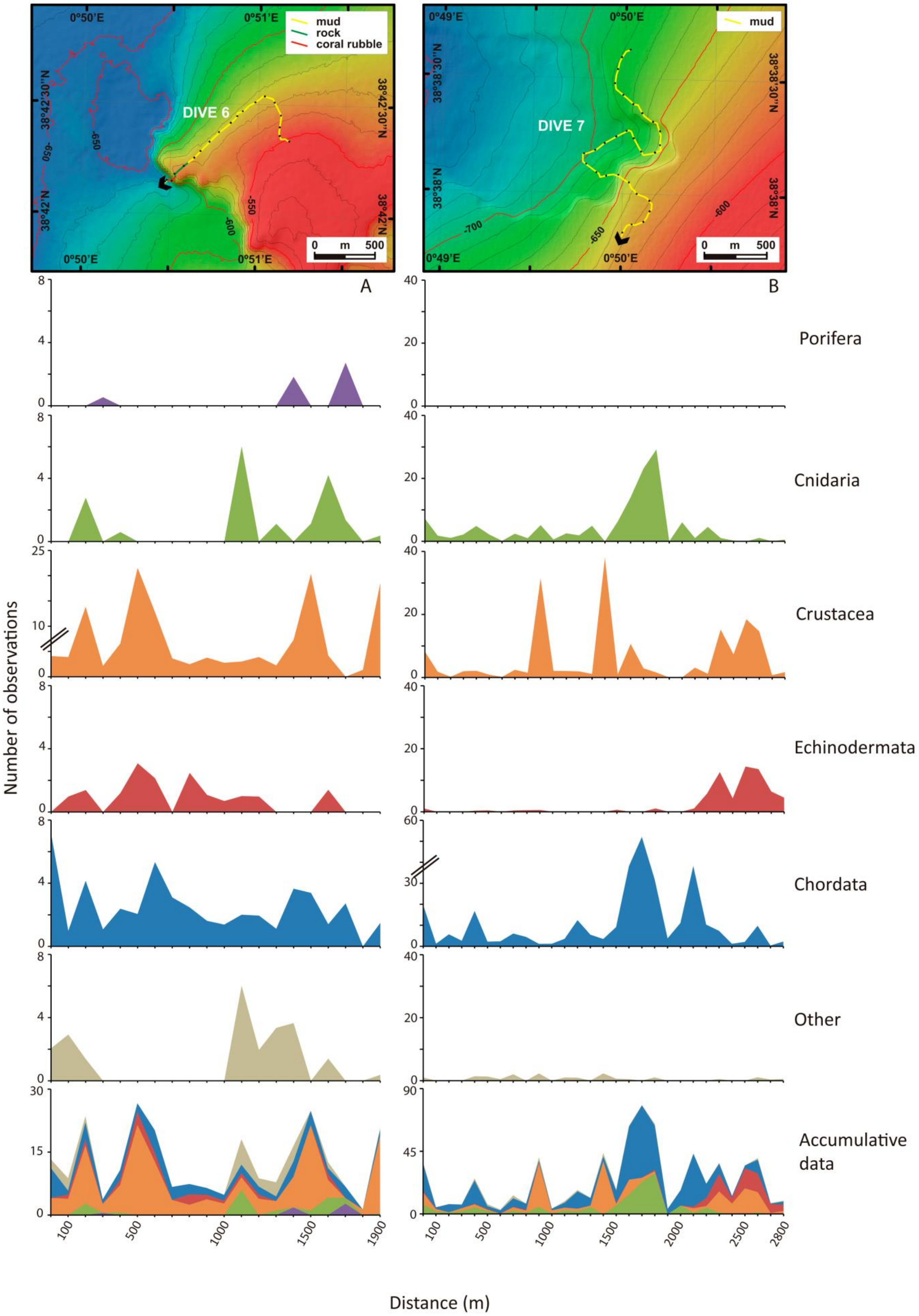

Figure 8. Eivissa Channel landslides. Number of faunistic observations plotted by taxonomical group each 100 m. A) Dive 6, Jersi landslide; B) Dive 7, Ana lanslide.

In the landslide habitat, the NMDS analyses (Fig 3D) conclude that substrate type did not significantly relate to taxa (see also Table 3). We also observed that Chordata and Cnidaria structures were similar and preferred deeper areas. Finally, the Echinodermata, Crustacea, and Porifera were close together in NMDS plots indicating a similar structure (i.e. species composition and abundances) and prefer shallower depths.

Anthropogenic impact within habitats

A noticeable level of anthropogenic impact was observed in all studied zones, with 158 recorded artificial artefacts of different kinds produced over an apparently large temporal scale. These items included plastic bags, cans, and bottles (Fig. 9A). Trawl marks were also consistently observed (Fig. 9B). Finally, lost or discarded fishing gear was also detected, including longlines (Fig. 9C) and remains of fishing nets (Fig. 9D).

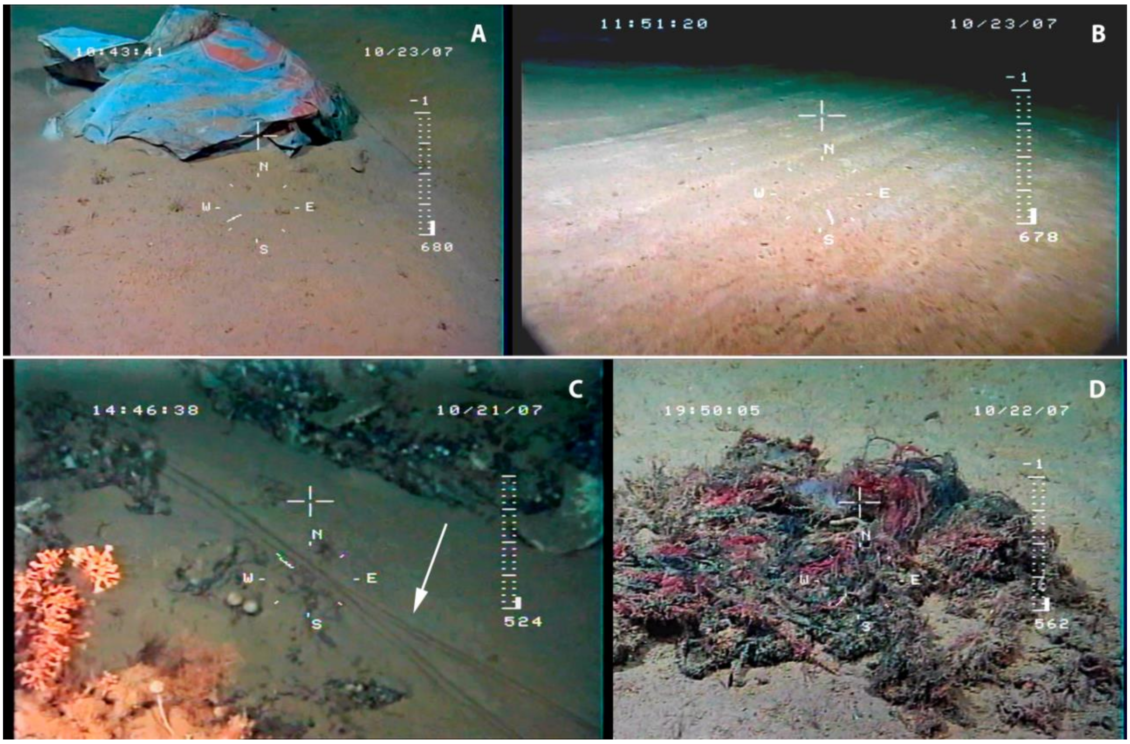

Figure 9. Different types of anthropogenic impact observed. A) Litter; B) Trawl marks; C) Longlines; D) Fishing net.

Overall, litter not classified as longlines or nets was the most abundant observation (39%), followed by trawl marks (30%) and longlines (28%), with lost or discarded nets being less abundant (3%) (Fig. 10). In the canyon head habitat, litter (plastic bags and bottles) represented 79% of the total observations, whereas longlines accounted for 14% and only 7% of the observations related to anthropogenic impacts were trawl marks. No fishing nets were detected (Fig. 10).

On the sea hills and their surrounding areas, 58% of the anthropogenic impact observed was represented by longlines, with a significant amount of other litter (22%), trawl marks (16%), and only 4% was constituted by fishing nets (Fig. 10). On the landslides, approximately half (45%) of the total anthropogenic observations were constituted by trawl marks and other litter (44%), being longlines (9%) and fishing nets (3%) less representatives (Fig. 10).

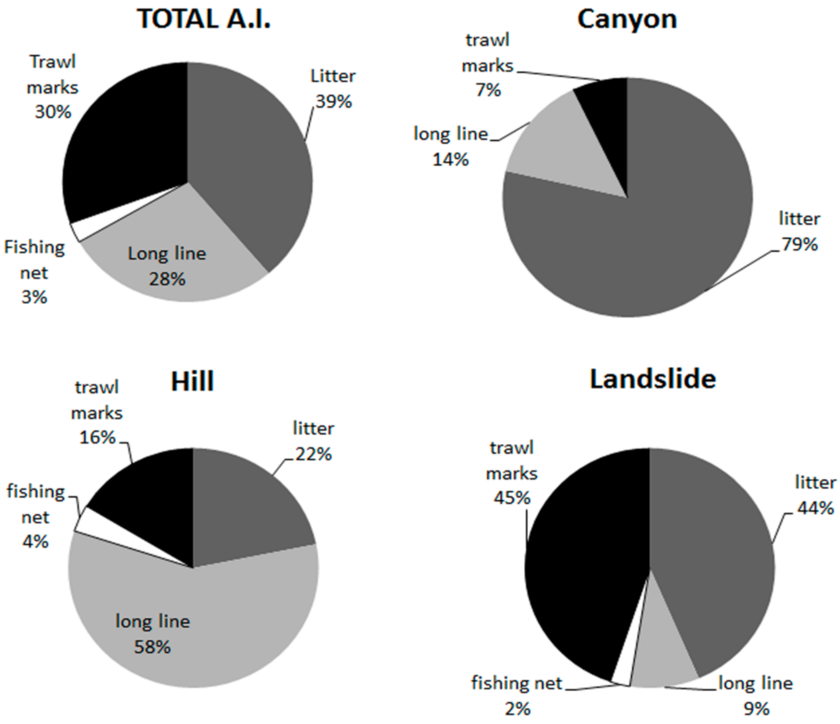

Figure 10. Percentage of total anthropogenic impact observed in the study and in each area.

Behavioural observations of identified species

Several behavioural observations were made for motile fauna during the ROV surveys. Within decapod crustaceans, individuals of the Family Galatheoidea were observed projecting forward their claws as the ROV approached, suggesting a marked territorial behaviour. Burrowing behaviour was also observed in an isolated individual of the Norway lobster (*Nephrops norvegicus*, Linnaeus, 1758) at 670 m depth (Fig. 11A). This animal showed a motile activity in relation to the occupation and emergence from a multiple-burrow system. That animal was observed as engaged in patrolling different burrow entrances, entering and exiting from them. Another behaviour displayed by decapods was related to camouflage. This was observed in six individuals of *Paromola cuvieri* (Risso, 1816), which were carrying white plastic bags and other artefacts on their carapace (Fig. 11B, C).

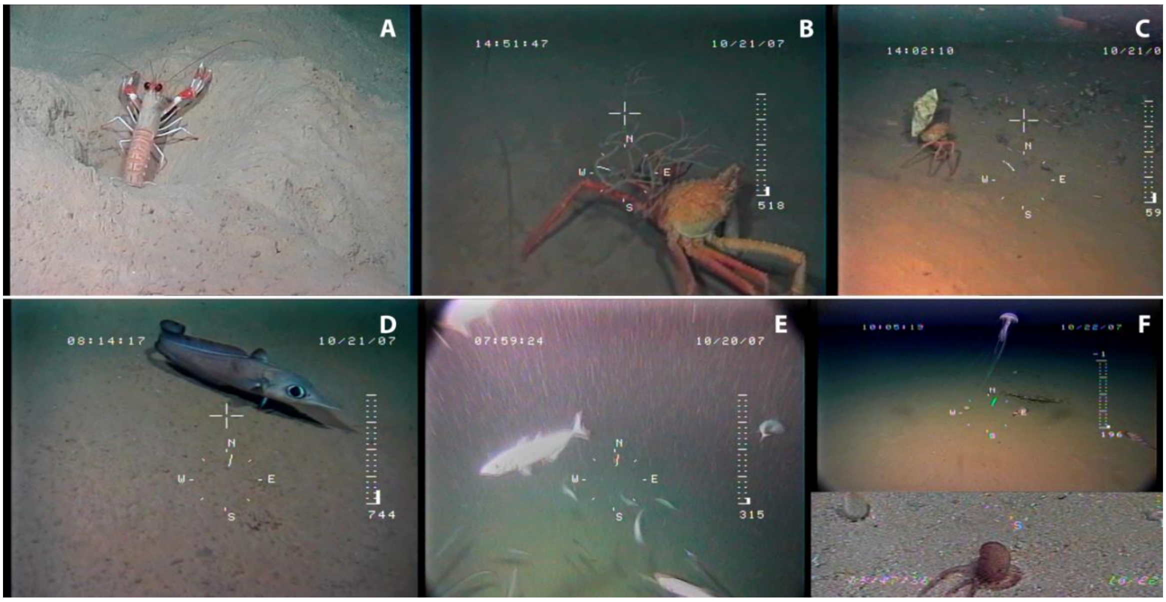

Figure 11. Behavioural observations. A) Territorial behaviour, from a Norway lobster, *Nephrops norvegicus*. B, C) Camouflage behaviour from *Paromola cuvieri*; C) The Macrourid *Trachyrincus scabrus* (Rafinesque, 1810), just before escape; D) *Trachurus sp.* Schooling; E) *Pelagia noctiluca* near the bottom. F) *Pelagia noctiluca*

Fish behaviour was also noted in relation to their reaction as the ROV approached. Evasion was typically observed in individuals of the Family Macrouridae (Fig. 11D), while other fishes (i.e. Order Scorpaeniformes) did not show alterations in their behaviour. Attraction to the lights of the ROV was never observed. Schooling behaviour was reported for *Trachurus trachurus* (Linnaeus, 1758), *Pagellus bogaraveo*, *Capros aper* (Linnaeus, 1758) and *Lepidopus caudatus* (Euphrasen, 1788) (Fig. 11E).

Finally, a peculiar observation was reported in relation to jellyfishes, mostly *Pelagia noctiluca* (Forsskål, 1775) and specimens from the Order Coronatae. These were observed swimming a few centimetres over the seabed. In the case of *P. noctiluca*, small groups were observed touching the seafloor over the top of the flat sea hill in the Eivissa Channel (Fig. 11F).

Discussion

In this study, we carried out for the first time *in situ* video-observations of the benthic communities inhabiting a set of geomorphologically diverse north-western Mediterranean Sea habitats. We produced spatially extended faunistic observations that indicate the presence of distinct species assemblages. Species composition was related to different seafloor characteristics, such as topography, substrate type, and depth. Other environmental factors, such as local hydrography or organic matter input may have been We observed similar faunistic composition in dives conducted in different areas of canyons, landslide, and sea hills. This suggests that substrate type and depth play a also playing a significant role in shaping the local faunal communities [58,59], but pertinent data were not gathered here strong role in driving species composition toward a convergence, in geographical regions hosting different topographic structures. That recurrent species composition across geography is of importance for the validation of canyons, open slope, sea hill, and landslides topographies as valid seascapes units [60]. In fact, analysed habitat features, play a key role in shaping benthic community composition and biodiversity at different geographic scales of continental margins, across different geomorphologies [59]. Clearly, a combination of topography and substrate seems to influence, as expected, the presence of filter-feeding animals. We showed how sponges, corals, gorgonians, and brachiopods are always strictly linked to hard substrate, being these either rocks or litter. Conversely, other groups of sessile fauna only appeared on soft sediments (i.e. anemone *Cerianthus membranaceus* and seapen *Pennatula* spp. (Linnaeus, 1758)). Gorgonians, sponges and corals appeared on hard substrate on hill areas where orientation and currents result adequate. Differently, other filter feeders were present in zones of both sporadic elevated hydrodynamism and turbidity, such as seapens on soft bottoms on canyon areas (see below).

Anyway, faunistic results are different for highly motile species such as fishes or crustacean decapods. For example, aggregations of motile macrozooplankton have been observed often above abrupt topographies such as sea hills/seamounts, submarine canyons and the steep slopes of shelf breaks, indicating that, beside the strong effect of currents on animals' motion, individual choice is oriented toward areas promoting high trophic architectures, which sustain richer communities [61]. We also observed that specific microhabitats, such as punctual rocks, may be important as topographic structures [62].

Depth was another parameter that shaped the zonation in our faunistic observations, constraining the presence of some species at certain locations, in a fashion that appeared to be independent from the local geomorphology or the type of substrate. Some shallow-water species (i.e. the Anthozoa *Pennatula rubra* (Ellis, 1761), the Asteroidea *Anseropoda placenta* (Pennant, 1777) and the Holothuroidea *Parastichopus regalis* (Cuvier, 1817), were not observed in deeper areas, even when suitable substrata was available. Similarly, deep-living species such as decapods of the genus *Plesionika* spp. or fishes belonging to the Order Stomiiformes and the Family Myctophidae were only observed below a depth threshold. Our data fit within species depth rages usually reported by trawling [63–65].

Another factor to be considered is water characteristics within the benthic boundary layer (i.e. the interface between the water column and bottom sediment [66]. This layer represents a high energy habitat, with several essential chemical components and suspended particles, which may sustain a specific associated fauna [67]. The high turbidity, organic matter content and hydrodynamism of the benthic boundary layer could represent a combination of conditions suitable for sustaining a high abundance of benthopelagic fauna such as shrimps and prawns. This high abundance of decapods was observed, for example, in the landslide areas. An increase in the abundance of jellyfishes was also observed in the benthic boundary layer, suggesting that this group may selectively target the benthic boundary layer faunas in a food-rich area. The abundance of small fauna in the benthic boundary layer [67] may induce the presence of predators or scavenging species, such as fishes and cephalopods, that were also detected in our ROV observations in close proximity to the seabed. Our results are discussed below separately for each geomorphologic zone.

Canyons

The majority of the observations on canyons corresponded to sessile fauna such as anemones, sea pens, sea fans, and tubular worms. All these taxonomical groups are suspension feeders and are common in canyons in the Balearic Sea [14,42,68]. Canyons increase heterogeneity of the shelf and slope muddy areas, by diversifying the types of available substrate (i.e. the combined presence of mud, sand or sandy sediment and rock). This results in the presence of a diversified associated fauna within small geographic scales [11,42,59,69–71]. The specific topographic and physical characteristics of these habitats deeply condition the faunistic composition and community structure of the canyon fauna, which are often different within the canyon itself and in the surrounding areas [14,24,61,72]. For example, the Blanes canyon experiences an internal down streaming flux of particles, three times higher than that reported on the surrounding open slope [58]. Additionally, some punctual processes of large magnitudes found on the northern Balearic Sea, such as dense shelf water cascading, produce a significant input of nutrients that affect the communities due to the resuspension of organic matter and enhance the recruitment of some deep-sea species [13,23].

The higher concentration of organic matter and associated presence of zooplankton aggregations in the head of some canyons [73] may produce a higher biodiversity and/or abundance of resident benthic fauna [42,68]. The high number of taxonomical groups in this area seems to be justified by the shallow depth [72,74].

Sea hills

Faunistic differences between the Gulf of Valencia and the Eivissa Channel hills were observed in the present work. These differences could be related to their topographic characteristics, and depth, in turn influencing substrate types, local hydrography, and food availability. The hill of the Gulf of Valencia, with its conical shape, presented a

Porifera community and hard coral fauna, related to the abundance of hard substrate, as well as to sediment pressure and hydrodynamism. On the other hand, the flat topped sea hill in the Eivissa Channel showed a dominance of motile fauna such as crustaceans and fishes, probably associated to the bioclastic sand. Another important factor to take into account when analysing the faunal community composition and structure of sea hills and seamounts is trawling activity [75]. Flat topped sea hills and seamounts may present a modified faunal composition from their original biodiversity caused by the impact of commercial fishing activity [76]. In fact, trawl marks were observed at the top of the flat hill. Trawl fisheries usually deeply change benthic communities in terms of species distribution, density and diversity [77].

In our study area, we observed evidences of a differential fishing activity on the two sea hills. There was a high amount of lost longlines (targeting fishes) tangled on the rocky substrate of the Gulf of Valencia rocky sea hill, while the flat topped Eivissa Channel sea hill presented a higher abundance of trawl marks (targeting mostly decapod crustaceans). These differences in fishing impact on the bentho-pelagic fauna may contribute to the observed differences in faunal composition between the two sea hills.

In general, we observed the presence of an elevated number of Phyla on the sea hills studied in comparison to the adjacent muddy seafloor. Sea hills are considered habitats of high biodiversity sustained by their peculiar geomorphologic conformation that strongly conditions local hydrography, with the consequent increase of water column nutrients input and retention [78,79].

Rocky substrate on sea hills are particularly suitable for colonization by sessile filter-feeding organisms, resulting in complex biogenic effects on the habitat, which modify their geomorphology (e.g. cold water corals), hence contributing to the establishment of high faunal diversity [80,81]. Nonetheless, recent studies provided data from some seamounts with the same benthic diversity and endemism that those reported on continental margins, which are traditionally considered poorer in overall biodiversity [29,82] and could be applied to sea hills in a future.

Landslides

On the Eivissa Channel, small submarine landslides and pockmarks have been reported [49]. These landslides are too old in geological time to affect still influencing community turnover at site colonization. Thus, we can consider them as mud plains or slight slopes with escarpments. Crustaceans and fishes dominated the faunal assemblages of both landslides. These results corroborate other studies employing other sampling strategies such as for example, trawling with otter-trawl and Agassiz trawl [21,63,83], which also found that these groups were the most abundant in terms of biomass. Motile fauna was the dominant group in the surveyed landslides, being sessile fauna only present over isolated hard substrates. A high proportion of predators (fishes and cephalopods) were observed in these areas. An interesting observation was that when crustaceans were dominant, fishes were rarely seen, and *vice versa*. This suggests a possible trophic competitive exclusion, among species of different phyla with similar ecological niche, leading to areas occupation by best performers.

Anthropogenic impact within habitats

In this study, we observed noticeable levels of human impact. A significant number of trawl marks was observed, along with litter that was present in all studied zones. In the Eivissa Channel, for example, we observed mobile litter (i.e. plastic bags) accumulated in depressions such as pockmarks. The Mediterranean is a closed sea, only open to the Atlantic through the shallow strait of Gibraltar, and its dimensions are smaller in comparison to oceans. It has been sailed also since the origin of history and hosts one of the ancient developments of humanity along its coastlines [84], which are nowadays highly populated. As a result, it has been affected by all kind of anthropogenic impact and for a longer time than other seas [39]. The impact of marine litter on deep-sea habitats is being addressed by several international initiatives [36,37,39,46,47,76,85]. These studies provide a first baseline on the distribution of marine litter and its potential effects on the habitat and fauna, such as suffocation, physical damage to fragile sessile fauna (e.g. sponges, cold water corals) or the ingestion of microplastics. Other studies have addressed the chemical contamination on deep-water fauna [44,45,86] and sediments [87]. The presence of lost or discarded fishing nets is also often observed [39,88], resulting in ghost fishing for long time periods. It has been shown that the repetitive trawls on canyon walls between 400 - 700 m produce a resuspension of sediment, which is mobilised towards deep areas with a potential significant impact on deep-sea communities [40,41].

The continuous trawling over the seafloor on the Catalan margin has had a ploughing effect of the seafloor, resulting in a change of the seabed geomorphology and characteristics [89]. In the Catalan margin, trawl marks have been observed down to 900 m, caused by fisheries targeting deep-sea species such as the red shrimp *Aristeus antennatus* (Risso, 1816) in the north-western Mediterranean Sea [90]. Recent studies in this region reported biodiversity and community composition differences between fished and non-fished areas, with a decrease of sessile species on impacted zones [42,68].

Behavioural observations of identified species

An analysis of species distribution according to different factors, including behavioural motility, has been conducted here. In this study, schooling behaviour of fishes was observed near sea hills, as reported in similar studies in other oceans [91]. Conversely, in the muddy open slope, isolated individuals were usually detected. The reaction of fishes to ROV approach varied depending on the species. As a first instance, all avoidance reactions could have been generated by a combination of strong enlightening from lamps, noise and by the ROV motion itself. In relation to passivity at ROV approach, some questions arise about why some deep-sea animals do not show avoidance to potential threatening stimuli [92]. Behavioural observations for fishes are becoming abundant as ROV studies increase, since species are well visible, being often the focus of these surveys [93–95]. Several studies in the Atlantic compared trawl data with ROV video-surveys in order to evaluate biases produced by both sampling methods [94,96]. These studies evidenced that fish reaction and response to both ROV lighting and net approach generates a different bias-dependent effect on observations.

In relation to the behaviour shown by crustaceans, camouflage was reported in *Paromola cuvieri*, as a common trait in several other species of crabs [97]. Although usually animals of this species use gorgonians as camouflage [98], in this study we had several observations of *P. cuvieri* carrying plastic bags. Another recent study also describes *Paromola* using plastic bags or other anthropogenic objects [99]. This behaviour in the Mediterranean populations could be the result of the availability of litter in deep-sea areas.

Another interesting behaviour observed was the aggregation of the pelagic jellyfish *Pelagia noctiluca* on the top of the Eivissa Channel flat topped sea hill at 200 m depth. This species is known to have nycthemeral migrations [100], and has been observed near the bottom, probably associated with the migratory zooplankton present over sea hills and seamounts [101]. The presence of *Pelagia noctiluca* over the seafloor could indicate that some fauna classified as fully pelagic can, in fact, enter the benthic boundary layer and plays a major role in the trophic structure and ecological processes taking place close to the seafloor and on the sefloor. Another interpretation could be that our observations were the result of some mass deposition of dead jellyfishes, probably resulting from some sort of schooling on the water column, which could be, potentially, a common behaviour in these animals [102].

Our faunistic results could be interpreted as contextual to the time of the day at those depth ranges where daily vertical migrations or nektobenthic displacements may occur for some species (i.e. at continental margin depths where light is still present). The effects of these movements on a non-temporally scheduled sampling are presently unknown and markedly different community perceptions may occur at different time scales [103,104]. Ethological characterization is therefore important in order to explain the observed species distributions based on the type of animal displacement, as well as to understand potential biases occurring with trawling from the reaction of animals to invasive collection procedures [35,105]. Unfortunately, direct behavioural observations are scarcer than faunistic data in deep-sea studies [106]. In relation to species distribution, as result of modalities of displacement, daily and seasonal movement of populations may occur along shelves and slopes with still poorly characterized patterns [107].

Conclusions

We observed that seafloor, which is characterised by a high variety of substrates, from muddy areas with low-inclination slopes to steep rocky outcrops, provide a variety of different micro-habitats to a variety of species. Although our ROV data did not allow for biodiversity analyses, the faunal observations conducted over two sea hills on the north-western Mediterranean indicate that there is a high variability of faunal groups, with different species/taxa associated to different topographic features (e.g., depth, substratum, and slope). Similar patterns of small-scale heterogeneity are found in canyons. These geomorphologies usually include steep slopes with rocky outcrops on the walls and relatively plain areas with sandy or muddy seafloor. This seafloor heterogeneity can result in an increased biodiversity [59]. These geomorphological and environmental factors result in the observed high variety of faunal communities and, potentially, high biodiversity. Furthermore, noticeable levels of human impact were reported from all explored areas. The ROV survey also allowed for behavioural observations of the megafauna, providing valuable information to understand the functioning of these communities.

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First in situ observations of the deep-sea carnivorous ascidian *Dicopia antirrhinum* C. Monniot, 1972 in the Western Mediterranean Sea

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Resumen

Dicopia antirrhinum C. Monniot, 1972 es una especie rara de ascidia de profundidad perteneciente a la Familia Octacnemidae, recogida entre los 1000 m – 2500 m de profundidad en aguas del Atlántico Europeo. Nunca antes del presente estudio se citaron individuos adultos de esta especie en el Mar Mediterráneo, donde únicamente se encontraron siete juveniles en 1975 a 500 m de profundidad cerca de la Isla de Malta. Se observaron ciertas semejanzas entre estos especímenes y los pertenecientes a la especie *D. antirrhinum,* sin embargo, la ausencia de características típicas de la especie en juveniles no permitieron su exacta clasificación taxonómica. Ningún otro miembro de la Familia Octacnemidae ha sido citado en el Mediterráneo. En el presente estudio, se describe pues el muestreo de un espécimen adulto de *D. antirrhinum* a 1100 m de profundidad en uno de los flancos del Cañón de La Fonera (también llamado Cañón de Palamós), en el Noroeste Mediterráneo; confirmando su presencia en el Mar Mediterráneo. Asimismo se observaron 5 individuos en su hábitat natural mediante un vehículo operado por control remoto (ROV). Nuestros datos resaltan la potencial presencia de individuos de la Familia Octacnemidae, hasta ahora ignorados, en cañones profundos del Mediterráneo Occidental. La importancia de estas observaciones deja patente la necesidad de incrementar el esfuerzo de muestreo mediante nuevas tecnologías, como ROVs, en hábitats ecológicamente relevantes como cañones, con el fin de obtener una visión más precisa de la biodiversidad del Mediterráneo profundo.

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Note

First in situ observations of the deep-sea carnivorous ascidian *Dicopia antirrhinum* Monniot C., 1972 in the Western Mediterranean Sea

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ABSTRACT

Dicopia antirrhinum C. Monniot, 1972 is a rare species of deep-sea ascidian belonging to the Family Octacnemidae, reported at depths of 1000–2500 m in European Atlantic waters. Adult individuals have never been reported before in the Mediterranean Sea, where only seven juvenile specimens were found in 1975 at 500 m water depth in the Central basin (Malta). The affinities of these specimens with *D. antirrhinum* were noted, but lack of some typical characters of the species in juveniles prevented a definite taxonomical identification. No other member of the Octacnemidae has ever been found in the Mediterranean. In this study we describe the sampling of an adult specimen of *D. antirrhinum* at around 1100 m water depth on the flank of the La Fonera (Palamós) canyon, Northwestern Mediterranean, confirming their presence in the Mediterranean Sea. We also observed 5 individuals of this species on their natural habitat with a Remotely Operated Vehicle (ROV). Our results highlight the potential occurrence of Octacnemidae, the presence of which has been largely overlooked, in several deep-sea canyon areas within the Western Mediterranean basin. These observations are important because they indicate the need for increased sampling effort with new technologies, such as ROVs, in ecologically relevant habitats such as canyons, in order to obtain a more accurate picture of deep-sea biodiversity in the Mediterranean Sea.

1. Introduction

Deep-sea ascidians are markedly different from their more abundant shallow-water counterparts. These differences relate to the dominance of solitary forms inhabiting soft bottoms as opposed to the commonly rock-dwelling and often colonial species in littoral waters (Havenhand et al., 2006; Monniot and Monniot, 1978). Another common trend in deep-sea ascidians is the adaptation to the scarcity of particulate matter, which led to partly or totally macrophagous feeding in some members (Tatián et al., 2011) of this otherwise filter-feeding group (Monniot and Monniot, 1978, 1991). Although far from reaching the abundance and diversity of shallow-water ascidian faunas, the diversity of deep-sea ascidians is surprisingly high (Monniot, 1998; Monniot and Monniot, 1973, 1991), reaching a maximum at great depth (4000–5000 m) (Monniot and Monniot, 1978). Individuals of some species have been found even down to 8000 m depth (Sanamyan and Sanamyan, 2002).

Many ascidian families have deep-sea representatives, but some groups (i.e., Octacnemidae) are exclusively found at great depths, and they feature some of the most notable morphological adaptations (Monniot and Monniot, 1991). The Family Octacnemidae, in particular, comprises deep-sea species that can reach high abundances in the continental slope and abyssal plains over a depth range from 500 to over 8000 m depth. This family has adopted a partly macrophagous diet using the oral siphon as a trap to capture prey. This siphon is often expanded into muscular lobes or lips capable of capturing motile prey (Monniot, 1998). The branchial sac is reduced to a variable degree, indicating a different mix of micro/macrophagous feeding, as confirmed by fatty acid analysis (Ogawa et al., 2005). At present, this family comprises 26 species belonging to 10 genera (according to the World Register of Marine Species and Ascidian World Database).

In the Mediterranean Sea, the diversity of deep-sea ascidians seems to be lower than that of nearby Atlantic waters and they are found at relatively shallower depths. Out of 50 strictly bathyal and abyssal species in European Atlantic waters, only 11 have been reported in the Mediterranean (Monniot and Monniot, 1990). The only previous record of Octacnemids in the Mediterranean was of seven juvenile individuals reported in Maltese waters at 500 m

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depth (Monniot and Monniot, 1975) and deposited in the Muséum National d'Histoire Naturelle. They were identified as *Dicopia* sp. because some diagnostic characters were lacking in juvenile forms, although their affinities with *D. antirrhinum* were noted (Monniot and Monniot, 1975, 1990). *D. anthirrhinum* is an Atlantic species (Monniot, 1972; Monniot and Monniot, 1974, 1985) that can be locally abundant and has been reported at depths between 1000 and 2500 m (Monniot and Monniot, 1990). No adult Octacnemid has been reported in the Mediterranean Sea until now (Coll et al., 2010), in spite of intense sampling effort in some areas, such as the Catalan margin, where scientific surveys with trawl nets or Agassiz sledges have been conducted over two decades in order to study the fauna inhabiting canyons and adjacent muddy plains areas (Ramírez-Llodra et al., 2008, 2010; Sardà et al., 1994, 2004).

New video-imaging techniques are providing a valuable tool to explore the behavior and the ecology of deep-sea fauna (reviewed by Aguzzi et al., 2010, 2012). Video surveys with Remotely Operated Vehicles (ROVs) have been increasingly performed in the past few years in the Northwestern Mediterranean, in a wide range of different deep-water geomorphological environments such as canyons, open mud plains, and submarine landslides. On the Catalan margin, previous ROV video surveys did not reveal the presence of Octacnemids, probably due to the shallow nature of the dives (lower shelf and upper slope), where deep-sea coral communities occur (Orejas et al., 2009). In this study, we report outcome of the PROMARES ROV survey on the Catalan margin (Northwestern Mediterranean), where individuals of the genus *Dicopia* were observed *in situ* for several minutes prior to the collection of a specimen in La Fonera (Palamós) canyon.

2. Materials and methods

Video observations were carried out with the Remotely Operated Vehicle (ROV) *Liropus* 2000 from the Spanish Oceanographic Institute (IEO) during the PROMARES survey, from 29th June to 15th July 2011. Seabed exploration was conducted onboard R/V *Sarmiento de Gamboa* on the Catalan margin and its deeply incised canyons (Cap de Creus, La Fonera and Blanes) (Fig. 1A). Footage of *D. antirrhinum* was obtained with the front-pointing video camera (HD Kongsberg OE14-502) and two small cameras (OE14376) on the extensible arm.

Navigation data (latitude, longitude and depth) were noted during each video records, making it possible to precisely locate all animals within previously-acquired swath bathymetry maps of the region (Lastras et al., 2011; Fig. 1B).

When an animal was seen, the ROV survey was interrupted in order to obtain a detailed photographic record of it. The ROV is equipped with an extensible arm (HLK-47000), allowing the

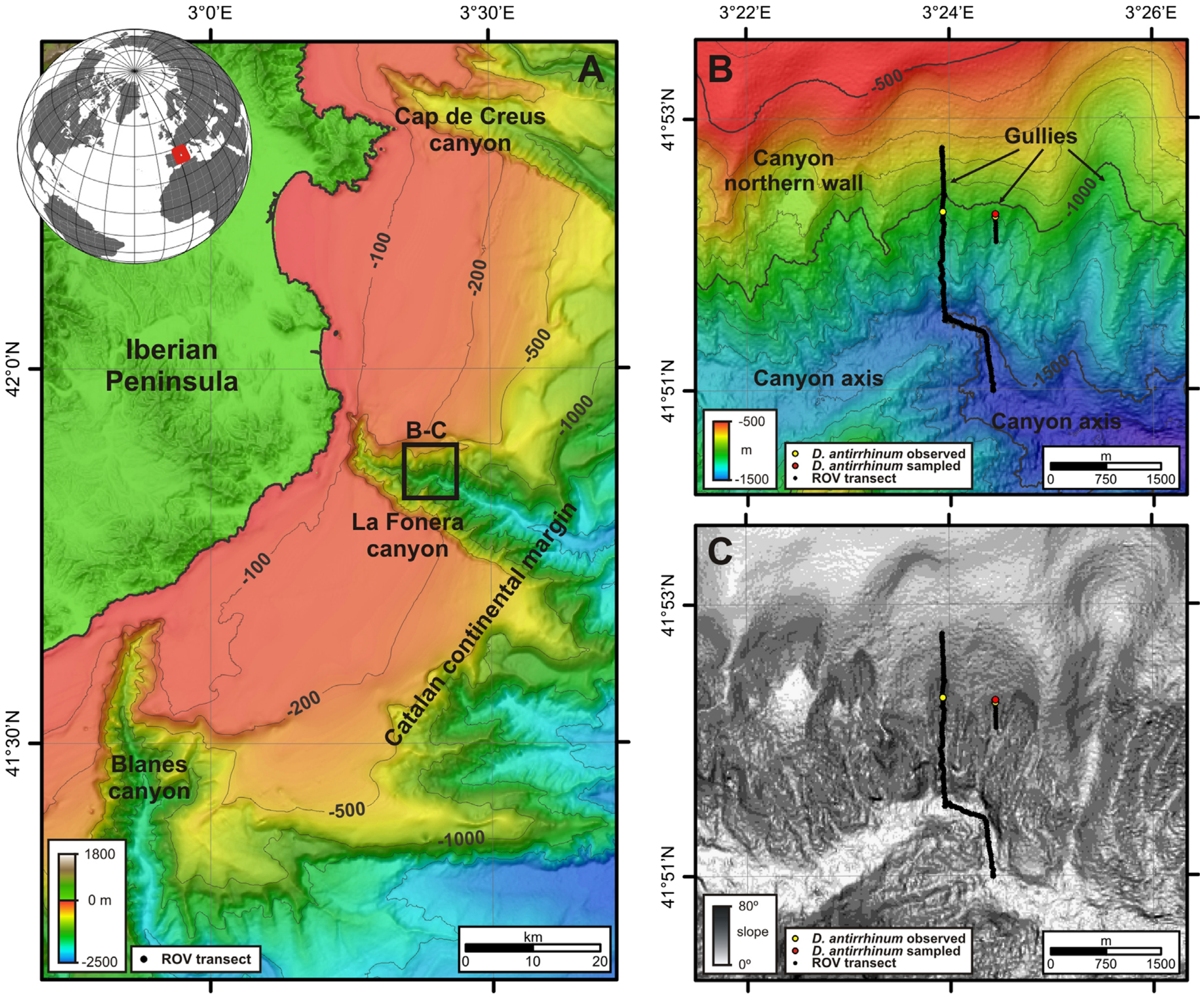

Fig. 1. Study area. (A) Location of La Fonera canyon on the Catalan margin and in the Western Mediterranean Sea. (B) Detailed bathymetric map and location of the sites where *Dicopia* individuals were observed and sampled during ROV transects. (C) Detailed slope map and sampling location.

collection of specimens. This was used to recover a single individual of *Dicopia* which was placed in a lightproof box on the ROV. Immediately following the ROV dive, the specimen was preserved in 10% saltwater formalin, prior to examination in the home laboratory.

3. Results

3.1. Underwater observation

A total of five adult individuals was observed in two different locations in the La Fonera canyon (see Fig. 1B and C). First, two individuals were reported on the northeastern canyon flank at 1106 m depth. Later, between 1072 and 1085 m depth on the same flank of the canyon, three other specimens were observed; two of them only a few centimeters apart and the third one separated by approximately 35 m. Fig. 2A–C shows selected images of two individuals. The individual collected (Fig. 2D) corresponds to the last specimen observed by the ROV (Fig. 2E). When examined after recovery, fragments of consolidated mud were found to be attached to the base of the peduncle, suggesting that the captured specimen was originally anchored to this harder material beneath a thin layer of soft mud.

Our video filming showed the behavioral activity of *Dicopia* over short temporal scales (i.e., min). The animals seem to close the oral siphon when disturbed, while at the same time they presented a slow opening and closing of the atrial siphon (Fig. 2E).

3.2. Morphology

The specimen captured measured 5.8 cm in height, and has a thin and translucent tunic covered with muddy material (Fig. 3A and B). The body is not stalked, but tapers posteriorly and ends in a flat area

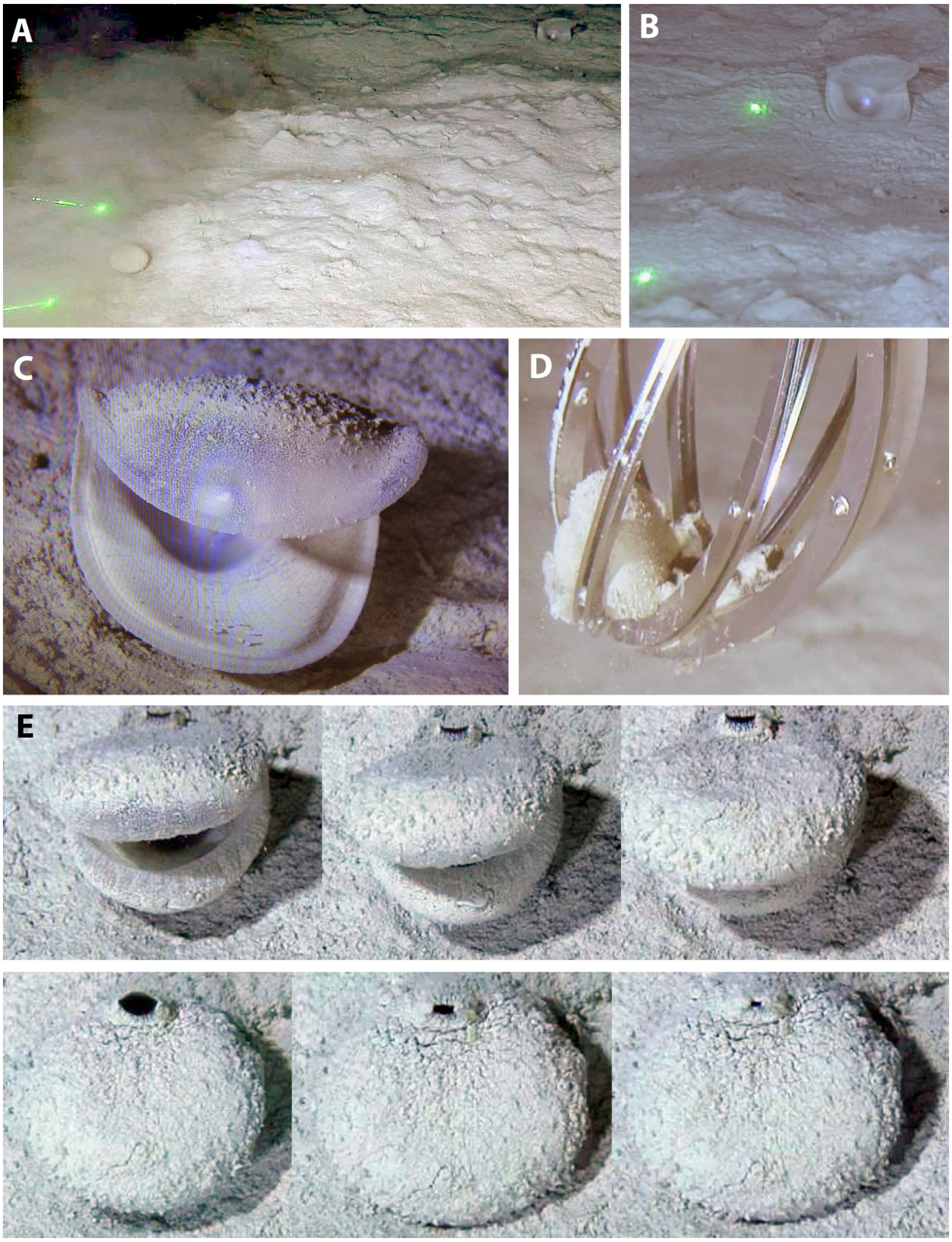

Fig. 2. (A) Two individuals detected during ROV video-surveys within the La Fonera canyon, fully closed and fully open. Laser points are 10 cm apart. (B and C) Detail of the fully open specimen. (D) Capture of an individual by the ROV arm. (E) Complete behavioral sequence of closure of the oral siphon.

with tunic rhizoids anchoring the animal to hardened mud. The oral siphon is enlarged forming a big horizontal slit surrounded by two lips. The atrial siphon is small and placed dorsally. Once the tunic is removed, the body wall is thin and has discrete muscular fascicles, consisting mainly of dense circular muscles around the rims of the lips, longitudinal muscles ventrally and dorsally (ending in the base of the body), and oblique muscles from the corners of the lips to the digestive system (Fig. 3C–E). The atrial siphon has some circular and radial muscles (Fig. 3F). Internally the oral siphon (i.e., the lips) is covered everywhere by small, flattened papillae.

The branchial sac is cone-shaped, limited anteriorly by a membranous velum topped with numerous filamentous tentacles. The velum is higher posteriorly. A triangular neural ganglion is anterior to the velum. The aperture of the neural gland is oblique. The pharyngeal groove is adjacent to the base of the velum and makes a slight indentation to surround the neural gland opening. The branchial wall is a three-dimensional network of sinuses, overlain by a perforated membrane with no ciliation (Fig. 3G). There are no imperforated areas in the branchial cone. The dorsal lamina is very low, while the endostyle is well developed.

The digestive system is compact and postero-dorsal. The esophagus opens at the bottom of the branchial cone and is followed by a slightly enlarged stomach and an ascending intestine. An ovarian mass lies in the gut loop, while a testicle is spread over the stomach and has a duct ending in a papilla close to the anus.

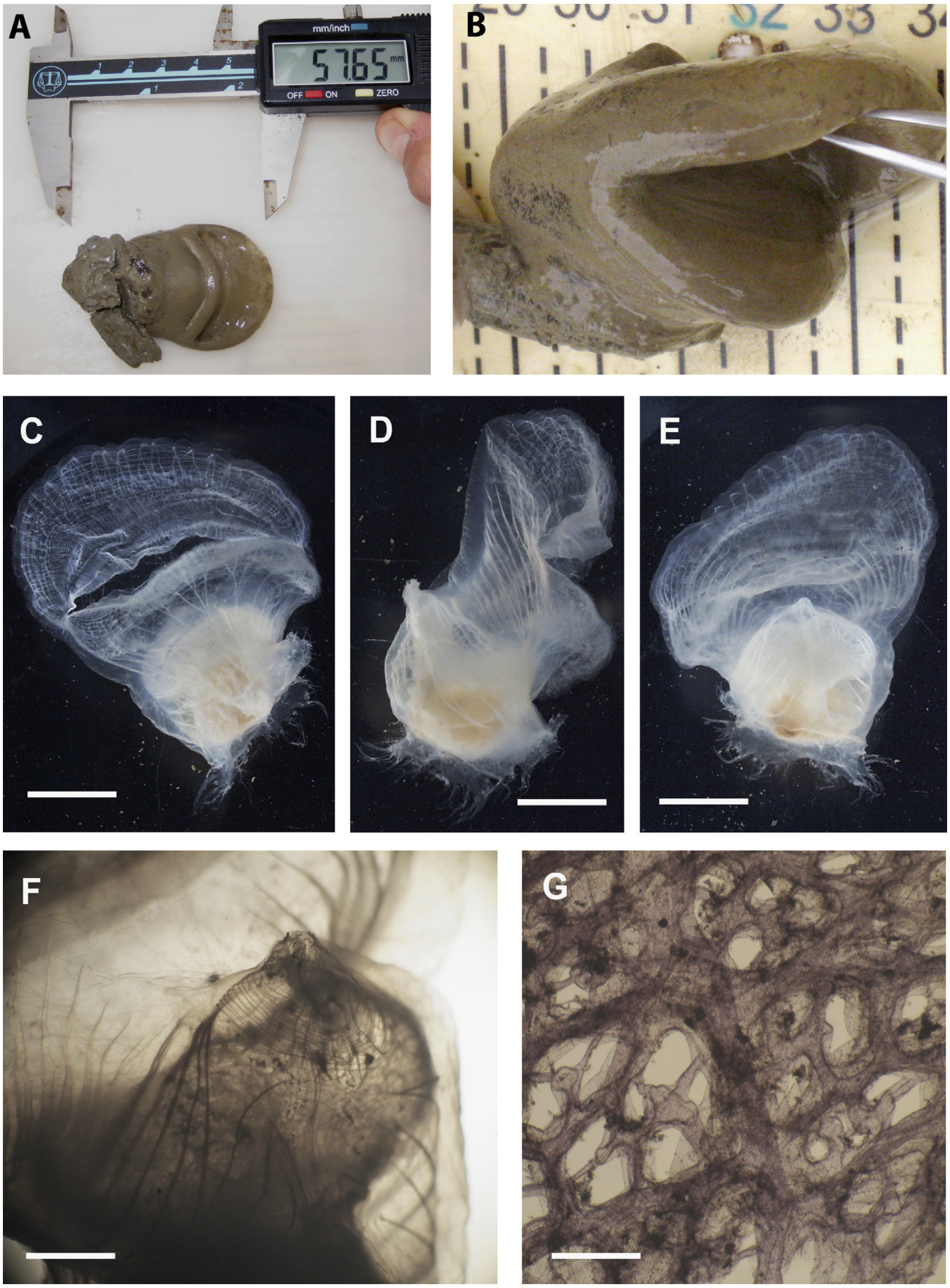

Fig. 3. Different morphological features of the collected specimen of *Dicopia antirrhinum*. (A and B) Ventral and lateral morphology of the animal with tunic intact. (C–E) Morphology of the animal without tunic: ventral (C), lateral (D), and dorsal (E) views. (F) Enlarged view of the atrial siphon. (G) Detail of branchial structure. Scale bars: C–E = 1 cm; F = 1 mm; G = 0.3 mm.

4. Discussion and conclusion

Three Octacnemid genera have an oral siphon in the shape of two lips: *Situla*, with a flat branchial lamina, and *Dicopia* and *Megalodicopia* with cone-shaped branchial sacs. The last two are distinguished by the presence of papillae in the inner side of the oral siphon and the absence of a muscular peduncle in *Dicopia* (Millar, 1988; Monniot, 1972; Sanamyan and Sanamyan, 2002), to which our specimens can be assigned. The morphological features of the present material agree well with the Atlantic species *D. antirrhinum*, as described in Monniot (1972), and Monniot and Monniot (1974). In particular, in the latter work specimens are described as attached by a flat surface with rhizoids as in the case of the individuals described here. The musculature of our specimens seems to be within the variability reported for this species. The other two *Dicopia* species described were Pacific forms; *D. fimbriata* Sluiter, 1905 has a very different shape and musculature and has papillae in the inner part of the tentacular velum (Monniot and Monniot, 1991; Sanamyan and Sanamyan, 1999), while *D. japonica* Oka, 1913 has a different shape and branchial wall (Millar, 1988).

In this study, we reported the presence of 5 adults of the deep-sea ascidian *D. antirrhinum*, previously represented only by possible juvenile specimens collected near Malta (Monniot and Monniot, 1975), and never filmed before. Few observations of living Octacnemidae have ever been made, a notable exception being *Megalodicopia hians* (Oka, 1918) which is displayed in Monterey Bay Aquarium (USA), Uozu Aquarium and Enoshima Aquarium (Japan) (Sanamyan and Sanamyan, 2012). Our specimens were observed at around 1100 m on two different ridges between gullies on the northern flank of La Fonera canyon, which suggest that deep canyon areas constitute the preferred habitat of this species in the Catalan margin and possibly beyond in the Mediterranean Sea.

The discovery of *Dicopia* on the northern flank of La Fonera canyon, but not on its southern flank may also indicate a preference for settings where current conditions favor encounters with free-swimming prey. La Fonera canyon in particular is crossed by the mesoscale northern current flowing in a southward direction, so that the leeward position of the northern canyon flank and the associated low-energy turbulence might favor such encounters.

Dicopia has been suggested to have a mixed diet (Monniot, 1972; Monniot and Monniot, 1978). This genus may passively capture organic detritus, as suggested for *M. hians*, where feeding habit were studied at all levels, from gut contents to fatty acid and isotopic analyses (Zhang et al., 2001; Okuyama et al., 2002; Ogawa et al., 2005). Unfortunately, an analysis of the diet of *D. antirrhinum* was not possible since only one specimen was captured. Moreover, this species has a reduced branchial sac in the form of a perforated cone, and no cilia were present in the stigmatal openings, all of which suggest weak filtration ability. On the other hand, the development of the oral siphon is an adaptation to macrophagous feeding (Monniot and Monniot, 1978). Individuals seem to be passive trappers of swimming zooplankton and micronekton like other Octacnemids (Havenhand et al., 2006; Lescano et al., 2010; Okuyama et al., 2002).

The presence of five individuals of *Dicopia* on a very small area of this deep-sea canyon once more highlights the role of these submarine structures as hotspots for biomass concentration and elevated biodiversity (Company et al., 2012; Danovaro et al., 2010; Ramírez-Llodra et al., 2010). The previous absence of *Dicopia* from other more accessible and much more extensively investigated slope zones could be the combined result of limited sampling in operationally complicated zones, the fragility of these kinds of species, which are usually recovered in a damaged and unrecognizable state in trawl samples, and the lack of taxonomical expertise to identify them.

Here we report for first time the presence in the Mediterranean Sea of adults of the genus *Dicopia* that can be confidently assigned to the species *D. antirrhinum*. This new record increased the lower depth limit of this genus in the Mediterranean Sea from 500 to 1100 m depth. This finding contributes to the knowledge of the poorly investigated Mediterranean deep ascidian fauna (Monniot and Monniot, 1990), in sharp contrast with the relatively well-known shallow-water Mediterranean ascidians (Coll et al., 2010)

Acknowledgments

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9. Appendix

9.1 Taxonomy of deep sea holothurians from Cantabrian waters

As an appendix of this Thesis some differences between Mediterranean and Atlantic holothurian species are described. On the framework of the project DOSMARES, three seasonal cruises were realized on the Cantabrian Sea. Three different depths (1500 m, 2700 m and 4700 m depth) were sampled by means of an Agassizz trawl. Resulting from these three cruises 15 species were obtained in front of the 11 species providing from the nine Mediterranean cruises.

The mention on the Table A1 of a higher number of species and individuals on the deepest sample (4700 m) was in accordance with other studies realized on north-Atlantic waters (Harvey et al. 1988; Billett 1991).

Table A1. Holothurians sampled on BIOCANT03. n= number of specimens

Species	n	station	Depth sampled
Mesothuria (Penichrothuria) verrilli	1	P3	1500 m
Molpadiodemas depressus	6	C8	4700 m
Molpadiodemas sp. 1	1	C8	4700 m
Molpadiodemas sp. 2	1	C8	4700 m
Pseudostichopus peripatus	1	C8	4700 m
Molpadia blackei	1	C8	4700 m
Molpadia musculus	1	C5	2100 m
Molpadia oolitica	2	C5	2100 m
Hedingia albicans	1	C5	2100 m
Deima validum validum	8	C8	4700 m
Amperima rosea	13	C5 - C8	2100 m - 4700 m
Peniagone diaphana	1	C8	4700 m
Benthogone rosea	2	C8	4700 m
Laetmogone violacea	3	C8	4700 m
Psychropotes longicauda	1	C8	4700 m

A superficial description of the species from the Atlantic cruises was provided in order to compare with some sister species from our samples described in Paper 2. The species present and described in the Mediterranean Sea paper are not re-described here, as also the specimens not classified until species taxon. Unfortunately no scale could be provided for these species figures. The relevant literature as these effect was based on studies from Western Atlantic (Mortensen 1927; Deichmann 1930; Gage et al. 1985; Pawson et al. 2001; O'Loughlin and Ahearn 2005) between others.

Order ASPIDOCHIROTIDA

Family Synallactidae Ludwig, 1894

Genus *Mesothuria* Ludwig, 1894

Subgenus *Mesothuria (Penichrothuria)*

Mesothuria (Penichrothuria) verrilli (Théel, 1886)

Material: One specimen of this species was sampled at 1500 m depth.

Description: As commented on Paper 2, the most significant external difference between this species and *Mesothuria intestinalis* was the calcareous ossicles present in the integument. These deposits in *M. verrilli* (Fig. 9.1A) are tables with a short and strong central spine (Fig. 9.1B) while in *M. intestinalis* this spine was longer and thinner (see Paper 2). The end of these spines is a characteristic feature to classify both species. In *M. verrilli* this end of spine is constituted by four simple teeth pointing to the exterior (Fig. 9.1) in front of the crown present on the top of the tables in *M. intestinalis* (see Paper 2).

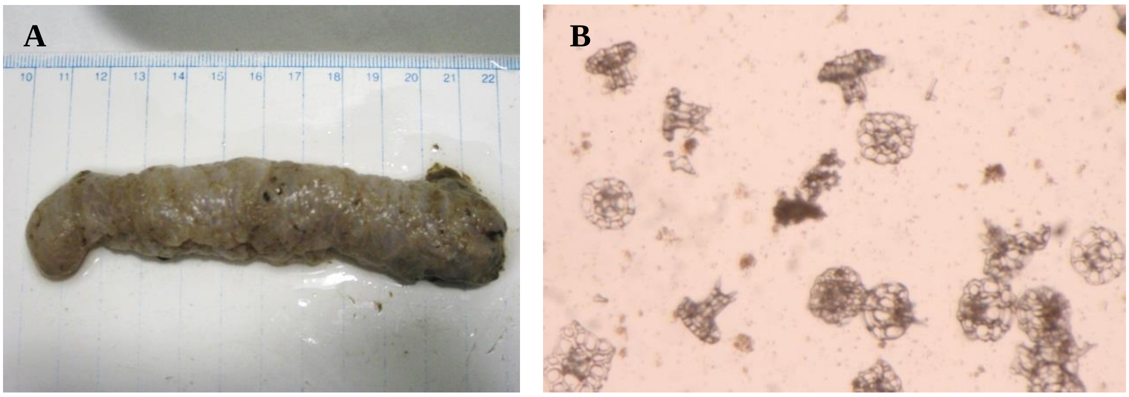

Figure 9.1. A*) M. verilli*. B) Calcareous ossicles of *M. verilli*

Genus *Molpadiodemas* Heding, 1935

Molpadiodemas depressus (Hérouard, 1902)

Material: Six specimens sampled at 4700 m depth.

Description: Body shape flattened ventrally and slightly vaulted dorsally. It presents a ventral and anal furrow. The integument is white and cartilaginous (Fig. 9.2A). A lateral brim is observable. Longitudinal muscles are flat and attached to the body wall. The gonads are also flat and branched. Ossicles present on tentacles as spiny rods, absent on gonads (Fig. 9.2B).

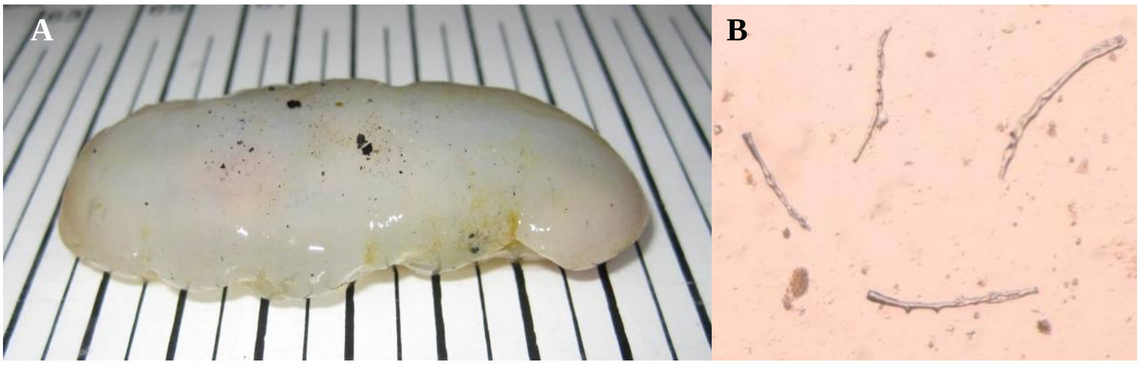

Figure 9.2. A) *M.depressus*; B) Ossicles of *M. depressus*

Genus *Pseudostichopus* Théel, 1886

Pseudostichopus peripatus (Sluiter, 1901)

Material: One specimen of this species was sampled at 4700 m depth.

Description: Body elongate with grooves and entirely covered by globigerinae (Fig. 9.3A). Longitudinal muscle presents a cylindrical shape. Abundant tube feet were observed. The ossicles present on tentacles are more or less spiny rods. In gonad, the ossicles are small spicules Y-X shaped (Fig. 9.3B). No ossicles were observed in respiratory trees.

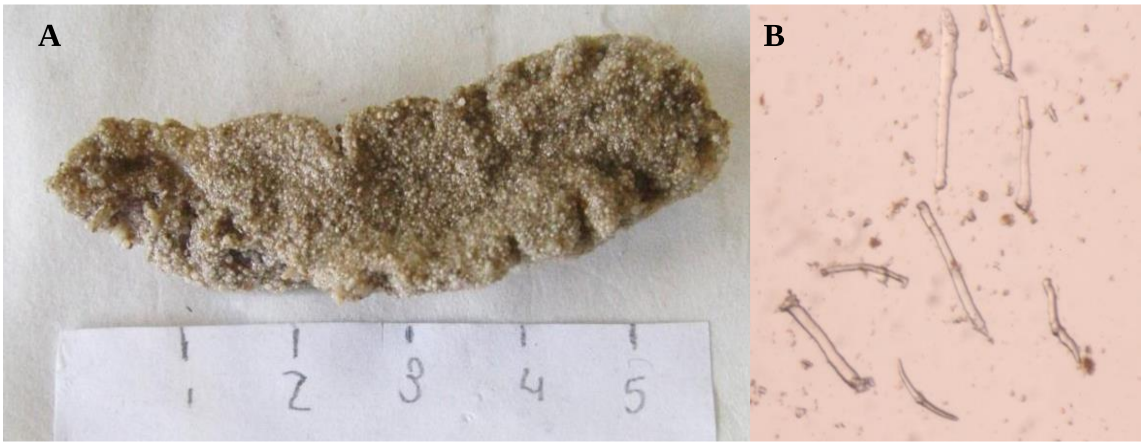

Figure 9.3. A) *P. peripatus*; B) Ossicles of *P. peripatus*

Order MOLPADIIDA

Family Molpadiidae Müller, 1850

Genus *Molpadia* (Cuvier, 1817) Risso, 1826

Molpadia blakei (Théel, 1886)

Material: One specimen of this species was sampled at 2100 m depth.

Description: This species presented the sausage shape typical from this genus (Fig. 9.4A). The tail is smaller than in other molpadiids (see Paper 2). The absence of phosphatic bodies results in a paler colour than in other species of the same genus. It was characterized by the presence of small and fragile tables constituted by three large holes and a central spine composed by three rods fused (Fig. 9.4B), being this separation visible at the base of the spine.

Figure 9.4. A) *M. blakei*; B) Ossicles of *M. blakei.*

Molpadia oolitica (Pourtalès, 1851)

Material: Two specimens of this species were sampled at 2100 m depth.

Description: This species presented also the typical sausage shape (Fig. 9.5A), but with a tail longer than the previous species. The phosphatic deposits were extremely abundant, for this reason the specimen was of a dark violet colour. The most abundant ossicles are tables with high number of holes. Racquet-shape plates and anchors are also present (Fig. 9.5B).

On both species a clear difference in external shape and ossicles with *Molpadia musculus* (see Paper 2) was observed.

Figure 9.5. A) *M. oolitica*; B) Ossicles of *M. oolitica*

Family Caudinidae Heding, 1931
Genus *Hedingia* Deichmann, 1938
Hedingia albicans (Théel, 1886) Deichmann, 1938

Material: One specimen sampled at 2100 m depth

Description: External shape (Fig. 9.6A) very similar to *H. mediterranea* (see Paper 2), sausage shaped with extremely large tail. Ossicles basically constituted by very irregular tables with a spiny central spine (Fig. 9.6B). Tables smaller and with more irregular plates contour than in *H. mediterranea*. As suggested in Paper 2, for these two species a molecular study could be the best way to designate them in one or two species.

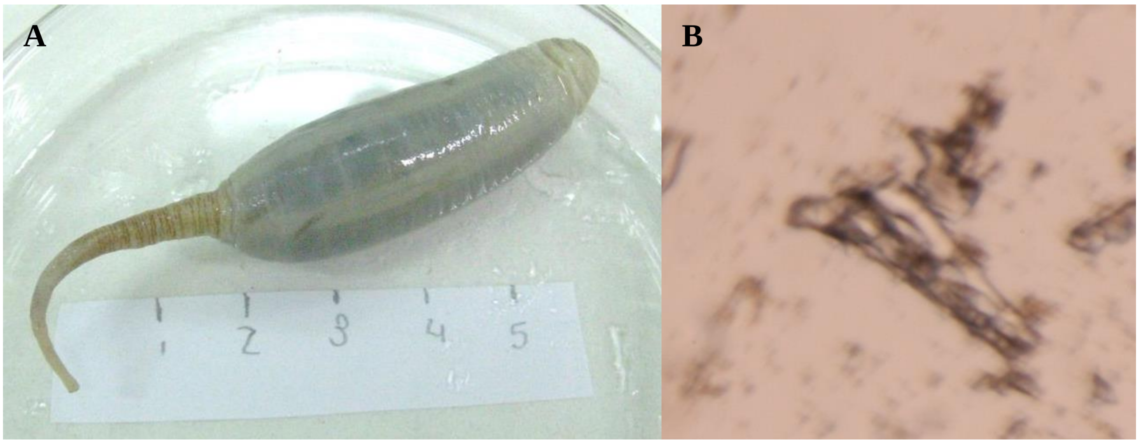

Figure 9.6. A) *H. albicans*; B) Ossicles of *H. albicans*

Order ELASIPODIDA
Family Deimatidae Théel, 1882
Genus *Deima* Théel, 1879
Deima validum validum Théel, 1879

Material: Eight specimens sampled at 4700 m depth.

Description: This species presented a ‘pigged’ shape (Fig. 9.7A). The body is ventrally flattened and dorsally vaulted, with large tube feet and six dorsal and six lateral large papillae on each side. Pink in colour. Ossicles in skin basically large perforate plates, providing a ‘parchment like’ integument (Fig. 9.7B).

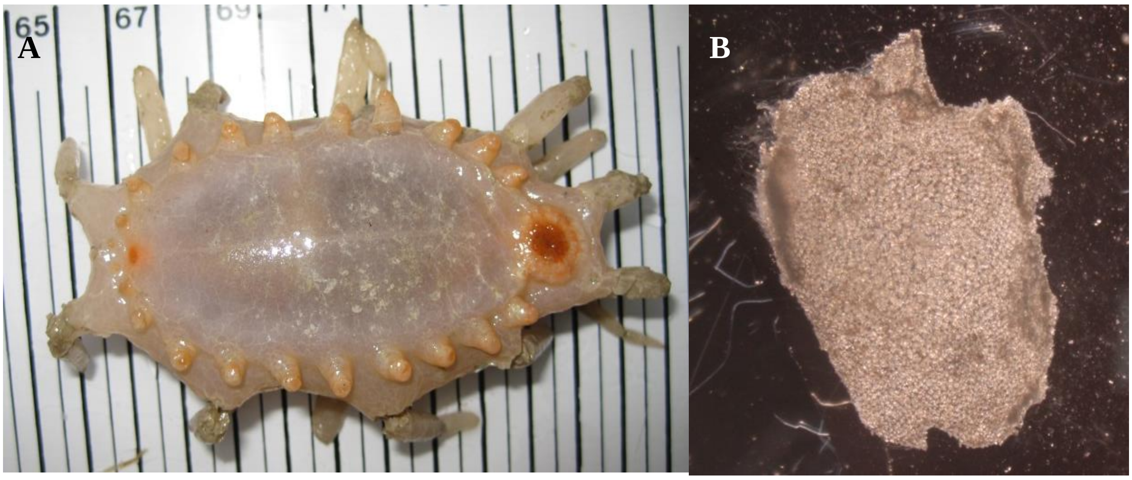

Figure 9.7. A) *D. validum*; B) Ossicles of *D. validum*

Family Elpidiidae Théel, 1882

Genus *Amperima* Pawson, 1965

Amperima rosea (R. Perrier, 1896)

Material: Seven specimens were sampled at 2100 m depth, and six at 4700 m depth.

Description: Specimens are usually small, non-exceeding 30 mm. The body shape is from elongated to more egg shaped. In all individuals ten tentacles were present. A four lobed velum was observed (Fig. 9.8A).

Behind the velum a pair of small papillae appears. Tube feet arranged in irregular row, low in the anterior part of the body (one) and numerous in the posterior (four) with a naked area between the anterior and the posterior tube feet. Ossicles basically constituted by triradiate deposits, C shaped ones and wheels (Fig. 9.8B).

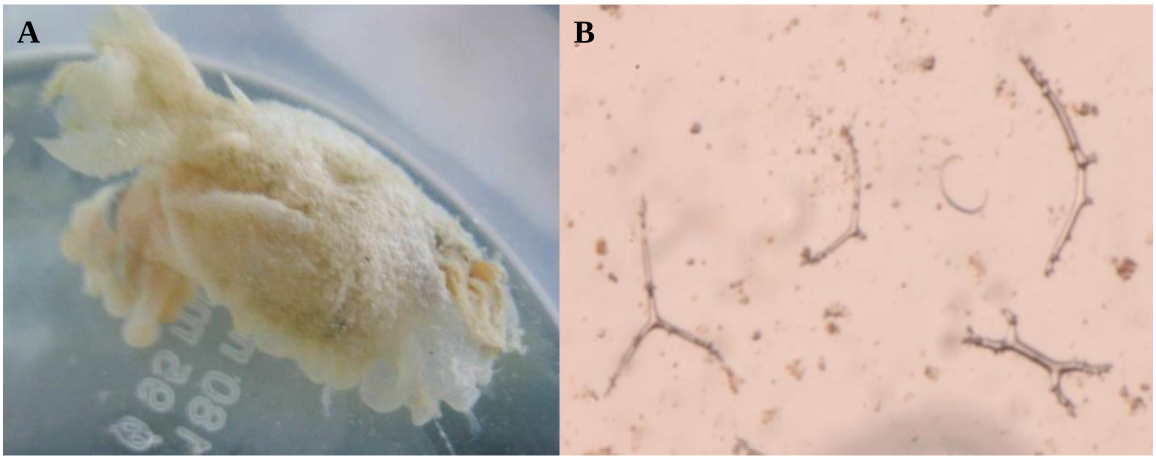

Figure 9.8. A) *A. rosea*; B) Ossicles of *A. rosea*

Genus *Peniagone* Théel, 1882

Peniagone diaphana (Théel, 1882)

Material: One specimen sampled at 4700 m depth.

Description: Species presenting a flat and rounded shape. The mouth is present in the ventral side of the body. Four pairs of tube feet at the end of the body are signalled (Fig. 9.9A). Anus is located in the dorsal area of the posterior part of the body. The ossicles are very abundant and presents a four radiated shape. Edges of the deposits are usually spiny (Fig. 9.9B).

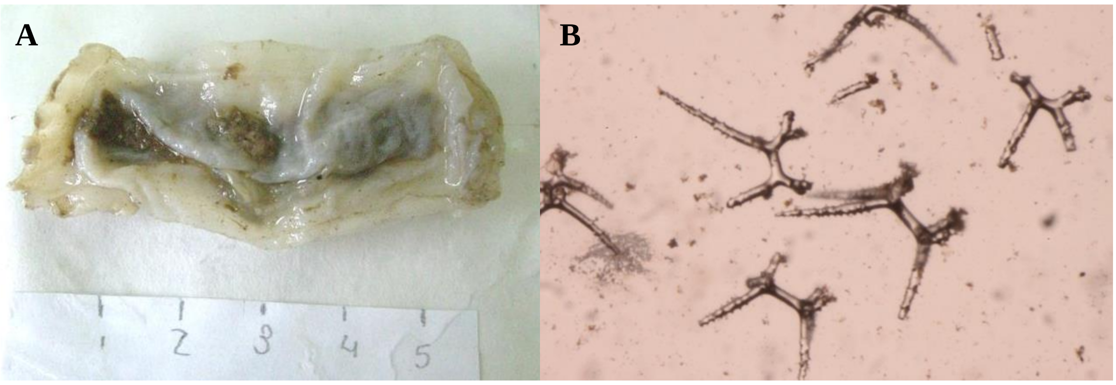

Figure 9.9. A) *P. diaphana*; B) Ossicles of *P. diaphana*

Family Laetmogonidae Ekman, 1926

Genus *Benthogone* Koehler, 1895

Benthogone rosea Koehler, 1895

Material: Two individuals of this species were sampled at 4700 m depth.

Description: This species presented a large body more or less cylindrical of about 100 – 150 mm. The ventral part presents two series of papillae. The mouth was surrounded by non- retractile tentacles (Fig. 9.10A). The colour of the tegument was pale pink and gelatinous, secreting high quantity of mucus. The skin ossicles of this specie were abundant and basically constituted by vaulted wheel (Fig. 9.10B)

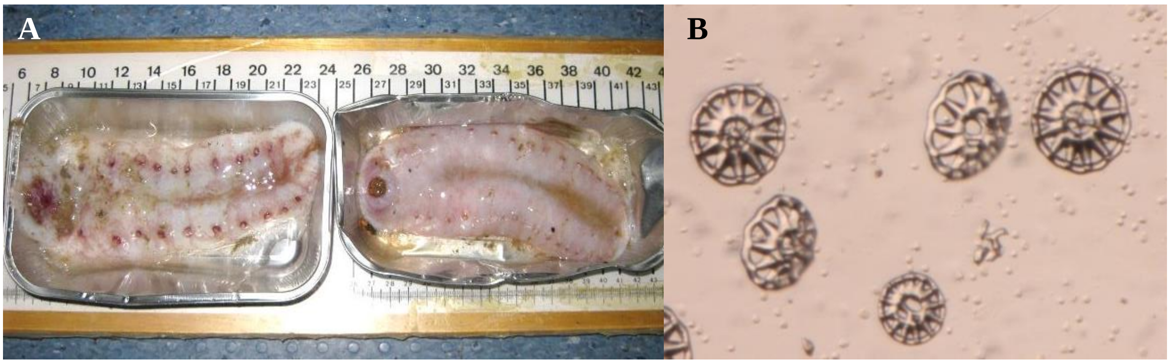

Figure 9.10. A) *B. rosea*; B) Ossicles of *B. rosea*

Genus Laetmogone Théel, 1879

Laetmogone violacea Théel, 1879

Material: Three specimens were sampled at 4700 m depth.

Description: Small specimens of about 20 mm (Fig. 9.11A). The body is flattened ventrally. Colour pale violet and transparent, due to the lack of the tegument by the trawl, with dark violet pieces of skin where colour was preserved. Fifteen tentacles were reported. Eleven pairs of podia regularly disposed on each side. Two dorsal papillae rows, small and closely placed. Genital processes dorsal, behind the tentacles. Ossicles constituted by two sorts of wheels, rods and plates deposits (Fig. 9.11B).

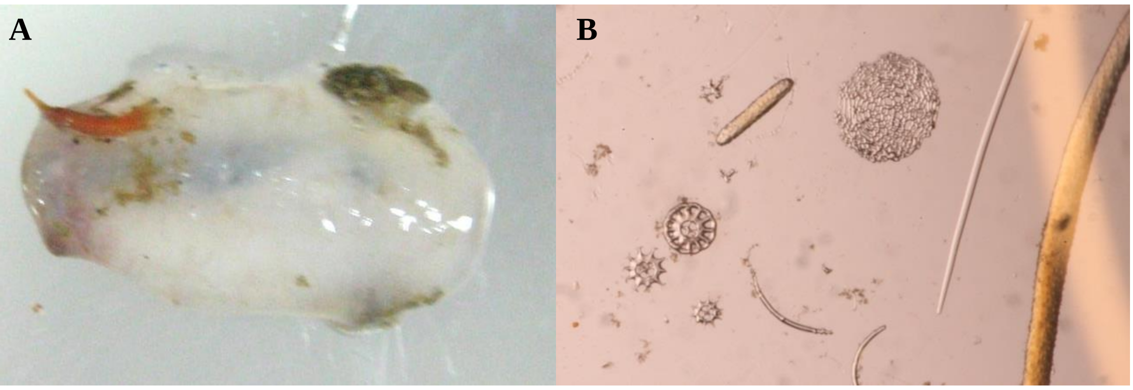

Fig. 9.11. A) *L.violacea*; B) Ossicles of *L.violacea*

Family Psychropotidae Théel, 1882

Genus *Psychropotes* Théel, 1882

Psychropotes longicauda Théel, 1882

Material: One specimen was sampled at 4700 m depth.

Description: Body flattened ventrally and vaulted dorsally. One long and flat characteristic appendage on the posterior part of the body (minimum as long as the whole body) (Fig. 9.12). A small brim on the anterior part of the body surrounding the mouth was observed. On the middle of the ventral side a double row of pedicels. Numerous pedicels on the external part of the ventral side too. Colour dark violet. Unfortunately this specimen was preserved for molecular analyses and the ossicles could not be observed, nonetheless the external morphology allow a classification.

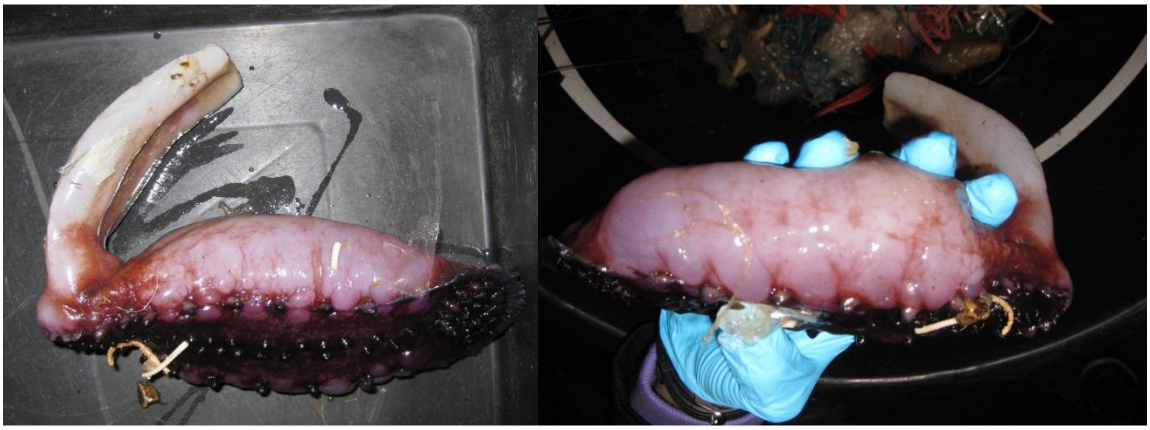

Fig. 9.12. *P. longicauda*

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Rare occurrences of the deep-sea *Phascolosoma turnerae* Rice, 1985 (Sipuncula: Phascolosomatidae) in the Mediterranean Sea.

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New records of *Phascolosoma turnerae* (Sipuncula: Phascolosomatidae) from the Balearic Basin, Mediterranean Sea

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Specimens of the deep-water sipunculan worm Phascolosoma (Phascolosoma) turnerae *were recently collected from the western part of the Mediterranean Sea. This species is characterized by hooks showing a peculiar anterior stout and long projection at their base. A key to all the* Phascolosoma *species found in the Mediterranean Sea is included.*

Keywords: *Phascolosoma turnerae*, new records, western Mediterranean, sipunculan worm

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INTRODUCTION

The genus *Phascolosoma* Leuckart, 1828, is one of the most species-rich genera within the phylum Sipuncula (Murina, 1984; Gibbs & Cutler, 1987; Cutler, 1994), with the majority of its species showing a preference for the warmer shallow waters of the world's oceans (Murina, 1975). However, a few phascolosomatid species are rare in their geographical distribution (Cutler, 1994), since they show preferences for deep cold waters instead.

Deep-sea communities in the Mediterranean are poorly known (Bazairi *et al.*, 2010). Recent studies conducted over the slope of the Balearic Basin (western Mediterranean) revealed the presence of deep-sea phascolosomatids (Cartes *et al.*, 2009). They were identified as *Phascolosoma turnerae* Rice, 1985 in a taxonomic checklist of the sipunculan fauna for the Mediterranean Sea (Coll *et al.*, 2010). The new phascolosomatids inhabited muddy bottoms in the Balearic Basin. From this area, a total of 7 other species of sipunculans have been collected (Cartes *et al.*, 2009; Mamouridis *et al.*, 2011; Tecchio *et al.*, 2013) by using different bottom trawls and box-corers at depths between 427 and 2265 m. This relatively high diversity of sipunculans was related to their ability in exploiting particulate organic matter of different quality (more or less degraded), arriving at bathyal depths, as inferred by the quite different stable isotope $\delta^{15}N$ found in *Aspidosiphon muelleri* Diesing, 1851 and *Sipunculus norvegicus* Danielssen, 1869 (respectively 5.39‰ and 9.57‰, Fanelli *et al.*, 2011), two dominant sipunculan species inhabiting the investigated area (Cartes *et al.*, 2009).

After a detailed anatomical study of the phascolosomatid specimens collected, we observed anatomical features unknown to any previously recorded Mediterranean species (Pancucci-Papadopoulou *et al.*, 1999) of the genus *Phascolosoma* Leuckart, 1828. Thus, the aims of the present study are: (1) to report this new faunistic finding for the Mediterranean Sea; and (2) to select reliable characters for the distinction of closely related species. The ecological information of those collected specimens is also discussed.

MATERIALS AND METHODS

Specimens of deep-sea phascolosomatids were collected during trawling operations of the RV 'García del Cid' in the north-western part of the Mediterranean Sea. Station names, coordinates, depths, sampling dates and number of specimens are listed as follows:

—Station BIOM3-OTSB4; 41°07.130″–41°07.841″N 2°22.497″–2°27.977″E; 1039–1103 m; 01/07/2007; 2 specimens.
—Station PROMETEO3 M-28; 41°07′60″N 02°52′16″E; 1500 m; 13 May 2009; 1 specimen.

Immediately after sampling, all samples were preserved in 4% borax-buffered formaldehyde, prepared using seawater. Once in the laboratory, the material was studied using standard dissecting techniques and both binocular and compound microscopes. The species identification was based mainly on the works of Cutler (1994) and Pancucci-Papadopoulou *et al.* (1999). Voucher material was deposited at the Institut de Ciències del Mar (CSIC).

RESULTS

SYSTEMATICS

Phascolosoma (Phascolosoma) turnerae Rice, 1985
(Figure 1A–F)

Trunk 23–44 mm long and 9–12 mm wide, light brown in colour (Figure 1A). Skin opaque to translucent. Papillae

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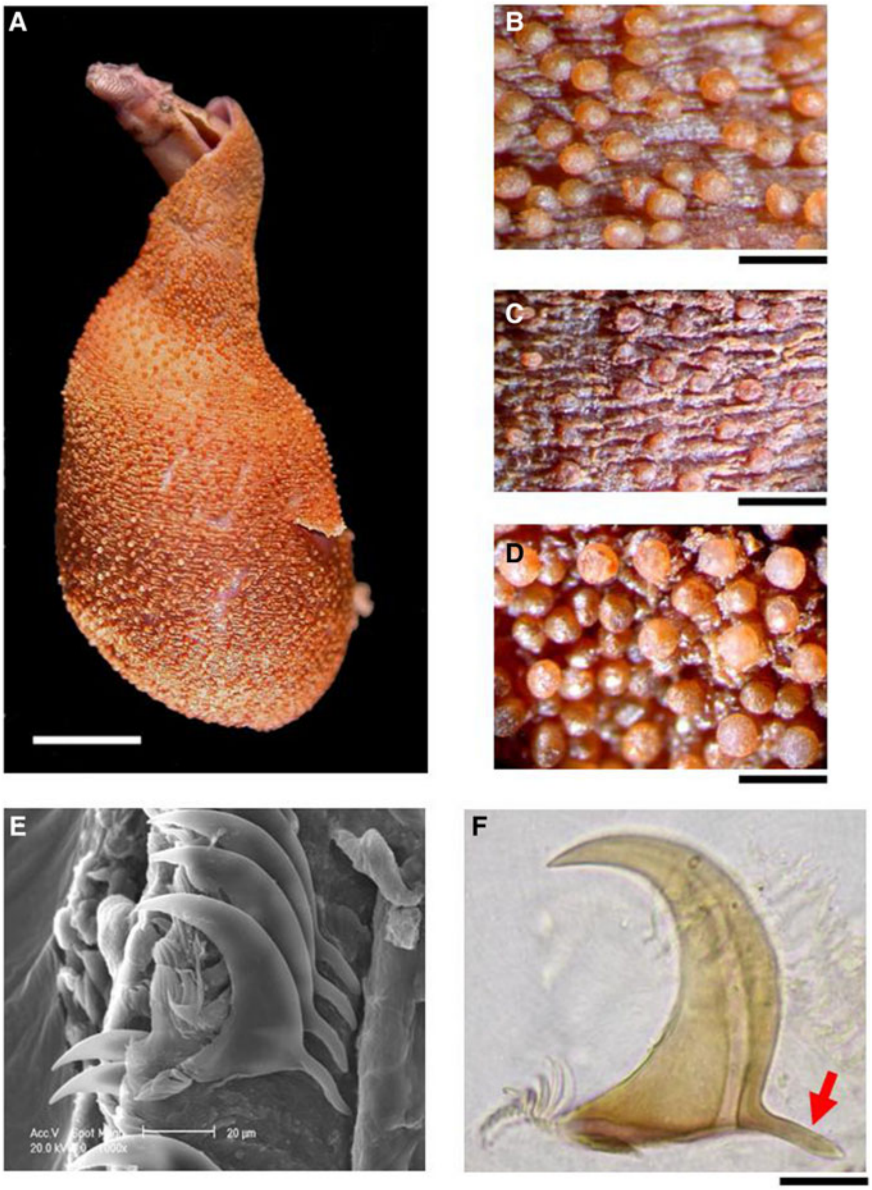

Fig. 1. *Phascolosoma (Phascolosoma) turnerae*: (A) external view; (B) papillae in the anterior part of the trunk; (C) papillae in the middle part of the trunk; (D) papillae at the trunk end; (E) scanning electron micrograph of a hook ring; (F) detail of a hook under the light microscope. Red arrow indicates the characteristic long basilateral extension of the hook. Scale bars: A, 5 mm; B and D, 0.5 mm; C, 0.3 mm; F, 20 µm.

distributed over the entire trunk, larger and more densely packed at both ends (Figure 1B, D). Introvert 25–42 mm long with some pigmented bands. About 28 digitiform tentacles unpigmented. About 90 hook rings, many incomplete. Under the microscope hooks appear slightly curved without a secondary tooth (Figure 1E). Height 50–75 µm. At the posterior base of the hooks there are long toes and a basal wart. A long clear streak with some expansion is noted. No triangles, but with a characteristic long basilateral extension (Figure 1F). Tubuliform papillae about 10 µm high are placed between the hook rings. Internally, the longitudinal musculature has about 30–34 anastomosing bands. Circular musculature also split in fine bands. Four retractors the ventral arising from 6–8 bands, whereas the dorsal arise from 7–8 bands. The distance from the ventral insertions to the trunk end is 5–10 mm. Gut organized in about 15 spirals. Spindle muscle present and attached posteriorly. Two nephridia, 11 mm long with the posterior 2 mm free. Nephridiopores open a little anterior to the anus. Gonads observed at the base of the ventral retractors.

DISCUSSION

A total of 5 species of *Phascolosoma* have been reported from the Mediterranean Sea at coastal waters varying from 0 to 230 m deep (Pancucci-Papadopoulou *et al.*, 1999). The collection of deep-water phascolosomatids in the mainland slope of the Balearic Basin was remarkable, since we were unable to relate them to any representative of the genus *Phascolosoma* for the Mediterranean Sea. According to Cutler (1994), the nature of the hooks appears to be species-specific within this sipunculan genus and a global key for the identification of all valid species was constructed by using basically the detailed study of the hooks under the light microscope. In this way, the presence of characteristic hooks with anterior prong-like extensions related our specimens to only the two species exhibiting this character: *Phascolosoma turnerae* Rice, 1985; and *P. saprophagicum* Gibbs, 1987. Gibbs (1987) differentiated the two species by: (i) the height of the hooks ($>$ 45–80 µm in *P. turnerae* vs 20–25 µm in *P. saprophagicum*); and (ii) the external shape of the hooks (sharply-pointed in *P. turnerae* vs bluntly-rounded in *P. saprophagicum*). Since our specimens showed sharply bent hooks, 50–75 µm tall, we have identified them with *P. turnerae*.

The faunistic finding is also interesting from an ecological approach. The species was described originally by Rice (1985) inhabiting submerged wood at bathyal depths in the Strait of Florida and the Gulf of Mexico. The large abundance of sipunculans boring the wood persuaded this author to note a biological association between this sipunculan species and the wood. Similarly, our BIOM3-OTSB4 specimens were collected (after 4.5 ha of trawling) together with a large piece (~25 kg) of a tree trunk, some tree leaves (from purple beech, *Fagus sylvatica*) and *Posidonia oceanica* remains, suggesting this ground located near Besós Canyon could be a depocentre area for terrestrial-shelf material. These terrestrial inputs are patchily distributed over the slope (authors, unpublished data), although they are more frequent in the mainland than in the insular slope of the Balearic Basin, with higher levels of total organic matter at 1000–1100 m (Fanelli *et al.*, 2013). δ^{13}C stable isotope signals on sediments close to the BIOM3-OTSB4 haul (between −23.6‰/−24.8‰ Fanelli *et al.*, 2013) also suggests an enrichment of terrigenous sediments there.

Almost simultaneously Edmonds (1985) described a new species, *Phascolosoma kapalum*, from deep bottoms off Australia. In this paper, there was no indication of wood occupation by the sipunculans. Later on, Edmonds (1992) compared specimens of *P. kapalum* with *P. turnerae* and decided that the two taxa were conspecific, the first name being a junior synonym of the last one. The finding of an extra specimen from a piece of rotting wood off the Great Barrier Reef (Australia) confirmed the character of a deep-sea wood dweller. However, in the review of the subgenus *Phascolosoma* achieved by Cutler & Cutler (1990), they identified further specimens from bathyal depths in the Gulf of Mexico, but this time the association was noted with tubeworm aggregations near a cold water seep. Other more ecological papers (Olu *et al.*, 1996, 2010; MacAvoy *et al.* 2005; Cordes *et al.*, 2006, 2007) reported the species also in mussel beds and sediments at the base of the tubeworm aggregations from different cold seep sites around the Gulf of Mexico, but also offshore the Orinoco River delta in South America (at 1950–2080 m deep in sediment samples).

Another rare phascolosomatid, *P. saprophagicum* Gibbs, 1987, was collected from bathyal depths close to Chatham Island (New Zealand). Several specimens were obtained from the crevices and between the attached mussels on a large piece of decaying bone corresponding to a whale skull. Both *P. saprophagicum* and *P. turnerae* are the only representatives of the genus colonizing bizarre ephemeral habitats in deeper waters. Moreover, both species share this unique basal projection at the anterior base of the hook. Whereas we can suspect the existence of some genetic affinity between the two species, only DNA has been sequenced by now from *P. turnerae* (Kawauchi *et al.,* 2012). Consequently, *P. turnerae* and *P. saprophagicum*, remain separate valid entities, as was proposed in the last monograph about the phylum (Cutler, 1994).

Recently, a comprehensive and detailed study on the reproductive biology and life-history of *P. turnerae* was published (Rice *et al.*, 2012), since the authors were able to maintain several specimens in captivity for long periods of time. Interestingly, other kinds of habitats were reported for the species, such as authigenic carbonate rocks and bundles of plant fibres, which inspired the authors to design artificial collectors of deep-sea phascolosomatids. The species was additionally found in bathyal depths close to the Bahamas and Barbados, showing by now a disjunct geographical distribution of two remote areas: the western Atlantic and south-western Pacific Oceans. To explain this gap, Rice *et al.* (2012) suggested the possibility of a wider distribution than currently known for *P. turnerae*. This hypothesis is today more plausible, with the discovery of *P. turnerae* in bathyal depths of the Mediterranean Sea (Bienhold *et al.*, 2013; our findings). In this way, these locations within the Mediterranean Sea represent an intermediate spot between the two remote areas where the species was previously collected. The presence of *P. turnerae* in the Mediterranean Sea has also implications in a potential connection of disjunct metapopulations of *P. turnerae* from both sides of the Atlantic Ocean. Dispersal trajectories of planktonic larvae were simulated in a recent study of this wood-dwelling species (Young *et al.*, 2012). Its larvae were capable of reaching the mid-Atlantic off Newfoundland, a distance of more than 3000 km, during a long drifting period. Consequently, the possibility of a genetic exchange from west to east in the North Atlantic Drift current is even more probable, specimens of *P. turnerae* having been collected in the Mediterranean Sea.

Finally, we were unable to note a direct association of *P. turnerae* with sunken wood in the western part of the Mediterranean Sea. However, Bienhold *et al.* (2013) did recently by deploying wood colonization experiments offshore the River Nile delta at a depth of 1690 m (eastern Mediterranean Sea). In our case, *P. turnerae* was the only sipunculan species collected in both hauls, while other sipunculans are relatively abundant from other sampling sites (Cartes *et al.*, 2009; Mamouridis *et al.*, 2011; Tecchio *et al.*, 2013) over the investigated slope. In general, surface deposit-feeder invertebrates were poorly represented in the first reported haul (only 1 holothurian, *Mesothuria intestinalis*), while the rest of invertebrates were—based on stable isotope analyses in the same area (Fanelli *et al.*, 2011, in 2013)—both filter feeders (1 bivalve *Abra longicallus*; 1 holothurian *Ypsilothuria bitentaculata*) and carnivores (1 seastar *Ceramaster grenadensis*). This suggests that inputs of fresh organic matter, as found deeper (e.g. over 1600 m, Cartes *et al.*, 2009), are low within the trawled area, indirectly reinforcing the idea that *P. turnerae* would have as food source ephemeral material derived from terrestrial inputs, such as remains of wood.

KEY TO THE GENUS *PHASCOLOSOMA* LEUCKART 1828, FROM THE MEDITERRANEAN SEA [MODIFIED FROM PANCUCCI-PAPADOPOULOU *ET AL*. (1999) TO ACCOMMODATE THE NEW RECORD]

1a. Hooks under the light microscope exhibiting an anterior stout and long projection at the base of their convex side .... *P. turnerae*

1b. Hooks without an anterior projection at the base of their convex side 2
2a. More than 50 complete and incomplete rings of hooks over the full everted introvert 3
2b. Less than 50 complete and incomplete rings of hooks ..4
3a. Hooks with a posterior crescent area, many >75 μm tall; preanal papillae are smooth cones; pigment bands on introvert .... *P. stephensoni*
3b. Hooks with a granular triangle at their anterior base, but without crescents, most <75 μm tall; no pigment bands on introvert .... *P. granulatum*
4a. Large rounded hump on concave side of the hook; preanal papillae smooth, posteriorly directed, cone shaped .... *P. perlucens*
4b. Concave side of hook smooth or with small tooth 5
5a. Hooks with distinct triangle; narrow band of red cone-shaped preanal papillae .... *P. scolops*
5b. Hook triangle indistinct or absent; preanal papillae not distinct from dome-shaped trunk papillae
P. agassizii agassizii.

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