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CHARACTERIZATION OF COLD WATER CORAL COMMUNITIES IN THE SOUTHERN ADRIATIC

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1. Introduction

1.1. Cold Water Corals in the Mediterranean Sea

The Mediterranean Sea is a temperate basin whose modern marine fauna has been reshaped after the dramatic ecological consequences for its resident biota in the terminal Miocene at the time of the "Messinian salinity crisis", ca. 6 million years ago (e.g., Taviani, 2002; Sabelli and Taviani, 2014; Vertino et al., 2014).

The Mediterranean Sea has been and still is home to Cold Water Corals (CWC thereafter) of Atlantic affinity (Vertino et al., 2014). CWC live in total darkness below the photic zone between 200 and 4000 m from tropical to polar latitudes (Roberts et al., 2006). CWC are suspension feeding organisms, dependent by the contribution of organic matter in the form of particulate matter and zooplankton, preferentially settling topographic highs, seamounts, submarine canyons or other reliefs where the currents are strong and sediment supply low (Freiwald et al., 2009). (Freiwald et al., 2009). In the Mediterranean the most important CWC are the two framebuilding colonial scleractinians *Lophelia pertusa* (Linnaeus, 1758) and *Madrepora oculata* Linnaeus, 1758, which are often associated with the solitary species *Desmophyllum dianthus* (Esper, 1794), the black coral *Leiopathes glaberrima* (Esper, 1788) and other cnidarians.

The habitat-forming ability of corals have fascinated generations of marine biologists. The role of one species to provide habitat for others has been formalised through the concept of 'ecological engineering' (Jones et al., 1994; Wright and Jones, 2006). Different cold water coral species develop habitats of varying physical sizes and life spans. The skeletons of long-lived cold-water corals, can themselves provide structural habitat during their lifetimes that may also persist for some time after death. The complex three-dimensional architecture and spatial variability of CWC habitats provides niches for a diverse range of organisms. Also serving as nursery grounds, and are areas of spawning and feeding for several commercially important deep-sea species (Jensen and Frederiksen, 1992; Mortensen et al., 1995; Fosså et al., 2002; Husebø et al., 2002; Roberts et al., 2008; D'Onghia et al., 2010; Piroddi et al., 2010).

Although their growth rate is very slow (5 to 33 mm / year depending on environmental conditions: Mortensen and Rapp, 1998; Roberts et al., 2002), the CWC may build considerable

bio-constructions, thus generating true "coral reefs" in cold, deep waters. Coral recruitment, growth, framework production, sediment trapping and bioerosion all lead to a highly varied structural habitat that may persist for millennia. The best-studied CWC reefs are those formed by *Lophelia pertusa* thanks to its capacity to provide an array of microhabitats such as those categorized by Mortensen et al. (1995) as: (1) the smooth surface of living coral, (2) the detritus-laden surface of dead coral, (3) the cavities made by boring sponges within the coral skeleton and (4) the free space between coral branches. Dead coral framework is the most significant structural habitat in terms of associated species diversity (Roberts et al., 2009). Dead framework is found beneath live coral and on lower slopes of the reef. It is typically characterized by attached, suspension-feeding fauna including numerous sponges, actinians and other coral species (notably the Atlantic gorgonians such as *Paragorgia arborea* and *Primnoa resedaeformis*). Smaller epifauna include bryozoans, hydroids and barnacles. Where sediment becomes trapped in the coral framework niches appear for infaunal species such as polychaete worms (Roberts et al., 2009). Deep-sea coral rubble systems are also characterized by high biodiversity (Raes and Vanetreusel, 2005; 2006; Raes et al., 2008; Roberts et al., 2008). The presence of different types of *Lophelia pertusa* macrohabitats (e.g., framework versus coral rubble) is also an important factor enhancing beta diversity in the associated macrofauna (Henry et al., 2010).

A number of CWC provinces and sites are known to date in the Mediterranean Sea (summarized by Taviani et al., 2016). They are mostly distributed in the western and central part of the basin, while living CWC grounds are much less common in the eastern basin. Significant CWC grounds have been identified in the Alboran Sea, Catalan-Provencal-Ligurian canyons, off south Sardinia, Sicily Channel, Southern Adriatic and Northern Ionian Sea (Freiwald et al., 2009; Orejas et al., 2009; Angeletti et al., 2014; Taviani et al., 2016). The Santa Maria di Leuca CWC province is characterized by co-occurring *Madrepora* and *Lophelia* (Tursi et al., 2004; Taviani et al., 2005; Freiwald et al., 2009; Savini and Corselli, 2010; Mastrototaro et al., 2010) and is the most extended and developed in the Mediterranean Sea (Freiwald et al., 2009). Living and dead CWC cover an area of approximately 1000 km² (Taviani et al., 2005; Freiwald et al., 2009; Vertino et al., 2010; Savini et al., 2014), contributing to habitat heterogeneity in the deep northern Ionian

Sea. CWC biodiversity estimates have been derived from both visual surveys of the macro- and megafauna and by identifying and counting numbers of macro and meiofaunal species in samples. The need of the governance and, ultimately, protection of Cold Water Coral habitats is gaining increasing attention becoming an important issue on the political agenda of many national and international organizations. In fact, many these CWC habitats are vulnerable and could be destroyed in less than a generation if not safeguarded (Freiwald et al., 2004). CWC are menaced by multiple threats, natural and anthropogenic. The latter are mainly fishing malpractices, dumping and littering. Because CWC act as biodiversity hotspots, attracting also species of economic importance, they are targeted by fishing activities like bottom longline and trawling (Le Goff-Vitry and Rogers, 2005; Mortensen et al., 2005; Waller and Tyler, 2005; Edinger et al., 2007; D'Onghia et al., 2010). In addition, the corals are also exposed to damages derived from scientific research. Therefore, the exploration of these ecosystems requires the use of non-invasive sampling methods to limit a negative impact on biota. In this regard, the use of underwater cameras ROV (Remotely Operated Vehicle) is recommended since allow to get visual information and samples CWC habitats without causing major damage (Uiblein et al., 2003; Freiwald et al., 2009; D'Onghia et al., 2011; Capezzuto et al., 2012; Angeletti et al., 2014; Taviani et al., 2016).

Most information on the deep benthos of the Albanian deep water derives from fishery activity. In 2013 the oceanographic cruise Cocomap13, by mapped and sampled the Albanian margin, acquiring the first geomorphologic data along the continental slope of Karaburun Peninsula. This exploration, contemplating also ROV dives, failed to find any sizable live or dead CWC bioconstruction in this area (Report on habitat maps of shallow and deep water areas at different scales (T40, WP11). D11.10 CoCoNet). A limited number of bottom samples did, however, provide a small quantity of dead CWC, that have been considered in the present thesis.

The present study regarding the Albanian CWC and associated biota consists of specific research activities focused on the four main topics listed below.

Sessile benthic fauna associated with sub-fossil Cold Water Coral from off Albania

This section presents a preliminary description of the fauna associated with sub-fossil coral framework in two sites recently found in off Albania. Macrofauna was recorded during two experimental fishing surveys conducted in March 2015 and May 2016 in two deep areas of the Albanian coast, between the depth of 190 to 230 m. Sub-fossil coral samples of *L. pertusa* and *Dendrophyllia cornigera* were analyzed to identify the associated fauna. A total of 82 species were identified, 2 Foraminifera, 22 Porifera, 6 Cnidaria, 25 Mollusca, 14 Annelida, 1 Arthropoda, 11 Bryozoa, 1 Echinodermata, including 75 species that are new records for the Albanian fauna. All such taxa have been previously reported at Santa Maria di Leuca and Bari Canyon. This study updates our knowledge on the biodiversity of the deep Adriatic Sea, with special reference to the virtually unknown Albanian waters. New information obtained for deep sea species further confirms the important contribution of CWC frameworks to deep sea biodiversity.

Morphological variations of Mediterranean *Lophelia pertusa*

In order to assess if the morphological variation of the coral *L. pertusa* follows a specific pattern based on environmental (or others) variables, a study was performed combining two different morphometric approaches: classical linear external morphology and use of three-dimensional coordinates landmarks. The main aims of this study are to test the existence of distinct morphological groups within the quasi-cosmopolitan species *L. pertusa* in the Mediterranean and to identify, if any, relationship between morphological groups (both in modern and fossil corals) and (paleo) oceanographic conditions.

Applications of X-ray Computed Tomography in the morphometric study of the Adriatic *Lophelia pertusa*

The purpose of this work was to compare four colonies of sub-fossil coral *Lophelia pertusa*, coming from the Mediterranean and East Atlantic. Sub-fossils are important archives that document suitable conditions in the past for coral growth. The aim of this analysis was to investigate whether there are really morphological differences between these four *Lophelia* colonies, both in the morphology of the corallites and in their spatial organization. Computed

tomography made possible to measure some variables chosen according to the species' characteristics.

Dating the Albanian Adriatic cold water corals

This part of my study deals on precise U/Th dating of dead corals specimens sourced coming from Albanian waters. Samples were obtained off Albania by the oceanographic CNR CoCoMap13 cruise in the southeastern Adriatic Sea and one *Lophelia pertusa* colony caught during experimental fishing surveys in north of the Vlora Bay. The purpose of this research is to date the precise age of coral fossils and then rebuild the geological events that have caused their death in the south-eastern part of the Adriatic.

2. Currently known distribution of Cold Water Coral habitats in the Adriatic

2.1. Introduction

One of the earlier account on CWC in the Mediterranean and their importance was presented by Pérès and Picard (1964). However, the presence of live CWC in the Adriatic waters would be documented much later (Freiwald et al. 2009), following the discovery of lush CWC growth by fishery operations on the eastern side of the Ionian Sea (Tursi et al., 2004, Taviani et al., 2005). The so-called "white corals" (Pérès and Picard 1964) play a fundamental role in the deep sea biome. The main habitat framebuilders, *Lophelia pertusa* and *Madrepora oculata*, provide niche to benthic and nektonic organisms, enhancing a complex and articulated trophic web. The importance of these species is important even *post-mortem* as their carbonate exoskeleton remains provide habitats to many organisms (Remia and Taviani, 2005; McCulloch et al., 2010; Buhl-Mortensen et al., 2010; Malinverno et al., 2010; Mastrototaro et al., 2010; Taviani et al., 2015). Because of their recognized ecological and ecosystemic services (Henry and Roberts, 2007; Foley et al., 2010, etc.), a number of oceanographic campaigns across the Adriatic have been conducted for their mapping and description (Zibrowius, 2003, Tursi et al., 2004; Taviani et al., 2010, D'Onghia et al., 2012). The scattered information derived from the various oceanographic missions devoted to the exploration of CWC grounds, has been conveyed in a general map reporting CWC sites discovered thus far in the Adriatic Sea, see Figure 2.1. The map was constructed on the basis of the presence / absence of CWCs in the living or fossilized state..

The asymmetric distribution of these habitats in the southern Adriatic is evident (Taviani et al., 2015). The presence of CWC is evident on both sides of the Adriatic, but there is a clear asymmetry of these habitats (Taviani et al., 2015). One of the most important sites for number of coral colonies and associated fauna, is Bari Canyon (Freiwald et al., 2009; Angeletti et al., 2014; D'Onghia et al. 2015; Taviani et al., 2015).

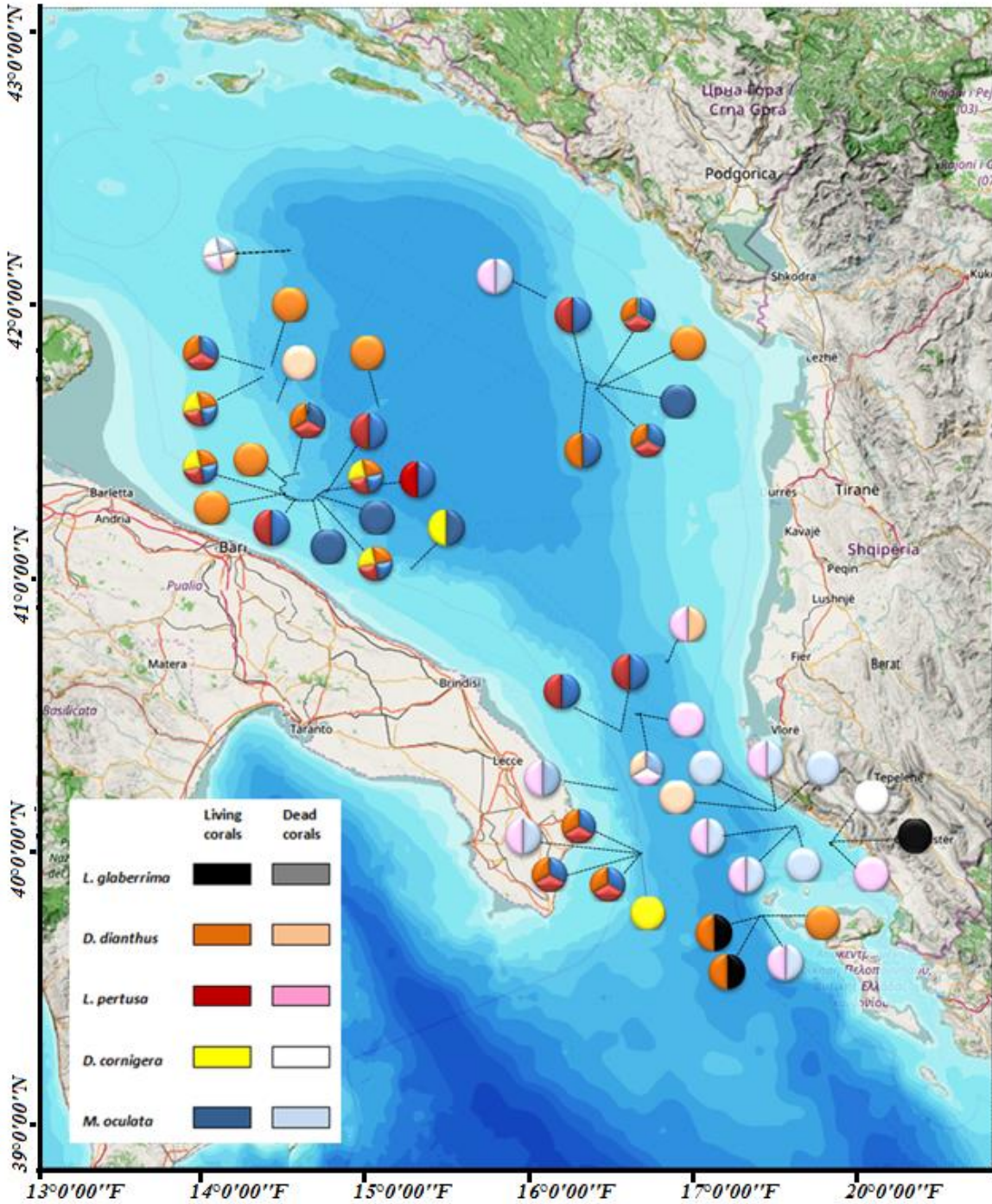


Figure 2. 1. Currently known distribution of cold water coral habitats in the Adriatic (A list of station coordinates taken into account for this map is attached to the end of the chapter, (Considering the density of the stations, the map has been simplified): modified from Taviani et al (2016).

2.2. Main traits of the southern Adriatic deep-water circulation

The Southern Adriatic basin is characterized by a complex hydrology. The southern Adriatic basin is connected to the Northern Ionian Sea through the Strait of Otranto, which is the area in which it is channeled an annual flow of water masses of 35 million m³, with a typical cyclonic circulation (Artegiani et al., 1997).

The basin is affected by North Adriatic dense waters, i.e. NADW: North Adriatic Dense Water, ADW or Adriatic Deep Water and Levantine Intermediate Waters (LIW) Fig 2.2. In particular, the dense and cool NADW flows north to south along the western continental shelf while the warmer and salty come from the Northern Ionian Sea through the Otranto Strait and flow north-south along the eastern Adriatic coasts of Albania, Montenegro and Croatia (Manca et al., 2002; Aagaard et al., 1985). The North Adriatic cool and dense deep water masses, whose formation responds to bora wind regimes, flow along the Italian coast (western Adriatic side) reaching down off the Gargano peninsula 2-4 months after their formation (Trincardi et al., 2007; Rubino et al., 2010). A branch of NADW entering in the Bari Canyon, is accelerated, reaching speeds exceeding 60 cm s⁻¹. At the exit of the canyon, the current disperses laterally, producing fields of mud waves on a surface of 80 km² (Trincardi et al., 2007; Canals et al., 2009). These water masses are the cause of the presence of a higher thermal and saline regime in the southern part of the Adriatic basin compared to its western part (Artegiani et al., 1997). The surface current present along the western coasts (see fig 2.2), drives the water masses from the Adriatic to the Ionian (Zore-Armanda 1968, 1969). Thanks to the presence of these streams, the Southern Adriatic basin, is characterized by the mixing of the Adriatic waters, colder and less salty, and the Ionian waters, with higher temperature and salinity (Bregant et al., 1992; Vilibic et al., 1995). The average salinity of the Adriatic basin amounts to 38.5 ‰, with Levantine Intermediate Waters (depth 100-500 m) that have slightly higher salinities (38.75 ‰; 13.7 ° C), followed by the Adriatic deep waters ADW (38.65 ‰; 13.3 ° C) temperature (Poulain, 2001). The surface temperature of the basin varies from 28-29 ° C in summer to 9-11 ° C in winter. The trophic conditions show an oligotrophic medium Southern Adriatic basin, with average concentrations of nitrogen and phosphate ranging between 25 g / l and 35 g / l, and between 7 g / l and 12 g / l (Casavola, 1999).

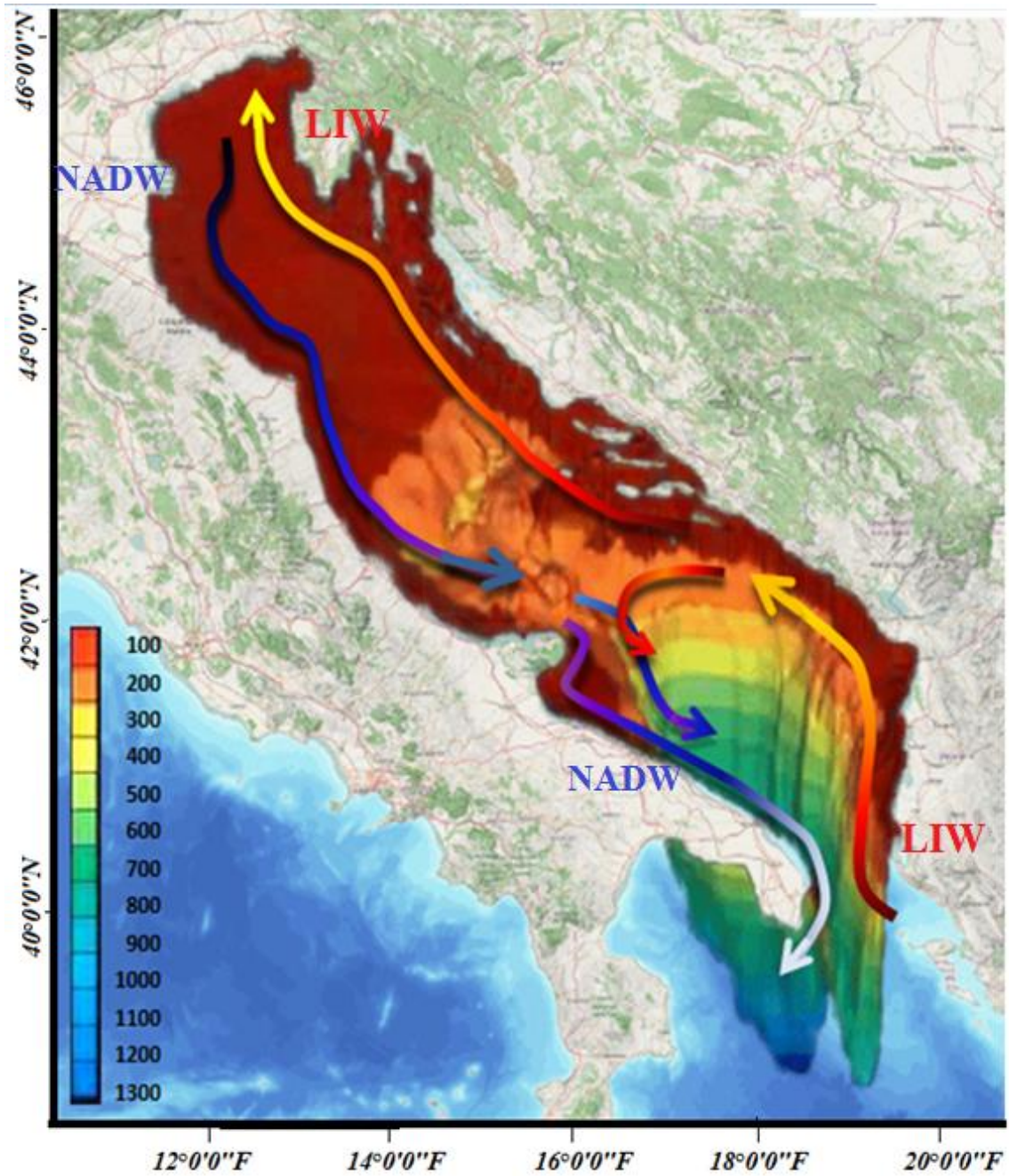


Figure 2. 2. Bathymetric map of the Adriatic Sea reporting also thermohaline circulation. NADW: North Adriatic Dense Water; LIW: Levantine Intermediate Waters
Image modified from:<http://engineering.dartmouth.edu/adriatic/index.html>)

2.3. The western margin of Adriatic

Currently known sites for the presence of CWCs in the southwestern part of the Adriatic are Bari Canyon, Gondola Slide and Dauno Seamount (Freiwald et al., 2009; Bo et al., 2012; Sanfilippo et al., 2013, Angeletti et al., 2014; Taviani et al., 2015). The latter are also the most important white coral sites so far discovered throughout the Adriatic. Two other sites less explored but equally important in this part of the Adriatic are the sites of Tricase and Otranto. The data on these two sites comes from different sea bottom sampling (Angeletti et al., 2014).

2.3.1. Description of the coral area of Bari Canyon

One of Mediterranean canyons characterised by the presence of CWC, episodic cascading processes and habitat heterogeneity is located off the city of Bari in the southern Adriatic Sea (central Mediterranean) (D'Onghia et al., 2015). Is a complex structure which continues for 10 kilometers wide and 30 km long, consisting of two main branches separated by a relief. Its depth ranges from 200 to 1700 meters, where the southern branch is deeper and wider (Ridente et al., 2007; Trincardi et al., 2007, D'Onghia et al., 2015).

The Bari Canyon serves as the main source of food and oxygen for many benthic organisms, carrying sediments and nutrients through the North Adriatic Dense Water stream. The canyon serves as a deviation for a part of the Adriatic Dense Water stream, which spends from the continental shelf down to the bathyal zone. The latter runs southward to the Strait of Otranto in the Ionian Sea, following the isobates at an intermediate depth of between 400 and 700 meters (Vilibic and Orlic, 2002, Angeletti et al., 2014).

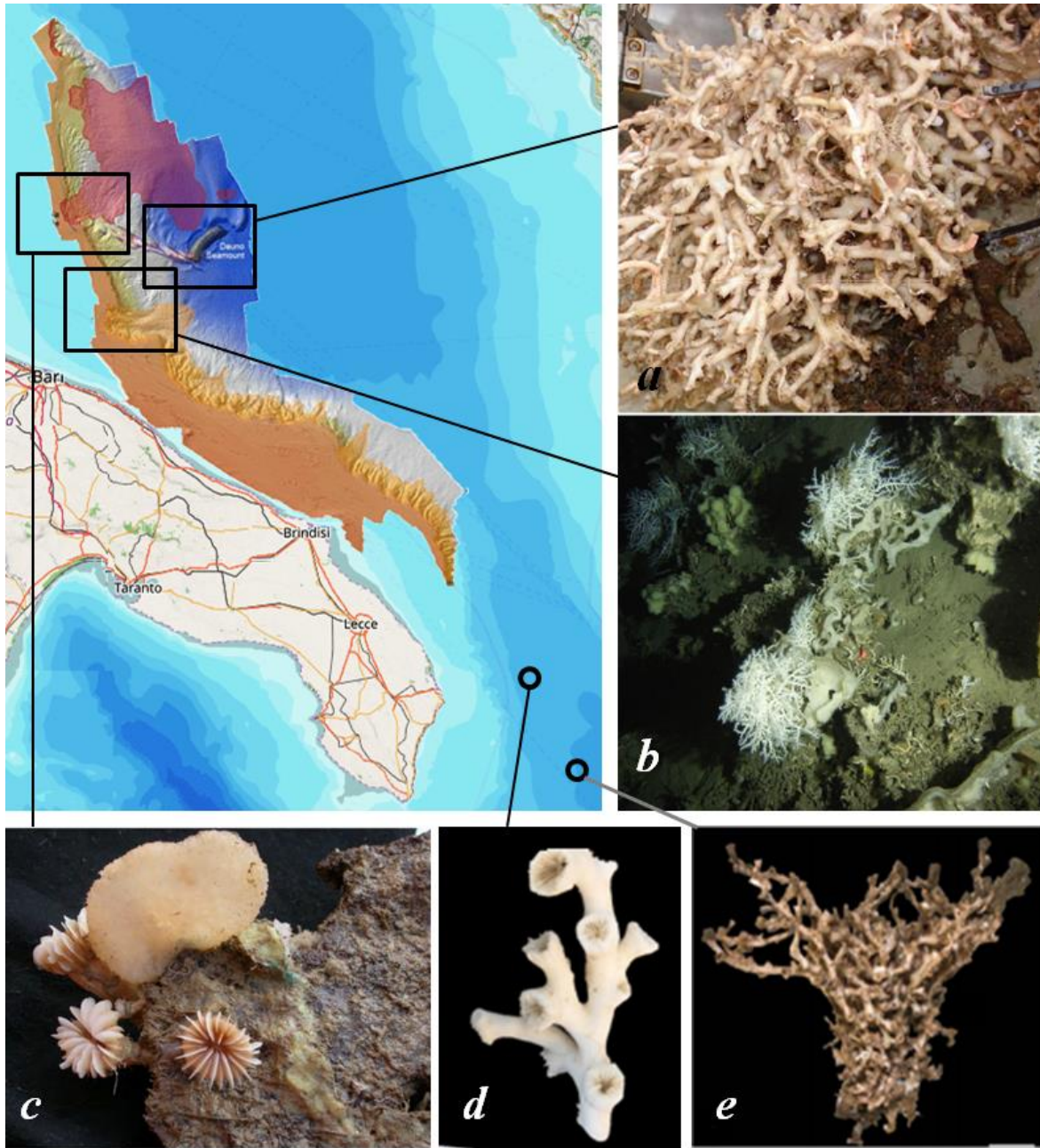


Figure 2. 3. Currently known distribution of Cold Water Coral habitats in the south-western Adriatic Sea, **a)** Dauno Seamount (Freiwald et al., 2009); **b)** Bari Canyon (Taviani et al., 2015); **c)** Gondola Slide (Freiwald et al., 2009); **d)** Otranto slope (from Bari university samples); **e)** Tricase (Angeletti et al., 2014). Bathymetric map produced by the ISMAR-CNR, Bologna (Tricardi et al., 2010).

This site is receiving increasing attention and is probably the most studied CWC site in the Mediterranean Sea after Santa Maria di Leuca (Trincardi et al., 2007; Freiwald et al., 2009; Sanfilippo et al., 2013; Angeletti et al. 2014; D'Onghia et al. 2015; Taviani et al., 2015).

The canyon's morphology is characterized by a very variable morphology, This habitat presents the environmental characteristics suitable for the coral development. The rocky bed of this Canyon is dominated by the *Lophelia-Madrepora-Desmophyllum* association (fig 2.3), which offer shelter to many other species, such as sponges, serpulids and crustaceans. The fan-shaped colonies of *M. oculata* and *L. pertusa*, present between 450 and 500 meters deep, are often associated with the sponge *Pachastrella monilifera* and solitary corals such as *Caryophyllia calveri* and *D. dianthus* (Angeletti et al., 2014; D'Onghia et al., 2015; Taviani et al., 2015). These associations of cnidarians and sponges are mainly aligned along the canyon wall, adapting to dominant current directions in order to maximize their feeding efficiency and mechanical (Bo et al., 2012; Angeletti et al., 2014; Taviani et al., 2015). A total of 111 species occurs at this CWC site, many of them also recorded at Santa Maria di Leuca (D'Onghia et al., 2015). According to Angeletti et al. (2014), this may suggest a connectance between the two CWC sites.

2.3.2. Dauno Seamount

The Dauno Seamount is a structural high in the southern Adriatic, extending for more than 20 km in the east in the water depth range of 700-1100 m (Viana and Rebesco, 2007; Trincardi et al., 2007). The Dauno Seamount, possibly related to the tectonic activity of the Gondola fault system (Tremontana et al., 1995), is characterized by a relief of about 400 m above the surrounding seafloor and steeper north flank, which may act as a hydrological barrier (Genin, 2004; Craig et al., 2010; Viana and Rebesco, 2007). The Dauno Seamount hosts a reduced CWC presence mostly exploiting the bedrock and hardgrounds mainly represented by *D. dianthus* (Freiwald et al., 2009). The corals occur between 930-780 m, and are often associated with the sponge *P. monilifera*.

2.3.3. Gondola Slide.

The Gondola Slide represents the most obvious mass wasting deposit in the area. It is characterized by large blocks, a 10-km-wide slide wedge branching on the shelf edge, and a total runout of more than 50 km. The upper portion of the slide, extending about 23 km from the slide scar and down to water depth of 800 m, is exposed on the seafloor (Minisini et al., 2006; Trincardi et al., 2008). The presence of suitable hard substrates (such as the slumped blocks and hardgrounds) coupled with persistent bottom currents, and the seasonal variability of nutrients, are the main factors which favor CWC growth of CWCs at the Gondola Slide (Trincardi et al., 2007; Freiwald et al., 2009).

2.3.4. Tricase

The Tricase slope has been recently identified as a CWC site (Angeletti et al., 2014; Taviani et al., 2015). The morphology of the margin is complex and encompasses several canyons, and a large valley at the base. The slope reaches 786 m water depth. The presence of live colonies of *Desmophyllum dianthus*, *L. pertusa* and *M. oculata* has been confirmed at c. 700 m. Most colonies of *L. pertusa* and *M. oculata* recovered here are subfossil but serve as substrate for many other organisms such as serpulids, sponges (*P. monilifera* and *P. compressa*) and cnidarians such as *Stenocyathus vermiformis* (Taviani et al., 2015).

2.3.5. Strait of Otranto

The Strait of Otranto is a key area for the entire Mediterranean deep circulation, since the Adriatic Sea is a major locus for the production of the basin bottom water (Poulain et al., 1996). The data on this site are the same as limited to grab samples. The samples were presented with thin pieces of hard-ground colonized by small colonies of *L. pertusa* and *M. oculata* (Angeletti et al., 2014). This site could be another stepping stone between the site of Santa Maria di Leuca and the CWC habitats of the southern Adriatic. The site is located along the peninsula of Salento, between the Italian and the Albanian margins, 775-778 m deep, at the base of a slope, which is probably under the influence of the North Adriatic Dense Water (Angeletti et al., 2015).

2.4. South-eastern Adriatic Sea

2.4.1. Croatian margin

The Croatian margin, influenced mainly by the Levantine Intermediate Waters (Manca et al., 2002, Aagaard et al., 1985) does not present any ascertained CWC occurrence (Angeletti et al., 2014; Taviani et al., 2015).

2.4.2. Montenegrin margin

The Montenegrin margin has been explored by about six oceanographic cruises which have also collected geo-biological information according to Angeletti et al., (2014) and Taviani et al. (2015). Hardground blocks emerging from a muddy seabed in canyons serve as propitious substrate to many sessile organisms, such as serpulids, sponges and molluscs. The presence of black coral *Leliopates glaberrima* colonies, and associated clusters of live *M. oculata* and cm-size *L. pertusa* is noticeable (fig 2.4). In addition, it has been documented the occasional presence of *Dendrophyllia cornigera*. The soft sediment is sparsely inhabited by the white fan-shaped sponge and gorgonian *Pachastrella monilifera* and *Paramuricea macrospina*, respectively, while hardgrounds are intensely colonized by sessile megabenthos, most noticeably, flashy colonies of the fan-shaped *Callogorgia verticillata*, reaching up to 1 m in height (Angeletti et al., 2014). *C. verticillata* colonies are preferentially oriented contrasting the dominant currents co-occurring, in forming a true gorgonian garden (Angeletti et al., 2014). The scarcity of hard substrates to the north of the Montenegrin margin also affects the absence of corals in this area.

2.4.3. Albanian margin

From the compiled reports on CoCoMap 2013 oceanographic campaign, one can appreciate a general picture of the geomorphology of the Albanian margin. The Albanian margin has several canyons, where the most prominent are concentrated in the south.

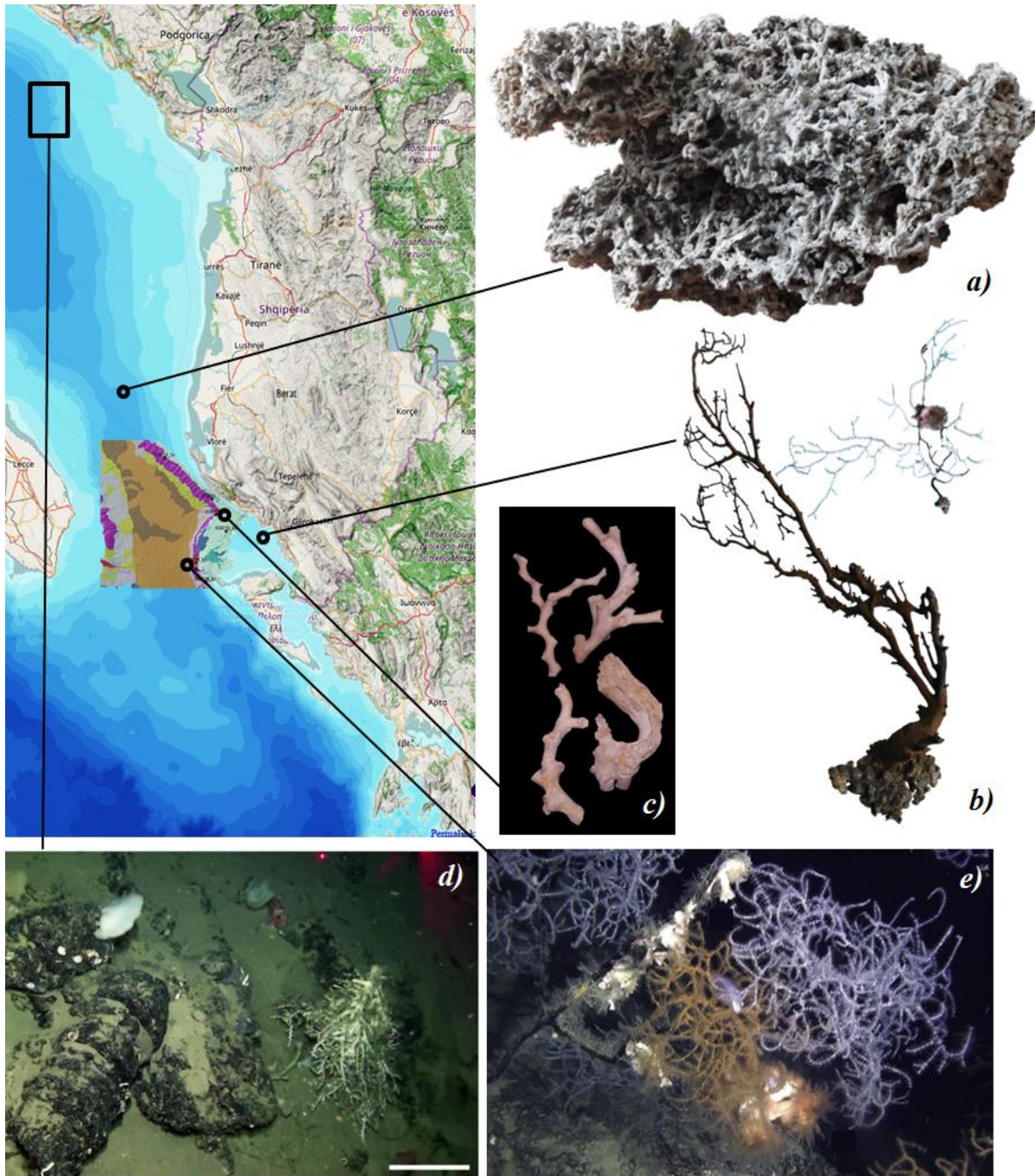


Figure 2. 4. Currently known distribution of cold water coral habitats in the south-eastern Adriatic Sea: a) Seman slope, b) *L. glaberrima* from Saranda margins, c) samples from Albanian margins CoCoMap13, d) Montenegrin margin (Angeletti et al., 2015); e) *L. glaberrima* colonies with *D. dianthus* from Greek margins (Taviani et al., 2015).

The northern canyons are floored by muddy deposits, while the southern ones expose partially undraped hard substrates (Taviani et al., 2015). Although several canyons have been explored, no CWC habitat has been found, with the exception of subfossil *Lophelia pertusa* and *Desmophyllum dianthus* (Angeletti et al., 2014). The sessile fauna in the Albanian shelf at the depth of 120 m, south part of Karaburun peninsula is characterized by the massive presence of the brachiopod *Megerlia truncata*, serpulids and encrusting sponges. During this PhD research, it was possible to identify two other white coral sites in the Albanian margins. In the first site (Seman slope), subfossil colonies of *Lophelia pertusa* occur (Fig 2.4), associated with live specimens of *Stenocyathus vermiformis*. The second site is located in Saranda, close to the island of Corfu; the seabottom in here is draped by rubble of highly-degraded *Dendrophyllia cornigera* (Fig 2.4), with branches often fouled by encrusting sponges. In addition, we have sampled live and subfossil *L. glaberrima* at this site.

2.4.4. Greek margin

The first data concerning the Greek Adriatic Sea have been obtained from the CoCoMap14 campaign, providing information on geomorphology and habitats along the coast of the island of Corfu / Kerkyra. The seabed in this area is quite similar to that of the Albanian margin, presenting hardened muddy lithologies often colonized by serpulids and, rarely, sponges plus small individuals of *D. dianthus*. Only in one out site, it was detected the presence of hardgrounds, often populated by *L. glaberrima* between 498 and 508 m (Fig 2.4). The colonies are of considerable size, sometimes meter in length, and are used as substrate for other species, such as *D. dianthus*.

2.5. Discussion

As it has already been seen in the preceding paragraphs, the distribution of Cold Water Coral habitats in the Adriatic has a clear asymmetry, dominating their presence in the western part. The most important sites for both colonies and habitat sizes are the Bari Canyon site, Gondola slide and Dauno Seamount (Trincardi et al., 2010; Angeletti et al., 2014; Taviani et al., 2015). In the eastern part of the colonies are sporadic, smaller, and often fossilized. The most important site in

the eastern part, now known, is from the canyon of Montenegro (Angeletti et al., 2014, Angeletti et al., 2015).

Looking more closely at the map of bathymetric, and the geomorphology of Adriatic Sea, you can clarify the explanation of this asymmetric distribution. The Cold Water Coral habitats often develop into canyons, slopes and underwater mountains. These geomorphological structures may be the primary cause of this asymmetric distribution of corals in the Adriatic. The presence of hard substrates, and precipitous topographies, create ideal conditions for the distribution of corals, such as the triad *Lophelia-Madrepora-Desmophyllum*, considering their need for a hard substrate for their settling (Zibrowius, 2003; Taviani et al., 2005, 2015; Rosso et al., 2010; Savini and Corselli, 2010; Vertino et al., 2010; Savini et al., 2016,). The reduced presence of live corals in the eastern part of the Adriatic, despite the presence of favourable substrate (sub-vertical canyon walls, hard substrates), seems to be related to a different hydrodynamic regime. Another determinant factor in the development of these habitats is the North Adriatic Deep Water cascading events, which bring nutrients and help to transport sediments, avoid sedimentation in coral branches. The constant presence of this stream forces coral development in one direction to maximize the consumption of the particles carried (e.g., McKenzie and Bernoulli, 1982; Allouc, 1986; Noé et al., 2006; Malinverno et al., 2010, Taviani et al., 2016).

Being *Madrepora* and *Lophelia*, two species that feed preferentially on fresh zooplankton (Naumann et al., 2015), these species are more dependant from a current regime as food particle provider (Taviani et al., 2015). On the contrary, the antipatharian *Leiopathes glaberrima*, is more opportunistic for its diet since capable to feed on dead organic matter (Carlier et al., 2009).

The presence of subfossil corals in the eastern part of the Adriatic Sea discloses that ecological conditions were suitable for their development in the past. The CWC disappearance in this part of the Adriatic Sea remains an open question,

These data describe some of the key factors affecting the development and distribution of CWCs, providing a picture of how important these organisms are and how vulnerable they are to the changes in the surrounding environment.

2.6. Conclusions

The explorations of the Adriatic Sea conducted in the last years offers a clue to evaluate the distribution of CWC habitats in the Adriatic Sea. As already shown in the preceding paragraphs, their distribution along the southern Adriatic coasts is a-symmetric, with strong dominance in the southern part. The engineers of these habitats are primarily *M. oculata* and *L. pertusa*, followed by *D. cornigera*, *D. dianthus* and the antipatharian *L. glaberrima*. The distribution of these corals is consistent with the path of the North Adriatic Deep Water, what suggests a role of this current in creating a suitable environment for their development (Langone et al., 2016, D’Onghia et al., 2010, 2012; Angeletti et al., 2014; Langone et al., 2016; Taviani et al., 2016).

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Annex

Table 2. 1. List of stations hosting the CWC discussed in the present study.

Nr Station	Site	Gear	Depth (m)	Start		Finish		<i>Lophelia</i>		<i>Madrepora</i>		<i>Desmophyllum</i>		<i>Dendrophyllia</i>		<i>Leiopathes</i>	
				Latitude	Longitude	Latitude	Longitude	L	D	L	D	L	D	L	D	L	D
CoCoMap 13-16	Albania	Grab	841	40. 31 68 597	18, 88 22280				x		x		x				
CoCoMap13-21	Albania	Grab	354	40. 0971 727	19. 4978433						x			x			
CoCoMap13-24	Albania	Grab	393	40. 1741777	19. 4776848	40. 1740363	19. 4776848				x						
CoCoMap13-25	Albania	Grab	507	40. 0887310	19. 4983957	40. 0990418	19. 4984548				x		x	x			
CoCoMap13-26	Albania	Grab	404	40. 1001860	19. 4985168						x						
CoCoMap13-27	Albania	Grab	334	40. 0963017	19. 4962880	40. 0963465	19. 4954285								x		
CoCoMap13-35	Albania	Grab	361	40. 0972387	19. 4976313	40. 0972397	19. 4977513				x			x			
CoCoMap13-36D	Albania	EpiDredge	369-308	40. 0972447	19. 499025	40. 0950748	19. 501790				x			x			
CoCoMap14-20	w Fano	Rov	535	39. 83952648	19. 35275159							x				x	x
CoCoMap14-21	NW Fano	Rov	440	39. 87226229	19. 34668647											x	
CoCoMap14-29	Fano	ROV	520	39. 77790467	19. 40105775							x				x	x
Altro-32 station:DR32	Montenegro	H. dredge	429	41. 38 59 70	18. 41 28 09	41. 38 57, 92	18. 41 46 47		x		x						
Altro-33 station:DR33	Montenegro	H. dredge	540-417	41. 38 52 01	18. 41 25 57	41. 38 55 34	18. 41 48 28				x						
Altro-39 station:DR39	Montenegro	H. dredge	430-450	41. 40 43 77	18. 36 32 97	41. 40 53 93	18. 36 25 32							x			
Altro-46 station:DR46	Montenegro	EpiDredge		41.40 39 65	18. 36 36 39	41. 40 05 10	18. 36 15 77				x			x			
CROMA 10 DR	Montenegro	EpiDredge		41. 51 74	18. 20 18	41. 54 85	18. 18 06		x			x					
fishermeof Bari	Montenegro	Longline	1000	41°53', 000	18°21', 000						x					x	
fishermeof Bari	Montenegro	Longline	1000	41°43', 000	18°28', 000												x
fishermeof Bari	Montenegro	Longline	800	41°33', 000	18°35', 000						x					x	
CrOMA 20 CR 35	Montenegro	Grab	484.9	41. 40 55 39	18. 36 70 31									x			
CROMA- CRM 37	Montenegro	Grab	62	42. 28 83 28	18. 42 44 94						x						
AP01	SMLeuca canyon	Grab	513	39,134,840	18,123,300			x	x	x	x	x					

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AP07	SMLeuca canyon	S. dredge	525-536	39,134,520	18,123,580	39,134,290	18,123,750	X		X		X			X		
AP08	SMLeuca canyon	S. dredge	530-528	39,134,500	18,123,300	39,134,490	18,123,670	X	X	X	X	X			X		
AP09	SMLeuca canyon	Grab	530	39,134,460	18,123,070				X		X	X					
AP15	SMLeuca canyon	Grab	535	39,134,600	18,123,120				X		X	X					
AP17	SMLeuca canyon	Grab	649	39,136,790	18,130,490				X		X	X					
AP29	SMLeuca canyon	S. dredge	765-790	39,128,000	18,124,200	39,127,380	18,124,460	X	X	X	X	X			X		
AP30	SMLeuca canyon	Grab	747	39,128,090	18,124,420				X		X	X			X		
fishermeof Bari	Bari	Longline	551	41°16', 836	17°16', 997					X							
fishermeof Bari	Monopoli	Longline	558	41°04', 769	17°33', 468					X					X	X	
1B1	Bari Canyon	Grab	280	41°17.4859'N	17°09.2288'E					X	X						
1/5/1900	Bari Canyon	Longline	551	41°16.9460'N	17°19.1280'E	41°16.8360'N	17°16.9970'E			X	X						
MS6	Bari Canyon	Rov	480-490	39136.72'	18130.49'			X	X	X	X	X	X				
MS4	Bari Canyon	ROV	630-640	39135.00'	18123.38'			X	X	X		X				X	X
2009 / Bari	SMLeuca canyon	Trawling	630-678	39 38, 69	18 38, 89	39 37,16	18 38, 98										
2010 / Bari	SMLeuca canyon	Trawling	800m	39 27. 28	18 23. 95	39 26, 68	18 24, 84										
2011 / Bari	SMLeuca canyon	Trawling	1104-1139	39 23, 99	18 21, 60	39 24, 93	18 19, 92										
CR80		R. dredge	606	39°45.719'	19°23.565'	39°45.683'	19°25.133'	X									
CR89		Epidredge	405	39°48.102'	19°22.489'	39°48.883'	19°23.950'	X		X							
CR92		R. dredge	723	39°52.203'	18°50.776'	39°53.233'	18°50.150'	X		X							
CR93		Epidredge	726	39°52.166'	18°50.654'	39°52.883'	18°50.383'	X		X							
CR94		Epidredge	542	40°07.495'	18°44.578'	40°06.900'	18°44.616'	X		X							
SE06_05		Grab	314	41°17.687'	17°08.313'							X					
SE06_13DR		R. dredge	423	41°22.030'	17°06.720'	42°22.700'	17°06.750'	X									
SE06_19		Grab	308	41°18.969'	17°05.104'			X		X							
SE06_48DR		Epidredge	705	41°43.098'	17°03.757'	41°43.083'	17°03.750'					X					
735		ROV	663	41°17.476'	17°16.623'	41°17.060'	17°16.630'	X		X							
739		ROV	997	41°33.073'	17°27.953'	41°32.920'	17°28.730'					X					

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745		ROV	557	41°17.824'	17°10.753'	41°17.553'	17°10.004'	X		X							
752		ROV	710	41°43.507'	17°02.794'	41°43.180'	17°03.650'	X									
BAR07_22BC		BoX corer	328	42°02.748'	16°51.324'			X		X		X					
SI08_15		Grab	560	41°29.573'	17°08.700'							X					
SI08_57		Grab	154	41°39.835'	16°53.341'												
SI08_58		Grab	147	41°40.026'	16°52.306'												
SI08_59		Grab	147	41°40.233'	16°51.286'												
A63		ROV	443	41°38.879'	18°40.443'	41°38.808'	18°41.494'	X									X
A65		ROV	441	41°38.800'	18°40.490'	41°41.056'	18°36.224'										X
A67		ROV	543	41°41.057'	41°41.057'	41°41.073'	18°36.343'			X							X
A68		ROV	468	41°41.069'	18°36.344'	41°17.267'	18°36.629'										X
A71		ROV	428	41°17.262'	17°16.613'	41°17.267'	17°16.629'										
A77		ROV	449	41°14.656'	17°19.981'	41°12.009'	16°56.907'										
A84		ROV	426	41°42.009'	16°56.955'	41°42.009'	16°56.906'										
A91		ROV	309	41°16.987'	17°17.545'	41°17.000'	17°17.525'			X							
A121		ROV	485	42°26.715'	17°26.715'	42°26.492'	17°31.928'										
A208		ROV	412	41°14.235'	17°17.053'	41°17.154'	17°16.664'			X							
A210		ROV	478	41°17.313'	17°17.117'	41°16.765'	17°15.935'			X							
MEMA12_10		BoX Corer	778	40°14.631'	18°48.424'			X		X							
MEMA12_11		BoX Corer	778	40°14.635'	18°48.430'			X		X							
MEMA12_27		BoX Corer	720	39°52.284'	18°53.020'			X		X		X					
MEMA12_26		Grab	720	39°52.260'	18°52.960'			X		X		X					
MEMA12_34		Grab	724	39°52.264'	18°52.972'			X		X		X					
MEMA12_36		Grab	786	39°53.468'	18°55.175'			X		X		X					
ALTRO31	Montenegro	ROV	426	41°38.815'	18°41.532'	41°38.896'	18°41.469'										
ALTRO35	Montenegro	ROV	480	41°38.885'	18°41.455'	41°38.885'	18°41.456'	X		X		X		X			
ALTRO36	Montenegro	ROV	480	41°38.885'	18°41.463'	41°38.906'	18°41.433'			X							
ALTRO37	Montenegro	ROV	428	41°40.768'	18°36.551'	41°40.713'	18°36.567'			X				X			

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ALTRO44	Montenegro	ROV	443	41°40.737'	18°36.558'	41°40.731'	18°36.548'		X		X							
ALTRO82	Montenegro	ROV	395	42°02.667'	18°23.693'	42°02.527'	18°23.641'											
COC13_8	Albania	Grab	230	40°10.453'	19°29.352'			X	X									
COC13_10	Albania	Grab	232	40°10.308'	19°29.539'				X									
COC13_11	Albania	Grab	247	40°04.814'	19°40.507'			X										
COC13_15	Albania	Grab	848	40°19.177'	18°53.497'			X										
COC13_16	Albania	Grab	841	40°19.011'	18°52.933'			X	X		X							
COC13-19	Albania	ROV	482	40°22.984'	19°10.054'	40°23.269'	19°10.182'											
COC13-20	Albania	ROV	299	40°05.690'	19°29.867'	40°05.688'	19°29.860'											
COC13-23	Albania	ROV	313	40°10.344'	19°28.840'	40°10.588'	19°28.563'											
COC13_24	Albania	Grab	393	40°10.450'	19°28.659'				X									
COC13_25	Albania	Grab	407	40°05.923'	19°29.903'			X	X									
COC13_27	Albania	Grab	334	40°05.802'	19°27.772'			X	X									
COC13_35	Albania	Grab	361	40°05.834'	19°29.857'				X									
COC13_36	Albania	Grab	308	40°05.834'	19°29.874'				X									
COC14_20	Off Greece	ROV	535	39°50.372'	19°20.504'	39°50.731'	19°20.540'			X	X							
COC14_21	Off Greece	ROV	440	39°52.336'	19°20.801'	39°52.427'	19°20.735'											
COC14_26	Off Greece	Grab	526	39°50.366'	19°20.477'						X							
COC14_29	Off Greece	ROV	520	39°46.674'	19°24.063'	39°46.670'	19°24.063'			X	X							
CROMA10	Montenegro	Epidredge	960	41°50.34'	18°20.628'	41°54.850'	18°18.060'	X			X							
CROMA14	Montenegro	grab	134	41°39.999'	18°58.736'													
CROMA16	Montenegro	Epidredge	478	41°40.660'	18°36.613'	41°41.085'	18°36.266'				X							
CROMA20	Montenegro	grab	485	41°40.553'	18°36.703'													

**3. Sessile benthic invertebrate
associated with sub-fossil Cold
Water Coral bioconstructions from
off Albania**

3.1. Introduction

Cold Water Corals (CWC), such as the frame-builders *Lophelia pertusa* and *Madrepora oculata*, have been documented from various parts of the Mediterranean basin including the Adriatic Sea (Taviani et al 2016 with references therein). There, most evidence is related to the its western side, whilst CWC appear to be much less common on the eastern sector and basically limited to Montenegrin waters (Angeletti et al 2014; Taviani et al 2016). This asymmetrical CWC distribution in the southern Adriatic has been hypothesized to be governed by hydrologic processes, including North Adriatic Dense Water cascading (Taviani et al 2016). Although the Adriatic Albanian margin is indented by a number of canyons, evidence of living megabenthic sessile fauna is almost lacking. This holds especially true for CWC which are documented to date only as subfossil remains of *L. pertusa* and *Desmophyllum dianthus* (Angeletti et al., 2014) found in the Albanian part of the Otranto channel. *L. pertusa* and *M. oculata* may form three-dimensional structures which provide substrate for various sessile benthic invertebrates, thus increasing the environmental biodiversity of CWC habitats, including their thanatofacies (Mastrototaro et al., 2010; Rosso et al., 2010). The subfossil coral frames have been documented to act as taphonomic traps, at times including representatives of the CWC-associated fauna and skeletal remains from adjacent habitats or subsequent muddy draping (Remia and Taviani 2005, Taviani et al., 2011, Angeletti and Taviani, 2011).

This paper provides information about the benthic macrofauna associated with subfossil coral frameworks and hardgrounds obtained from two sites offshore the Albanian coast.

3.2. Study area

Two surveys were conducted in March 2015 and May 2016 in two areas of the Albanian coast between 190 and 230 m depth on-board commercial fishing boats. The occurrence of dead (subfossil)CWC frames in that region was anecdotically known to fishermen since a while, promoting therefore an exploration to get samples suitable for a dedicated study. During the surveys, soft bottoms off the southern Albania coast have been sampled by trawling (Fig. 3. 1; Tab. 3. 1). Two surveys off Vlorë Bay were completed in 2015 and 2016 at stations AL15SE1, and AL16SE2. Sampling on the muddy bottom resulted in the catch of two large subfossil *Lophelia* colonies and hardground slabs and fragments fouled by serpulids and sponges.

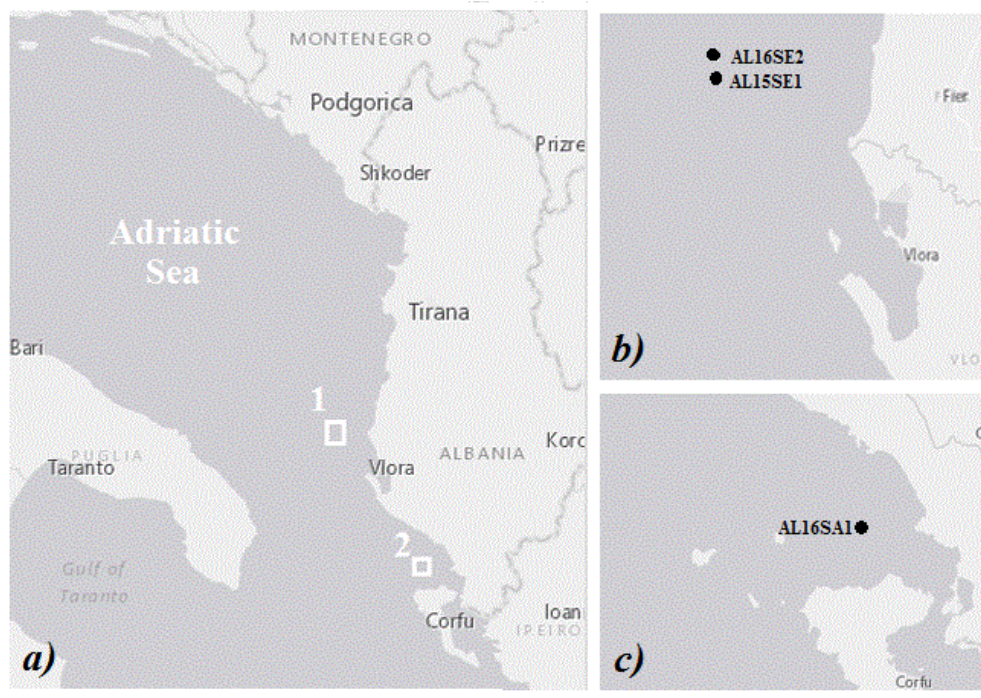


Figure 3. 1. Map of the 2 areas where sampling was carried out: a) geographical position of the two sampling areas in the Adriatic; b) Geographic location of sampling points of the first area AL15SE1 and AL16SE2, Geographic location of sampling station of the second area AL16SA1.

The second sampling site located, at the southern Albanian margin off Saranda, close to Corfu Island (Greece) was characterized by the predominance of corall rubble of *Dendrophyllia cornigera* (yellow coral) and hardgrounds fouled by serpulids and sponges. Fragments of *D. cornigera*

appear coated by oxides, bioeroded and partly covered by sediment. These subfossil remains were colonized by sponges and several specimens of the solitary scleractinians *Caryophyllia calveri* and secondarily by *D. dianthus* and *Stenocyathus vermiformis*. All living taxa were extracted from the substrate and fixed in 95% ethanol.

Table 3. 1. List of the geographic coordinates where sampling was carried out

Stations	Specie	Latitude	Longitude
AL15SE1	<i>Lophelia pertusa</i>	N 40° 48' 26.18"	E 18° 59' 24.47"
AL16SE2	<i>Lophelia pertusa</i>	N 40 50.730	E 18 59.970
AL16SA1	<i>D.cornigera and L.glaberrima</i>	N 39 55.938	E 19 48.248

Secondly, they have been identified in the lab to the lowest possible taxonomic level with the help of recent identification manuals. For species identification of sponges, slides of dissociated spicules were prepared and observed with an optical microscope (Leica DM500). Systematic listing and nomenclature adopted following WoRMS (World Register of Marine Species) available online at <http://www.marinespecies.org> and with special reference to Rosso and Di Martino (2016) for bryozoans.

3.3. Analysis of samples provided by the fishermen

In order to validate the information resulting from the fishermen's reports, it was carried out a detailed analysis of the coral samples, provided by fishermen, by the research team of the Biology Department of the University of Bari.

To each sample was made species identification and assessment of the state of the sample or of the fragment, if dead or alive at the time of collection. Subsequently, the individual samples were weighed and photographed. It has been carried out, also, the sorting of benthic associated species through the use of the stereoscope and have been made of the detailed photographs in order to ensure a precise determination of the found taxa.

The samples, finally, were numbered and labeled to allow from time to time and the recognition and identification of species. In particular, on each label have been reported, the sample species, the discovery site and the date on which it was caught.

3.3.1. Identification of sponges

Each sample of coral colonies was selected and analyzed by stereomicroscope to detect sponges. The sponge samples were first fixed in 95% alcohol for later inspection. For species identification, slides of dissociated spicules were prepared and observed with an optical microscope. This technique provides cleaner, permanent preparations, but the process involves noxious chemicals and should be undertaken only with suitable facilities (e.g. protective clothing, fume extraction). This process uses nitric acid instead of bleach. Fragments of sponge are placed in flasks, directly on glass slides, or directly on electron microscope stubs. Several drops of acid are placed on the fragment, gently heated over a flame until bubbling, and repeated until all organic matter is digested (this is easily ascertained by eye). The heat-accelerated digestion process produces various oxides, including nitrous oxide, and it is cautioned that these are noxious. It should also be noted that the acid is evaporated rather than burnt, so low heat is preferable (e.g. using an alcohol flame rather than gas). Once dry and cool, preparations can be mounted immediately without washing. Siliceous spicules are bonded directly onto the substrate by this technique, which makes it useful for both light and scanning electron microscopy. Spicule preparations obtained from both techniques are now ready for covering using a suitable mounting medium (e.g. Depex, Canada balsam, Euparal, Durcupon, etc.)(Hooper 2012).

Spicule morphology was examined by means of optical and scanning electron microscopes (SEM) using a Philips XL 20. For SEM analyses, dissociated spicules were transferred onto stubs and sputter coated with gold. Measurements were made for each spicule type in order to record their size ranges, reported as smallest length or width – (mean \pm standard deviation) – largest length or width. The classification was mainly based upon Hooper and van Soest (2002).

3.3.2. Identification of mollusks

The three dimensional structure of the coral, can provide to the formation of habitat for many organisms which after their death can be trapped in its branches (Freiwald and Wilson, 1998). The growth of the frame structure of the coral, related biostromal production may optimise entrapment, and ultimately the preservation of vagile visitors or stable exploiter visitors of such coral habitats (Angeletti and Taviani, 2011). The recurrent process of early submarine cementation is conducive to the lithification of the intracoral fine-grained matrix resulting in carbonate rocks (framestones) which encase both coral frames and skeletal remains of associated macrofauna, mainly molluscs, followed by brachiopods, echinoids and decapods. The rare shells of gastropods and bivalves that perhaps were present accidentally among the living corallites may also be rapidly cemented and become firmly attached to the living colony.

3.3.3. Identification of Polychaetes and Bryozoas

The samples collected from serpulidae and bryozoa were examined and identified by the research group at the University of Catania, Department of Geological Sciences.

The serpulidae association, represented by 14 taxa belonging to thanatocoenosis, continues to be one of the most representative groups among epibionts (Sanfilippo, 2007, Sanfilippo et al., 2017). Generally, from each colony, the most representative samples of the species were collected, depending on the state of conservation and the number of species present. The analyzes focused mainly on the morphology of serpulide calcareous tubes, their shape, and internal and external structure. Low magnification images were taken through a Zeiss Discovery V8A stereomicroscope equipped with an AxioCam MRC and Axiovision acquisition system in order to document the general tube morphologies. The ornamentation of the external tube surface of the serpulid tubes is variable within populations and may be quite elaborate (e.g., *Janita fimbriata*, Bianchi 1981), but most typically consists of longitudinal and transverse elements.

The bryozoans, are an other important skeletobiont community of coral colonies, both in terms of coverage and in number of species (Rosso and Sanfilippo, 2005). The calcareous exoskeleton of

most bryozoan species provides many architectural structures, usually so constant and specific that they enable a quick and reliable diagnosis. Furthermore, their colonial nature allows examination on only one specimen of numerous replicates of the same pattern of individual organization, observation in some zooids of traits absent or eroded in others, and, finally, evaluation of the degree of intraspecific variability (i.e., biometry) (Zabala and Maluquer, 1988). Thus, diagnosis is much more reliable than in other groups, which also have exoskeletons, but of non-colonial organization (mollusks, crustaceans, echinoderms, and so on). The examined specimens are aggregates composed of incrusting bryozoa, which develop small unilaminar colonies composed of few zooids. The erected colonies are little present, and are often small sized, and poorly branched.

3.4. Results

A total of 82 species has been identified and listed in Table 3. 2. The most represented taxa are Porifera, Mollusca and Annelida, represented by 22, 25 and 14 species, respectively.

Table 3. 2. List of identified species, associated with sub-fossil *L. pertusa* colonies and hard-grounds, from the two study areas.

Phylum	Order	Family	Species	
Foraminifera	Rotaliida	Homotrematidae	<i>Miniacina miniacea</i> (Pallas, 1766)	
	Miliolida	Nubeculariidae	<i>Cornuspiramia adherens</i> (Le Calvez, 1935)	
Porifera	Tetractinellida	Ancorinidae	<i>Jaspis incrustans</i> (Topsent, 1890)	
		Geodiidae	<i>Geodia nodastrella</i> Carter, 1876.	
		Pachastrellidae	<i>Pachastrella</i> sp. <i>Pachastrella monilifera</i> Schmidt, 1868	
		Vulcanellidae		<i>Poecillastra compressa</i> (Bowerbank, 1866)
				<i>Vulcanella gracilis</i> (Sollas, 1888)
			Siphonidiidae	<i>Siphonidium ramosum</i> (Schmidt, 1870)
		Polymastiida	Polymastiidae	<i>Polymastia</i> sp.
		Tethyida	Timeidae	<i>Timea</i> sp.
	Poecilosclerida	Microcionidae		<i>Antho signata</i> (Topsent, 1904)
				<i>Hymedesmia mutabilis</i> (Topsent, 1904)
			Latrunculiidae	<i>Sceptrella insignis</i> (Topsent, 1890)
		Desmacellida	Desmacellidae	<i>Desmacella inornata</i> (Bowerbank, 1866)
		Merliida	Hamacanthidae	<i>Hamacantha johnsoni</i> (Bowerbank, 1864)

		Hamacanthidae	<i>Hamacantha papillata</i> Vosmaer, 1885 <i>Hamacantha</i> sp.	
	Axinellida	Raspailiidae	<i>Eurypon clavatum</i> (Bowerbank, 1866)	
		Axinellidae	<i>Phakellia robusta</i> Bowerbank, 1866	
	Bubarida	Bubaridae	<i>Bubarisvermiculata</i> (Bowerbank, 1866)	
	Agelasida	Hymenhabdiidae	<i>Hymenhabdia typical</i> Topsent, 1892	
	Haplosclerida	Chalinidae	<i>Haliclona flagellifera</i> (Ridley and Dendy, 1886)	
	Verongiida	Ianthellidae	<i>Hexadella dedritifera</i> Topsent, 1913	
Cnidaria	Coronatae	Nausithoidae	<i>Nausithoes</i> pp.	
	Alcyonacea	Isididae	<i>Isidella elongate</i> (Esper, 1788)	
	Antipatharia	Leiopathidae	<i>Leiopathes glaberrima</i> (Esper, 1788)	
	Scleractinia	Caryophylliidae		<i>Caryophyllia calveri</i> Duncan, 1873
				<i>Caryophyllia cyathus</i> (Ellis andSolander, 1786)
				<i>Desmophyllum dianthus</i> (Esper, 1794) (subossil)
				<i>Lophelia pertusa</i> (Linnaeus, 1758) (subfossil)
	Guyniidae	<i>Stenocyathu svermiformis</i> (Pourtalès, 1868)		
	Dendrophylliidae	<i>Dendrophyllia cornigera</i> (Lamarck, 1816) (Subfossil)		
Mollusca	Vetigastropoda	Fissurellidae	<i>Emarginula adriatica</i> O. G. Costa, 1830	
		Trochidae	<i>Clelandella miliaris</i> (Brocchi, 1814)	
		Eucyclinae	<i>Putzeysia wiseri</i> (Calcara, 1842)	
	Neotaenioglossa	Rissoidae	<i>Alvania cimicoides</i> (Forbes, 1844)	
		Ranellidae	<i>Ranella olearia</i> (Linnaeus, 1758)	
	Ptenoglossa	Muricidae		<i>Orania fusulus</i> (Brocchi, 1814)
				<i>Pagodula echinata</i> (Kiener, 1840)
		Nassariidae	<i>Nassarius lima</i> (Dillwyn, 1817)	
		Fascioliariidae	<i>Fusinus rostratus</i> (Olivi, 1792)	
		Conidae	<i>Bela nuperrima</i> (Tiberi, 1855)	
	Heterostropha	Odostomiidae	<i>Chrysallida stefanisi</i> (Jeffreys, 1869)	
		Pyramidellidae	<i>Eulimella bogii</i> Van Aartsen, 1995	
	Cephalaspidea	Acteonidae	<i>Callostracon tyrrhenicum</i> (Smriglio and Mariottini, 1996)	
	Thecosomata	Cavoliniidae	<i>Cavolinia gibbosa</i> (d'Orbigny, 1834)	
	Solemyoidea	Yoldiidae	<i>Yoldiella philippiana</i> (Nyst, 1845)	
	Arcoidea	Arcidae		<i>Asperarca nodulosa</i> (O. F. Müller, 1776)
				<i>Bathycaraphilippiana</i> (Nyst, 1848)
	Pterioidea	Propeamussiidae	<i>Parvamussium fenestratum</i> (Forbes, 1844)	
		Pectinidae		<i>Pseudamussium peslutrae</i> (Linnaeus, 1771)
				<i>Karnekampia sulcata</i> (Müller, 1776)
		Spondylidae	<i>Spondylus gussoni</i> O. G. Costa, 1830	
Veneroidea	Chamidae	<i>Pseudochama gryphina</i> (Lamarck, 1819)		
	Semelidae	<i>Abra longicallus</i> (Scacchi, 1835)		

		Kellielliidae	<i>Kelliella miliaris</i> (Philippi, 1844)
		Veneridae	<i>Timoclea ovata</i> (Pennant, 1777)
	Pholadomyoidea	Cuspidariidae	<i>Tropidomya abbreviate</i> (Forbes, 1843)
Annelida	Sabellida	Serpulidae	<i>Serpulidae</i> sp.
			<i>Filogranula gracilis</i> Langerhans, 1884
			<i>Filogranula stellata</i> (Southward, 1963)
			<i>Filogranula annulata</i> (O.G. Costa, 1861)
			<i>Filograna</i> sp
			<i>Filograna</i> sp
			<i>Hyalopomatus madreporae</i> Sanfilippo, 2009
			<i>Hyalopomatus</i> sp
			<i>Janita fimbriata</i> (Delle Chiaje, 1822)
			<i>Metavermlia multicristata</i> (Philippi, 1844)
			<i>Placostegus tridentatus</i> (Fabricius, 1779)
			<i>Protis</i> sp.
			<i>Serpula</i> cf. <i>vermicularis</i> Linnaeus 1767
<i>Vermiliopsis monodiscus</i> Zibrowius, 1968			
Bryozoa	Cyclostomatida	Tubuliporidae	<i>Idmidronea</i> sp.
		Tubuliporidae	<i>Tubuliporina</i> sp.
		Crisiidae	<i>Crisiatenella</i> Calvet, 1906 <i>longinodata</i> Rosso, 1998
	Cheilostomatida	Calloporidae	<i>Ramphonotus</i> sp.
			<i>Copidozoum exiguum</i> (Barroso, 1920)
		Cribilinidae	<i>Puellina pedunculata</i> (Gautier, 1956)
		Romancheinidae	<i>Neolagenipora eximia</i> (Hincks, 1860)
		Smittinidae	<i>Smittina</i> cf. <i>crystalline</i> (Norman, 1867)
		Escharinidae	<i>Herentia hyndmanni</i> (Johnston, 1847)
		Celleporidae	<i>Turbicellepora coronopus</i> (Wood, 1844)
Arthropoda	Decapoda	Leucosiidae	<i>Ebalia nux</i> A. Milne-Edwards, 1883
Echinodermata	Cidaroida	Cidaridae	<i>Cidaris cidaris</i> (Linnaeus, 1758)

3.4.1. Porifera

A total of 22 sponge taxa were identified, 15 of which exclusively from stations AL15SE1 and AL16SE2, 6 taxa (*Jaspis incrustans*, *Pachastrella monilifera*, *Poecillastra compressa*, *Leiodermatium lynceus*, *Hamacantha* sp., *Sceptrella insignis*) also occur at station AL16SA1. All the taxa belonging to the class Demospongidae. The order Tetractinellida counts more than 31% of the detected species, while the remaining 10 orders (Polymastiida, Tethyida,

Poecilosclerida, Axinellida, Desmacellida, Merliida, Bubarida, Agelasida, Haplosclerida, Verongiida) only include a few taxa each.

All sponges were identified at species level, with the exception of five taxa (*Pachastrella* sp., *Polymastia* sp., *Timea* sp., *Hamacantha* sp. and *Haliclona* sp.) whose definitive taxonomic assignment requires further analysis. For some of the species, especially for lithistidae, scanning electron microscope was necessary for identification. As for *Siphonidium ramosum*, the spicules of which are shown in Figure 3.2

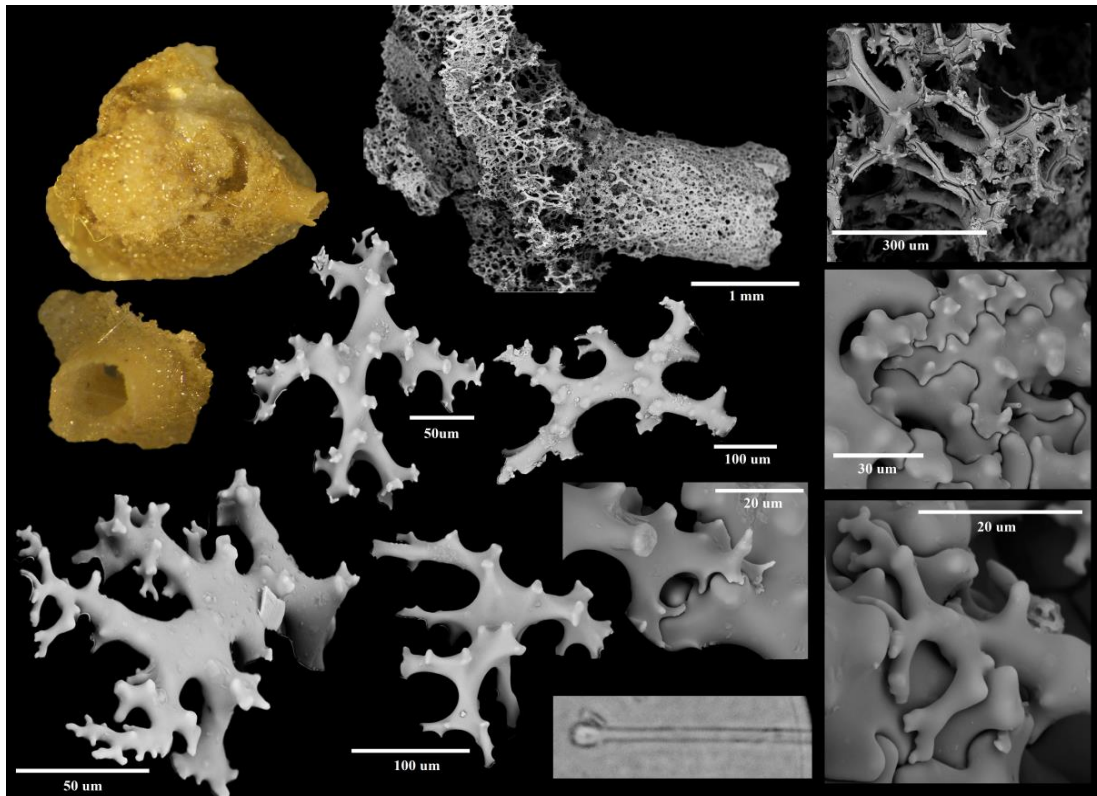


Figure 3. 2. Spicules of *Siphonidium ramosum*

Except for few taxa found in the samples in the massive form (*P. compressa*, *P. monilifera* and *L. lynceus*) sponges mainly occurred as small thin incrustations on the exposed surfaces and branches of subfossil coral colonies. Although the scleraxes of the *L. pertusa* and *D. cornigera* sub-fossil colonies were heavily affected by perforations, it was not possible to extract sponge tissue for the identification of the boring sponge species, probably owing to the samples' poor preservation state.

Most sponge listed here have a prevalent north-Atlantic distribution, being are at present known from a few Mediterranean sites (van Soest et al., 2017). All such taxa were already recorded from CWC grounds at Santa Maria di Leuca (SML) and the Bari Canyon and (11 for Bari Canyon and 14 for SML, respectively: Mastrototaro et al., 2010; D'Onghia et al., 2015). In particular, *Antho signata*, and *Geodia nodastrella* identified at SML, were first Mediterranean records of these taxa (Mastrototaro et al., 2010), to which we now add the Albanian findings.

3.4.2. Cnidaria

The subfossil *L. pertusa* colonies under scrutiny were intensely fouled by serpulids, sponges and bryozoans. Live *Caryophyllia calveri*, and *Caryophyllia cyathus* were also present on the surface (fig. 3.3). On the basal part of these *L. pertusa* colonies, solitary polyps of *Stenocyathus vermiformis* formed a canopy comprehensive of dead and living individuals (Fig 3.3). Dead specimens of *C. cyathus* and *C. calveri* were used as a substrate by juveniles belonging to the same species, as has been observed for the Eratosthenes Seamount (Galil and Zibrowius, 1998).

The bamboo coral *Isidella elongata* was ascertained on muddy bottoms at both site. Furthermore, we have noticed a number of *Stephaniscyphistoma*, the benthic stage of bathypelagic medusae of the genus *Nausithoe* (Scyphozoa: Family Coronatae), in analogy with other coral banks (Jarms et al., 2003; Mastrototaro et al., 2010). In the station, AL16SA1 rubble of *D. cornigera* colonized by *C. cyathus* occurred. Two live samples of *Leiopathes glaberrima* were collected during this sampling. The presence of *L. glaberrima* in this sector of the Mediterranean was firstly documented during the CNR oceanographic cruise CoCoMap 2014, off Saranda associated with *D. dianthus*. Solitary corals such as *D. dianthus*, *S.vermiformis* and *C. calveri*, settle on sub-fossil coralsubstrate.

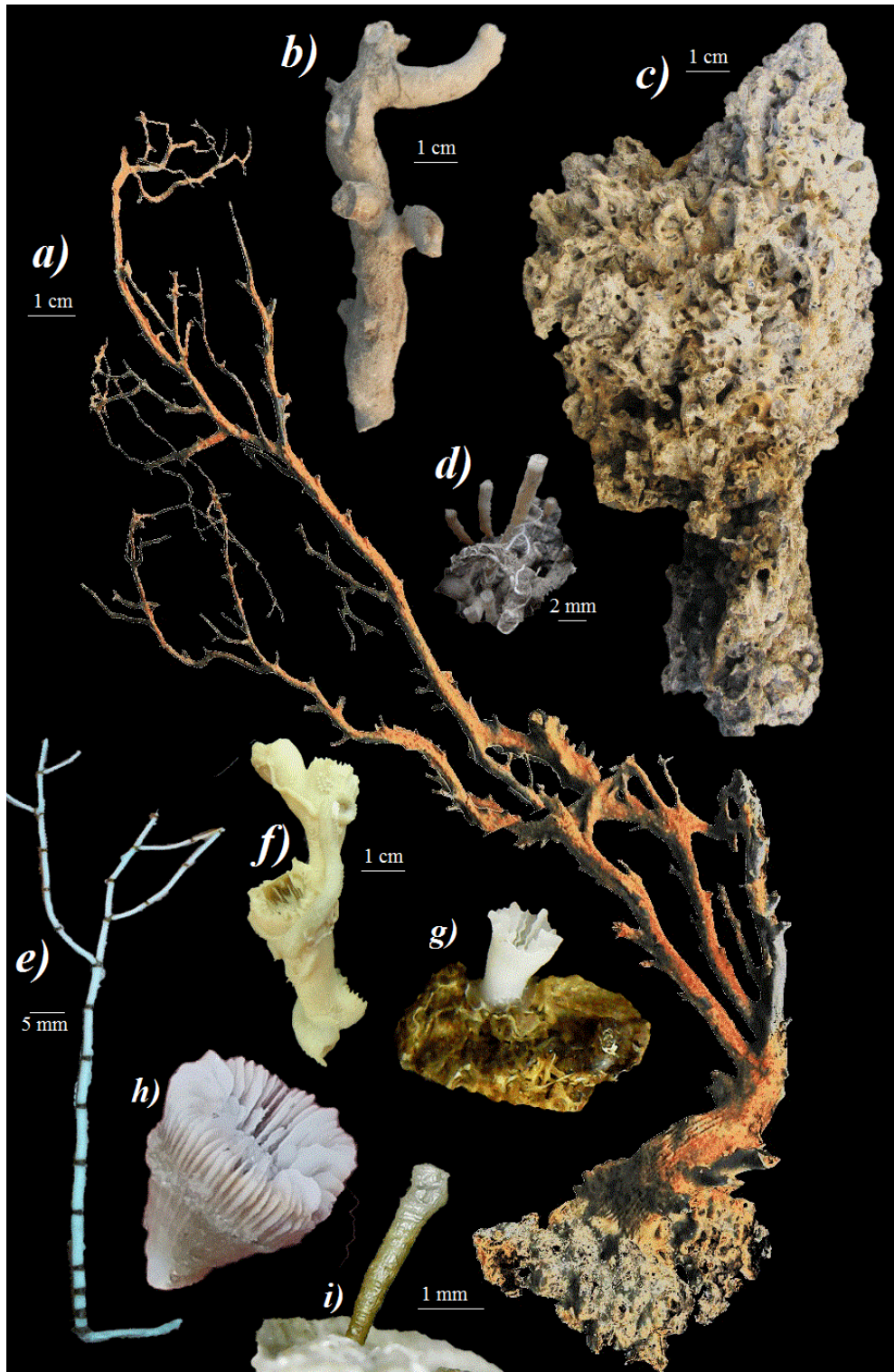


Figure 3.3. Cnidarian species identified a) *Leiopathes glaberrima*; b) *Dendrophyllia cornigera*; c) f) *Lophelia pertusa*, d) *Stenocyathus vermiformis*; e) *Isidella elongata*; g, h) *Caryophyllia calveri*; i) *Nausithoe* spp

3.4.3. Mollusca

The mollusk fauna consisted of empty shells from muddy bottoms and others (some alive) associated with hard surfaces such as *Lophelia* subfossil colonies, and hardgrounds. Some shells were cemented or encased into the subfossil coral frames, a case reported from other CWC situations in the Mediterranean Sea (Remia and Taviani, 2005; Taviani et al., 2005b; Angeletti and Taviani, 2014). No mollusks strictly associated with CWC habitats, such as corallivorous gastropods (Taviani et al. 2009), have been identified in our samples. A total of 26 species was identified belonging to Gastropoda (14 species) and Bivalvia (12 species). The arcid bivalves *Asperarca nodulosa* and *Bathyarca philippiana* were commonly found byssated on hard substrates. As a whole, the fauna includes typical epibathyal mollusks in the Mediterranean Sea (Oliverio, 2008; Negri and Corselli, 2016). The first site, AL15SE included the highest number of species, all already recorded from the deep Adriatic Sea (Di Geronimo and Panetta, 1973; Taviani, 1978; Negri and Corselli 2016). The mollusk fauna is comprehensive of few taxa exclusive of hard substrates but by a large number dwelling in mud such as *Clelandella miliaris*, *Alvania cimicoides*, *Nassarius lima*, *Abra longicallus* (Fig. 3.4).



Figure 3. 4. Molluscs associated with the *Lophelia pertusa* and *Dendrophyllia cornigera* subfossil colonies collected in AL15SE1 and AL16SE2, and AL16SA1: a) *Ranella olearia*, b) *Orania fusulus*, c) *Nassarius (Uzita) lima*, d) *Pagodula echinata*, e) *Putzeysia wiseri*, f) *Eulimella bogii*, g) *Emarginula adriatica*, h) *Timoclea ovata*, i) *Tropidomya abbreviata*, j) *Yoldiella philippiana*, k) *Spondylus gussonii*, l) *Kelliella miliaris*.

The presence of gastropods such as *Putzeysia wiseri*, *Alvania cimicoides*, *Emarginula adriatica*, *Clelandella miliaris*, and bivalves such as *Asperarca nodulosa*, *Bathyarca philippiana*, *Karnekampia sulcata*, *Spondilus gussonii*, is consistent with CWC habitats s.l., although not exclusive (Tursi et al., 2004; Rosso et al., 2010; Mastrototaro et al., 2010; Negri and Corselli, 2016).

3.4.4. Annelida

The annelid stock identified at stations AL15SE1, AL16SE2 is mainly composed by 3 living species i.e. *Metavermilia multicristata*, *Filigranula gracilis* and *Filigrana* sp. 1. As a whole, the annelid fauna includes both living and dead specimens, consisting of 14 species in AL15SE1 and 11 species in AL16SE2, respectively.



Figure 3. 5. Annelids associated with the *Lophelia pertusa* subfossil colonies collected in AL15SE1 and AL16SE2:
a) *Hyalopomatus madreporae*, b) *Filigranula* spp., c) *Serpulidae* sp.1, d) *Placostegus tridentatus*, e) *Janita fimbriata*, *Metavermilia multicristata* , f) *Metavermilia multicristata*.

The species occurring at both stations are *Filigranula gracilis*, *F. stellata*, *F. annulata*, *Hyalopomatus madreporae*, *Metavermilia multicristata*, *Janita fimbriata*, *Placostegus tridentatus*, *Serpula* cfr. *vermicularis* and *Vermiliopsis monodiscus* (Fig. 5). Two species belonging to the same genus, *Filigrana* sp.1 and *Filigrana* sp. 2, were found in both colonies (Sanfilippo, unpublished). Most taxa are widely distributed in the Mediterranean and East Atlantic (Castelli et al., 2008; Sanfilippo, 2009; Mastrototaro et al., 2010; D’Onghia et al., 2015). In the AL15SE1 colony, was observed an extensive coverage, consisting of aggregates of 3 species of *Filigranula* tubes. This framework was previously described also in the samples found in Bari Canyon (D’Onghia et al., 2015). The micro-serpulids *Vermiliopsis monodiscus* and

Hyalopomatus madreporae endemic to the Mediterranean (Zibrowius, 1968; Sanfilippo, 2009) have been both reported from SML and Bari Canyon CWC provinces (Sanfilippo, 2009; Mastrototaro et al., 2010; D’Onghia et al., 2015).

In summary, the censused sessile annelid macrofauna as a whole is typical of deep-circalittoral and bathyal habitats and share common traits with, for example, what present in the Apulian CWC grounds (Mastrototaro et al., 2010; Sanfilippo et al., 2013; D’Onghia et al., 2015).

3.4.5. Bryozoa

A total of 10 bryozoans have been identified on subfossil *Lophelia* colonies, most of which determined at species level. Cyclostomes are represented by 2 taxa only (*Idmidronea* sp. 1 and *Crisia tenella longinodata*), whereas cheilostomes prevail with 8 species. Only 4 species occurred also alive, namely *C. tenella longinodata*, and *Idmidronea* sp. 1, *Neolagenipora eximia* and *Herentia hyndmanni*. The species *Copidozoum exiguum*, *Smittina* cf. *crystallina* and *Puellina pedunculata*, were present with dead but fresh-looking colonies. The remaining species (*Ramphonotus* sp. and *Turbicellepora coronopus*) as well as fragments of additional species exclusively recorded from sediments are only represented by old-looking specimens.

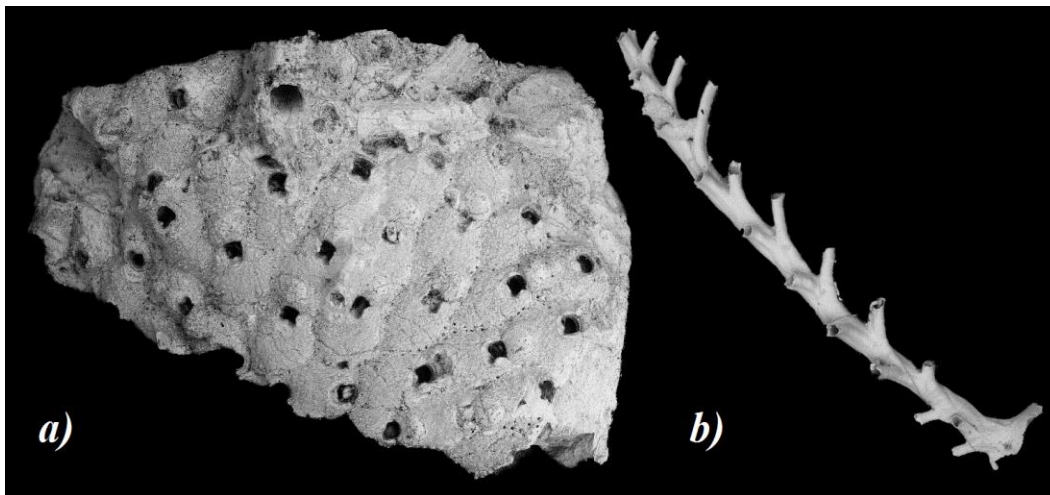


Figure 3. 6. Scanning Electron Microscope images of Bryozoans: a) *Neolagenipora eximia*; b) *Idmidronea* sp. 1

These latter, namely *Diplosolen obelium* and *Adeonella pallasii* and *Reptadeonella violacea* were omitted in the table 3.2. All taxa are documented by very few colonies, with predominant

encrusting morphotypes, including small unilaminar colonies and especially spots, i.e. colonies consisting of few zooids. Erect colonies are sporadic, small sized and poorly branched. Nearly all species were previously known as colonizers of hard substrates, including live and dead coral skeletons (Zabala et al., 1993; Mastrototaro et al., 2010; D’Onghia et al., 2015; Rosso and Di Martino 2016), but none of them seems specifically adapted or selective for colonizing coral skeletons. Most species were previously reported from the SML coral province and the Bari Canyon (Mastrototaro et al., 2010; D’Onghia et al., 2015). Both cyclostomes found with living colonies, first recognized as fossils from southern Italy (Rosso and Di Geronimo, 1998; Rosso, 2005), were already collected alive in the area either from the SML coral province (Mastrototaro et al. 2010), either from the Bari Canyon (D’Onghia et al., 2015). In contrast, *Puellina pedunculata*, *Smittina* cf. *crystallina* and *Herentia hyndmanni* were also been reported from canyons along the Catalonia slope by Zabala et al. (1993), besides collection in the Adriatic-northern Ionian Sea area (Mastrototaro et al. 2010; D’Onghia et al., 2015). *Neolagenipora eximia* is a first record for the area as living specimens (Fig. 3.6). This species was already known from the Mediterranean Sea, but restricted to its northwestern sector, from where it has been reported by Harmelin, (1976), Zabala et al., (1993) and Madurell et al., (2013). These populations were considered as relict in the basin by Di Geronimo et al., (1996) and Rosso and Di Geronimo (1998), owing to the main known distribution of *N. eximia* from the Atlantic and its occurrence in Pleistocene sediments of southern Italy (Rosso, 2005). This finding expands the present-day distribution of a species, once considered as nearly disappeared in the Mediterranean Sea.

3.5. Discussion

The large number of species associated with subfossil *Lophelia* colonies and in the *Dendrophyllia* rubble provides a further confirm that dead coral frameworks is the most significant structural habitat in terms of associated species diversity (Freiwald and Wilson, 1998; Roberts et al., 2009). Dead frames are typically characterized by attached, suspension feeding fauna including numerous sponges, actinians and other cnidarian species, and smaller epifauna including bryozoans and serpulids. Where the sediment becomes trapped in the coral framework

niches appear for infaunal species such as polychaete worms and molluscs. The predominance of suspension feeders seems to be linked to the trophic energy system in these ecosystems.

The knowledge of CWC grounds here discussed is due to commercial fishery which help disclosing the local presence of such habitats in Albanian territorial waters. There is clear need of further studies to fully evaluate their precise location, extent and biodiversity. Although, with a few exceptions, there is no specific fauna associated with living CWC banks (Jensen and Frederiksen, 1992; Mortensen and Fossaugh, 2006) nevertheless such habitats promote an enhanced biodiversity as seen, for instance, at SML, the Bari Canyon, and similar settings.

3.6. Conclusions

The occurrence of subfossil *L. pertusa* is documented for the first time in Albanian waters. The present study provides a first account of the macro- and megafauna deep-sea biodiversity associated with CWC and hardground habitats in the Adriatic Albanian waters. A total of 82 species were identified including 75 species that are new records for the Albanian fauna, adding to the general knowledge of the deep-sea benthos of the southeastern Adriatic Sea.

3.7. References

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4. Morphological variations of Mediterranean *Lophelia pertusa*

4.1. Introduction

4.1.1. *Lophelia pertusa* and Cold-Water Coral bioconstructions

Lophelia pertusa is a cosmopolitan azooxanthellate coral species that contributes to the formation of deep-water bioconstructions (Freiwald et al., 2004, Roberts et al., 2009). It is the most widespread species among the deep sea frame building corals (Zibrowius 1980; Frederiksen et al., 1992; Tyler and Zibrowius., 1992; Rogers, 1999; Freiwald et al., 2004; Taviani et al., 2005; Roberts et al., 2006).

The distribution of *L. pertusa* in the Atlantic and in the Mediterranean describes the ecology of this species and the environment in which it lives. According to Wilson (1979), *L. pertusa* requires hard substrates to settle down. The cases of its presence in muddy substrates have always been linked to the presence of small stone, shell or other hard objects to which the larva was initially settled (Wilson, 1979; Tudhope and Scoffin, 1995; Rogers, 1999). A second ecological factor influencing the distribution of *L. pertusa*, appears to be the presence of cold water flow (Frederiksen et al., 1992). Previous ecology studies of this species have shown its presence closely linked to deep-oceanic water. A constant or periodic flow of water must be present to bring food and oxygen to sessile corals and to remove sediment and wastes. The reason for a reliance on oceanic water for survival is unknown, but indicates that *L. pertusa* may be highly sensitive to salinity, density, temperature and other physical factors (e.g., Freiwald et al., 1997; Rogers, 1999). This species generally occurs in water of salinities between 32-37‰ and is not found below 6 °C. Also, recent observations on the vertical distribution of *L. pertusa* also suggest that occurrences coincide with the oxygen minimum zone (Freiwald, 1998; Creasey and Rogers, 1999).

CWC frame-builders are colonial organisms belonging to the order Scleractinia, able to secrete calcified skeletons that may form wide carbonate structures through time.

The bioconstructions engineered by *L. pertusa* can develop over many hundreds or thousands of years and form extensive carbonate structures, variously named in the literature as reefs, banks, carbonate mounds and coral build-ups (Freiwald, 2002). The widest known *Lophelia*-dominated bioconstructions in areas to the west and south of Norway, are among the most well-known and studied until now. The shallowest occurrence of live *L. pertusa* has been recorded at just 39m

depth in Trondheims fjord, mid-Norway, and the deepest records are 3383m from the New England Seamount chain and 2775 m off Morocco (Zibrowius, 1980). North Atlantic *Lophelia*-dominated carbonate mounds, preferentially occurring between 500 and 1000 m depth, vary in size and shape ranging from small, low relief ovoid features a few metres high and tens of metres across (Wilson, 1979; Scoffin et al., 1980; Masson et al., 2003; Foubert et al., 2005; Wheeler et al., 2005, 2006), to giant mounds hundreds of metres tall and a few kilometres across (Kenyon et al., 1998; De Mol et al., 2002; Huvenne et al., 2002, 2003; Akhmetzhanov, et al., 2003; Beyer et al., 2003). The upper parts of these carbonate structures are characterized by the youngest coral colonies and the densest aggregations of live *Lophelia*. The polyps of this coral work tirelessly keeping themselves clear of sediment, leaving the upper levels of the reef clean and pristine.

Live corals represent indeed only the "tip of the iceberg", below lies the reef framework formed by skeletal remains of previous coral generations and lithified sediment. Over time this skeletal framework traps sands and muds to form a robust cold-water coral reef - ultimately these portions of the reef become home to the highest number of species (Roberts et al., 2006, 2009).

L. pertusa forms bush-like colonies measuring up to several metres in width and consisting of thousands of polyps (reef). The tentacles are the most obvious feature of the polyp, surrounding its single opening which serves both as a mouth and anus (Zibrowius, 1980; Rogers, 1999; Cairns, 1994, 2000). As the colony grows, adjacent branches tend to join together ("anastomose") considerably strengthening the carbonate framework. This species is known to occur overall the Atlantic, in some areas of the Mediterranean Sea, in the Gulf of Mexico and the Caribbean Sea (Freiwald et al., 2004, 2009; Roberts et al., 2009 and reference therein). It is also present in a few locations in the Indian Ocean and the Pacific Ocean (Freiwald et al., 2004).

4.1.2. Mediterranean *Lophelia-Madrepora* bioconstructions

Lophelia pertusa and *Madrepora oculata*, have been documented from various sites of the Mediterranean region occupying a large variety of habitats such as canyons, steep cliffs, mud volcanoes, seamounts, mound-like features and tectonic ridges (Fabri et al., 2014; Fink et al., 2012; Freiwald et al., 2009; Lo Iacono et al., 2012, 2008; Orejas et al., 2009; Taviani et al., 2005;

2011; Vertino et al., 2010; Angeletti et al., 2014; Savini et al., 2014; Taviani et al., 2015; Mastrototaro et al., 2010; D' Onghia et al., 2015). Most of the reported occurrences refer to fossil remnants while living occurrences of *L. pertusa* and *M. oculata* are less abundant and exhibit rather scattered distribution patterns compared to those known from the North Atlantic (Fosså et al., 2002). It is certain that Atlantic-type deep-sea corals including the scleractinian triad *Lophelia-Madrepora-Desmophyllum* have been established in the Mediterranean basin since at least the Early Pleistocene (Taviani et al., 2005; Vertino et al., 2014). Still-submerged dead coral assemblages are widespread in the entire basin between c. 250-2500 m depth; the majority is aged at the late Pleistocene by AMS-¹⁴C and U/Th dating (McCulloch et al., 2010; Taviani et al., 2011). The present situation (post-glacial) is complicate in the sense that a number of living CWC stocks inhabit the Mediterranean, but sectors of the basin have not fully recovered with respect to a previous basin-wide Pleistocene distribution (Taviani et al., 2005; 2011; Fink et al., 2012).

The Mediterranean basin represents an excellent archive of past and modern CWC growth whose study may help to understand taxonomic, biogeographic, ecological, and evolutionary patterns of modern deep coral bioconstructions since their colonization in the latest Pliocene-Early Pleistocene (Taviani et al., 2005; Vertino et al., 2014) to the present day.

Modern Mediterranean cold-water coral populations are dominated by *M. oculata*, however fossil occurrences on-land and off-shore hint at the dominance of *L. pertusa* during the Pleistocene (Vertino, 2003; Vertino et al., 2014; Fink et al., 2015). The study of the spatio-temporal evolution of *L. pertusa* in the Mediterranean is therefore of high importance for a better understanding of the modifications that have characterized the deep-sea fauna of this basin at the boundary Pleistocene – Holocene.

4.1.3. The skeleton of *L. pertusa*

Lophelia pertusa is a species highly variable morphologically; however, there are almost no studies focusing on its phenotypic variation. Usually, corals do not meet the criteria of conventional species concepts due to extreme phenotypic plasticity and/or instances of geographical restriction or hybridization along the genealogical history of the species (Kaandorp and Kubler, 2001). The plasticity of these morphological variations depends on a number of ecological factors which often are intertwined. The distribution, size, shape and orientation of cold-water corals, such as *L. pertusa*, seems to be influenced by the presence of a suitable substrate and by the presence of a cold water flow, which provides food for the colony and keep the polyps clean from fine sediment (e.g. Freiwald et al., 2004; Vertino et al., 2010; Angeletti et al., 2014; Taviani et al., 2015; Fink et al., 2015). Hydrodynamic factors seem to play an important role in defining shape and direction of cold-water corals. Previous studies on branched corals report that polyps, in order to optimize its capacity of catching food brought by the water flow, grow in the direction of water currents (Kaandorp and Kubler, 2001).

The morphology of *Lophelia* seems to depend also on its position within a bioconstruction. Regarding the size of the skeleton of *L. pertusa*, it may not solely be an expression of optimum conditions for growth but it can also be a function of longevity (fig 4.1).

In order to assess if the morphological variation of *L. pertusa* follows a specific pattern based on environmental (or others) variables, this study combines two different morphometric approaches: classical linear external morphology and use of three-dimensional coordinate landmarks. The main aims of this study are to test the existence of distinct morphological groups within the cosmopolitan species *L. pertusa* in the Mediterranean and to identify, if any, relationships between morphological groups (both in the modern and fossil corals) and (paleo)oceanographic conditions. In particular, in this study different morphological characteristics of several specimens of *L. pertusa*, both modern and fossil, collected from two different marine areas, Santa Maria di Leuca coral province and off Albania have been examined, also we have used an Atlantic fossil colony to identify if there are morphological variations between Mediterranean and the Atlantic *Lophelia*.

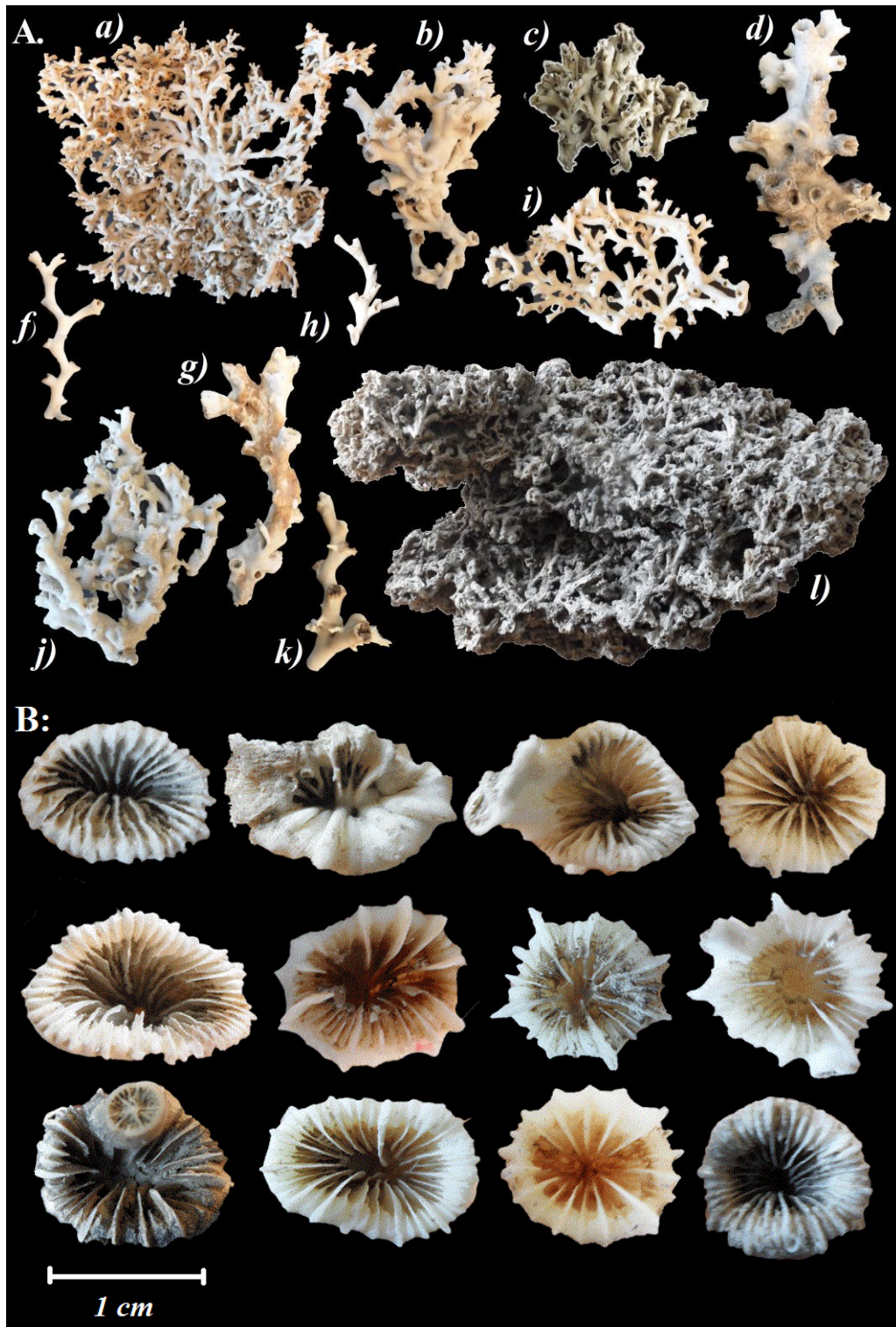


Figure 4. 1. A: Images of *L.pertusa* colonies measured for morphometric comparison. a, b, d, f, g, i, j, k: colonies from the coral area of Santa Maria di Leuca (Italy) (Coral Fish 16.10.2010-ST6-C1; Coral Fish 16.10.2010-ST6-C3-

lotto1; Corallo Bianco 14.02.01-St3; Corallo Bianco 14.02.01-St6; Corallo Bianco 08.10.04-ST64; 22.06.03-ST15-RIME1; 19.07.01-ST4-RESHIO2; Campagna corallo 16.02.01-ST8; Campagna corallo 16.02.01-ST15) **c**; RV Belgica GENESIS Cruise 09/14c; **h, l**; colony collected off-shore Albania (CoCoMap 13-16, AL16-SE2).
B: Morphological variability of *Lophelia pertusa* calice observed in some of the colonies we measured.

4.2. Material and methods

4.2.1. Morphometric analyses of scleractinian corals

The concept of morphometrics has a long history and can be defined as a "more or less interwoven set of largely statistical procedures for analysing variability in size and shape of organs and organisms" (Reyment, 1980). Landmark-based geometric morphometric methods are powerful tools in the study of size and shape. These methods allow to describe the shape of rigid structures considering a set of variables that can be used for statistical hypothesis testing, and to generate graphical representations of shape differences as deformations (Adams, 1999). The study of shape variation and its covariation with other variables, is an integral part of organism biology. By quantifying morphological variation, it is easier to identify the relationship between morphology and ecology (Losos, 1990; Ricklefs and Miles, 1994) and thus make more informed inferences on the evolution of organisms.

Morphological variation in scleractinian corals occurs on a series of hierarchical levels: within corallite, within colony, within local environment (population), and within species (Johnson and Budd, 1993; Budd et al., 2010, 2016). Genera and species of azooxanthellate colonial corals are distinguished by the skeletal architecture of the colony (shape, size, arrangement and degree of integration of corallites) and skeletal features of individual corallites (e.g. shape, size, number and arrangement of septa and central calicular structures) (Zibrowius, 1980; Vertino, 2003; Cairns and Kitahara, 2012). So far very few studies have been carried out on the morphometry of azooxanthellate scleractinian corals even if this methodology is essential to define and interpret the extreme morphological variability of these organisms (Johnson and Budd, 1993; Budd and Stolarski, 2009; Budd et al., 2010, 2016).

4.2.2. Material examined

The material analysed in this study consists of Mediterranean modern and subfossil colonies of *L. pertusa* collected in several campaigns carried out by the Department of Biology of the University of Bari Aldo Moro on the white coral habitats off-shore Santa Maria di Leuca (SML), from 2001 to 2010 (Fig. 4.1, Tab. 4.1). Moreover, two sub-fossil *Lophelia* colony aggregates collected off-shore the Albanian coast and, for comparison, a late Pleistocene colony collected from the Bay of Biscay (NE Atlantic) and held at the Renard Center of Marine Geology, Department of Geology, University Gent, Belgium, were examined (geographical location in fig. 4.2). All measured samples were classified as alive (at the time they were found) or modern corals and sub-fossils. Which was possible by the presence of dry tissue (coenosarc) in corallites. All samples consumed by time and often coated by Mn-Fe oxides and apparently belonging to pre-Modern have been classified as subfossils. All fresh-looking dead samples were excluded from the study as we did not know how to classify them.

Table 4. 1. List of specimens, relative cruise; coordinates, depth.

Sample code	Location	LAT (N)	LONG (E)	Depth (m)	Samples
Coral fish 16.10.2010/ ST 6 C1	Santa Maria di Leuca	39.34.827	18.11.716	528	Live
Coral fish 16.10.2010/ ST 6 C3/lotto 1	Santa Maria di Leuca	39.34.827	18.11.716	528	Live
Corallo Bianco 14.02.01/St3	Santa Maria di Leuca	39.27.59	18.23.23	809	Live
Corallo Bianco 14.02.01/St6	Santa Maria di Leuca	39.36.86	18.31.39	662	Sub-fossil
Corallo Bianco 08.10.04/ST 64	Santa Maria di Leuca	39.50.520	17.39.450	740	Sub-fossil
22.06.03/ST 15 /RIME 1	Santa Maria di Leuca	39.36.030	18.23.170	473	Live
19.07.01/ST 4 /RESHIO II	Santa Maria di Leuca	39.34.230	18.42.500	567	Sub-fossil
Campagna corallo 16.02.01/ST 8	Santa Maria di Leuca	39.37.42	18.38.80	665	Sub-fossil
Campagna corallo 16.02.01/ST 15	Santa Maria di Leuca	39.25.010	18.24.64	910	Sub-fossil
RV Belgica GENESIS Cruise 09/14c	Atlantic	43. 51.7600'	08. 43.9119'	440	Sub-fossil
Albania 2015 Seman 1	Albania	40° 48' 26	18° 59' 24	190	Sub-fossil
Albania 2016 Seman 2	Albania	40° 50'730	18° 59'970	210	Sub-fossil
Albania 2016 Seman 3	Albania	40° 50'730	18° 59'970	210	Sub-fossil

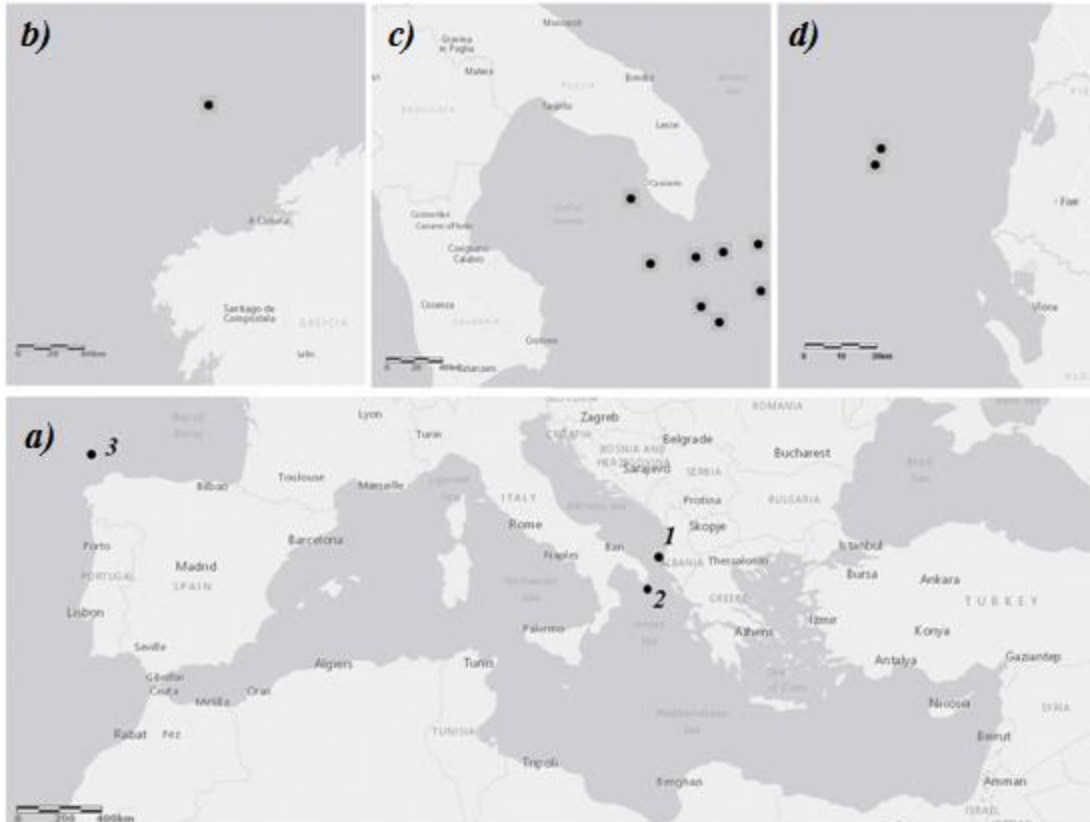


Figure 4. 2. Map of the geographical position of origin of every *Lophellia* colony analyzed a. Geographical location of the sites of samples origin, in the Mediterranean and in the Atlantic; b. Atlantic colony RV Belgica GENESIS Cruise 09/14c; c) SML colonies; d) Albanian colonies.

4.2.3. Methodology

Following the procedure indicated by Addamo et al. 2015, 2016, samples were carefully cleaned from mud or dry tissue (coenosarc) if present, so that corallite's calicular features were visible. Measurements of the SML specimens were directly performed on the coral skeleton using a caliper. Instead, the colonies collected in the NE Atlantic and off-shore Albania were examined through computed tomography (the procedure is explained in the next chapter). Due to the extreme variability of the shape of the colony of *L. pertusa* and the impossibility to collect entire colonies, it was decided to perform morphometric analysis on the corallites of the collected specimens. Corallite abundance by area, colony preservation state and position within the colony are summarised in Tab 4.2.

Table 4. 2. Frequency of the analysed corallites by area, position of the corallite within the colony, and live or fossil condition of the colony at the time of collection.

	Variables	Frequency	Percent	Valid Percent	Cumulative Percent
Area	Santa Maria di Leuca	171	61%	61%	61%
	Atlantic	28	10%	10%	71%
	Albania	79	28%	28%	100%
	Total	278	100%	100%	
Colony condition	Living	119	43%	43%	43%
	Sub-fossil	159	57%	57%	100%
	Total	278	100%	100%	

Twelve morphometric characters were measured in 278 corallites (Fig. 4.3, Tab. 4.3), including (GCD) Greater Calicular Diameter, (LCD) Lower Calicular Diameter (SN) Septa Number, (H) Height, (L) Length, (TT) Theca thickness; (GCDS) Greater corallite diameter section, (LSD) Lower corallite diameter section, (α) Angle pedicel-calice, (FL) Fossa length, (GFD) Greater fossa diameter (LFD) Lower fossa diameter, (MW) Maximum dominant septa width . For samples from SML, all characters were measured using the electronic caliber, and for other samples was used the MyVGL Studio software which view analyses and visualizations for tomography images (see the procedure in more detail in the next chapter).

Table 4. 3. Characters included in the macromorphological analysis in *Lophelia pertusa* colonies.

Character	Abbreviation	Description
Greater calicular diameter	GCD	The longest calice diameter measured between two opposite points of the external wall.
Lower calicular diameter	LCD	The shortest calice diameter measured between two opposite points of the external wall.
Height	H	Distance between the upper calicular surface and the base of the corallite
Length	L	Linear measure along the outer wall of the corallite between the corallite base and the distal most calicular edge
Theca thickness	TT	Thickness of the corallite theca measured in correspondence of GCD and LCD.
Angle pedicel-calice	α	Angle measurement between two corallites
Greater corallite diameter section	GCDS	The longest linear measurement of the diameter of a peduncle section.
Lower corallite diameter section	LCDS	The shortest linear measurement of the diameter of a peduncle section.

Some of the parameters measured during work were found to have a high margin error, although they were not considered for statistical analysis. The data obtained from the measurements were analyzed using SPSS 24.0 (IBM, 2016).

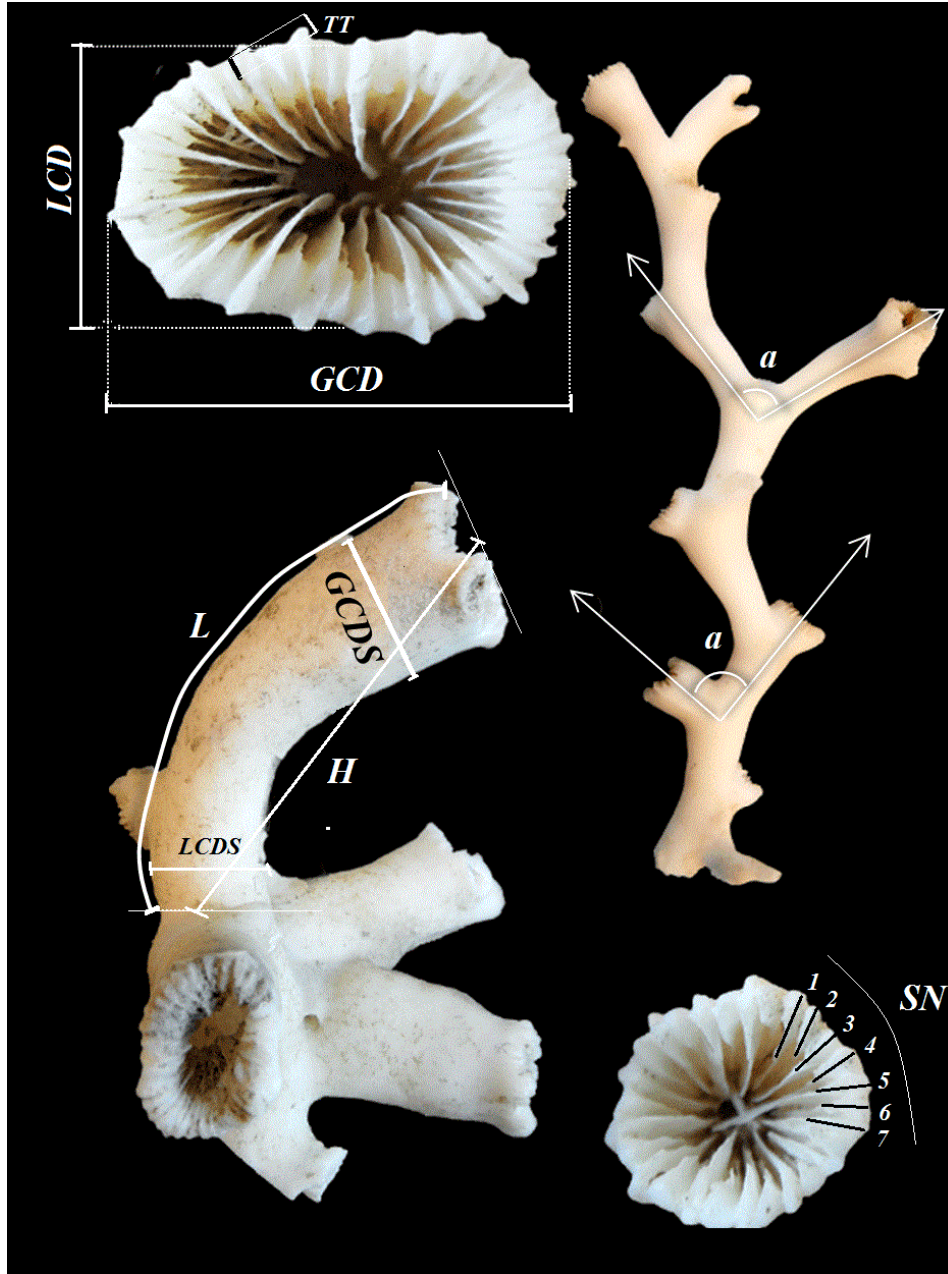


Figure 4. 3. Morphometric parameters of *Lophelia* corallites measured in this study.

A rough matrix coordinated by 278 corallites measurements and 9 morphometric characters was prepared for the statistical processes described below. To decrease the margin error, each value is the average of three measurements made for each of the morphological characters, and also the morphological characters with low error margin were chosen. Statistical exploratory procedures were performed such as frequency analysis and correlation of the nine morphometric characters for all measurements. Before performing a multivariate analysis, variables were examined by preparing the frequency histograms for nine morphological characters for all the examined samples. In addition, correlation analysis and core component analysis (PCA) were performed to examine the relationship between the nine morphological characters for comparison.

A statistical representation by boxplots was made, of variability for each morphological character between live and fossil corals. The canonical discriminatory analysis (CDA) was performed to compare the different morphometric characters between the colonies' origin areas.

4.3. Discussions

4.3.1. Descriptive Statistics

The descriptive statistic of morphometric characters measured in corallites is represented in the following Tab. 4.4. For morphometric comparison, only seven morphometric characters were used, which had a lower margin of error.

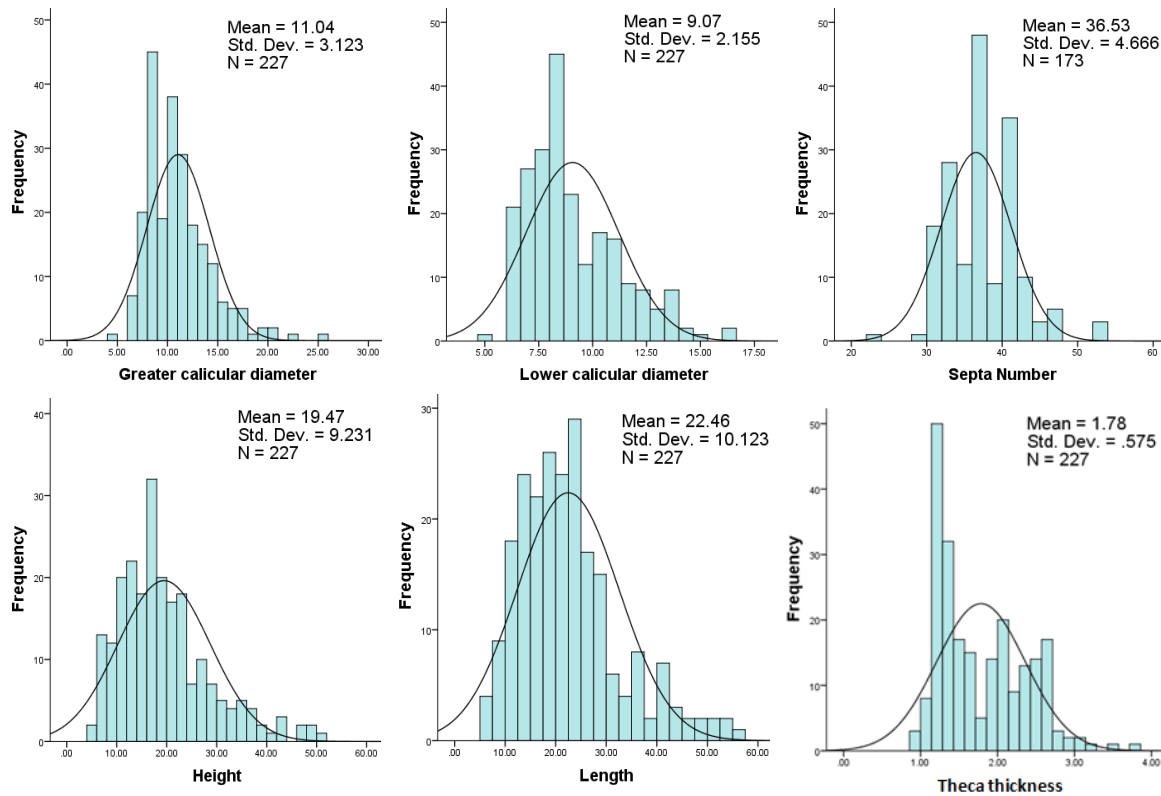
Table 4. 4. Statistical description of the morphometric characters considered for the study.

	GCD	LCD	SN	H	L	TT	α
Valid	278	278	224	278	278	278	278
Missing	0	0	54	0	0	0	0
Mean	11.0422	9.0678	36.53	19.4726	22.4586	1.7848	45.7045
Std. Error of Mean	0.20726	0.14305	0.355	0.61267	0.67192	0.03817	1.37496
Median	10.59	8.5	36	17.57	20.9	1.6	41.29
Mode	8.62 ^a	7.07 ^a	36	15.82 ^a	9.30 ^a	1.24	35.55 ^a
Std. Deviation	3.12263	2.15526	4.666	9.23076	10.12347	0.5751	20.71584
Variance	9.751	4.645	21.774	85.207	102.485	0.331	429.146
Range	20.78	11.78	29	44.39	49.11	2.88	98.79
Minimum	4.92	4.82	23	10.85	15.36	0.94	8.71
Maximum	25	16.6	52	50.24	55.47	3.82	107.5

As it is also represented in the table, the Greater Calicular Diameter varies from 25 mm to 4, 92 mm, while Theca thickness ranges from 3.82 mm to 0.94. All the parameters are represented in millimeters instead the angle α is represented in degrees.

4.3.2. Frequency histograms.

Frequency histograms in Fig. 4.4, confirm that the overall range of morphological variation among the corallites is considerable. In all 9 variables there is a continuous general variation, eight of these variables, fig. 4.4 (excluding angle pedicel-calice) are normally distributed at the level of significance of 0.005 according to the Shapiro-Wilk test. As the Frequency histograms show, the most common Greater and Lower calicular diameter is respectively 11.4 and 9.07mm. The most common septa number was 36, and there was also an even number of septa in each of the corals, which suggests that the septa are born in pairs during the growth of the corallites. The morphometric character with lower standard deviation is Theca thickness.



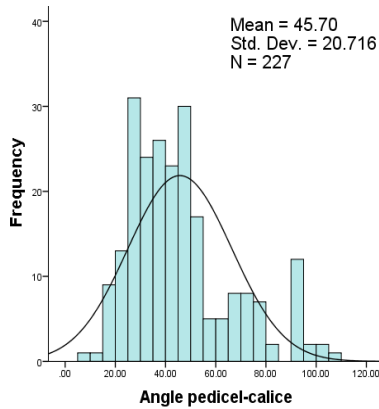


Figure 4. 4. Frequency histograms for nine morphological characters for the examined corallites. The histograms show the values of the measurements (expressed in mm with exception of septa number angle of pedicel) on the x-axis, and the frequency distribution of these values on the y-axis.

The correlation coefficients of Pearson were calculated to examine the linear correlation between each pair of morphometric variables.

Table 4. 5. Simple correlation matrix of 7 morphometric characters measured in 278-224 corallites of *L. pertusa*. The table indicates the correlation of Pearson, the probability by t-test and the number of calculated values taken. Asterisks indicate values showing significant linear correlation between morphological characters at significance level 0.01 using t-test.

		GCD	LCD	ST	H	L	TT	α
GCD	Pearson Correlation	1	.786**	.294**	.694**	.707**	.699**	0.001
	Sig. (2-tailed)		.000	.000	.000	.000	.000	0.098
LCD	Pearson Correlation		1	0.142	.698**	.689**	.724**	-0.037
	Sig. (2-tailed)			0.062	.000	.000	.000	0.054
ST	Pearson Correlation			1	.242**	.263**	.283**	0.019
	Sig. (2-tailed)				0.001	.000	.000	0.087
H	Pearson Correlation				1	.960**	.687**	-.211**
	Sig. (2-tailed)					.000	.000	.000
L	Pearson Correlation					1	.664**	-.212**
	Sig. (2-tailed)						.000	0
TT	Pearson Correlation						1	0.072
	Sig. (2-tailed)							0.023
α	Pearson Correlation							1
	Sig. (2-tailed)							

** Correlation is significant at the 0.01 level (2-tailed).

The correlation matrix showed that six of the seven morphological characters measured are strongly correlated to the significance level of 0.01 using the t-test: Greater Corallite Diameter, Lower Corallite Diameter, Height, Length, Theca Thickness. All characters are positively correlated to each other with the exception of the angle at which in some cases it is negatively correlated with some parameters such as length and height, this result suggests that the growth of the length of the corallite decreases the angle between the latter and the branch.

Strong ratios at the same level of significance with t-test are indicated between the diameter of the corallites and their length and height. The high correlation coefficients between each pair of six morphological characters on the correlation matrix suggests that there is a strong association between multiple characters.

4.3.3. Morphometric differences between sub-fossil and modern *Lophelia* corals

While measuring the skeletal features for morphometric purposes, it was noticed a significant difference between modern living and subfossil to fossil corals. To test this observation, a series of statistical analyses, which we explain in the following paragraphs, was conducted.

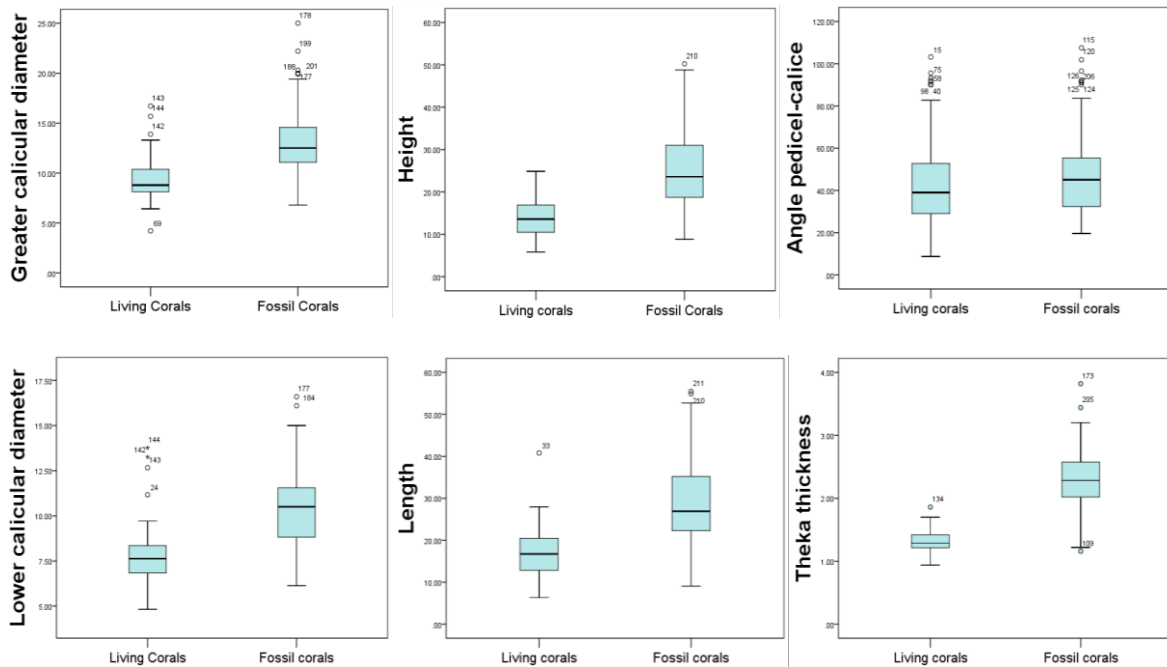


Figure 4. 5. Box plots of distribution of morphological characters throughout fossil and modern *Lophelia pertusa* coral samples. Distance expressed in mm and angles in degrees.

Variations in six morphological characters between the two populations (sub-fossils and living) also appear in box plots, fig 4.5. From the fig. 4.5 results in a significant difference in five of the six morphometric characters, in which modern corals exhibit lower parameters than the sub-fossil samples. The parameter that shows the highest differentiation between modern and fossil corallites is the theca thickness. Only 6 morphometric characters were used for the comparison given the lack of all the data in the other parameters.

Discriminant analysis.

The Canonic discriminant analysis, fig 4.6, using the six morphological characters selected was performed to find linear combinations of characters that better match the differences between the two populations (living and fossil corals).

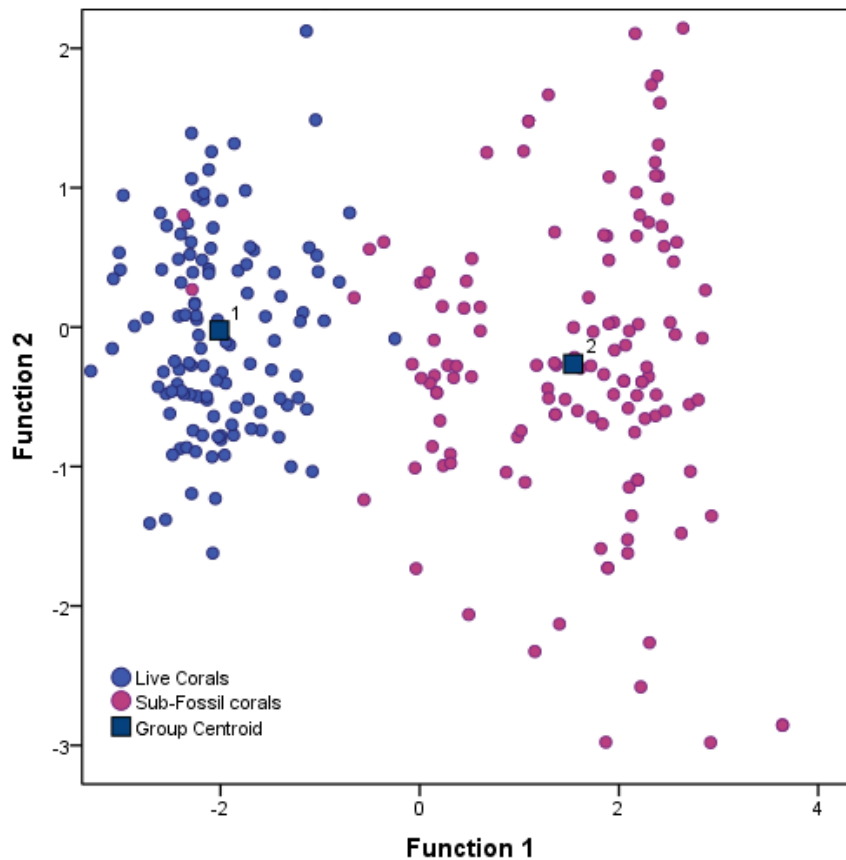


Figure 4. 6. Plot of the first two functions from the discriminant analysis of the six morphological data, classified by sub-fossil and living corals of Mediterranean.

Two canonical discriminant functions 1 and 2, which respectively explain 95.4% and 3.6%, of the variance were obtained, fig 4,6. The canonical discriminatory scores for each coral were also calculated using the coefficients of the canonical discriminating function, which shows the discrimination between the two populations based on canonical discriminating function1.

This result hints at a clear differentiation between modern (or living) and fossil Mediterranean populations of *L. pertusa* based on the 6 selected morphometric characters: Greater calicular diameter, Lower calicular diameter, Height, Length, Theca thickness, Angle pedicel-chalice. In order to confirm this distinction, it was also carried out a multivariate analysis (MANOVA) Tab. 4. 6.

Table 4. 6. Multivariate statistics (MANOVA) among the fossil and living populations of *Lophelia pertusa*

		Value	F	Hypothesis df	Error df	Sig.
1:Living coral	Pillai's Trace	.726	97.116 ^b	6.000	220.000	.000
	Wilks' Lambda	.274	97.116 ^b	6.000	220.000	.000
2:sub-fossil	Hotelling's Trace	2.649	97.116 ^b	6.000	220.000	.000
	Roy's Largest Root	2.649	97.116 ^b	6.000	220.000	.000

All multivariate tests Tab. 4. 6. (Wilks' lambda, Pillai's trace, Hotelling-Lawley trace and Roy's largest root) were also significant (P <0.0001) in multivariate statistics for the differences between the fossil and modern corals.

4.3.4. Morphological comparison between SML and Albanian *Lophelia*

The Canonical discriminant analysis between the sub-fossil *Lophelia* samples of Santa Maria di Leuca coral provinces and the prominent samples from off Albania show that the two provinces do not resemble. Canonical discriminating functions 1 and 2 which respectively explain 81.1% and 18.9% of the total variance and canonical correlation of about 0.7and 0.4 indicate a clear difference between the two populations. The most significant variables for this comparison are Greater Corallite Diameter, Theca Thickness, and Height with a significance p <0, 005. Plot of the first two functions from the discriminat analysis SML and Albanian sub-fossil corals is shown in Fig. 4.7, where the position of the two populations is clearly compared to each other.

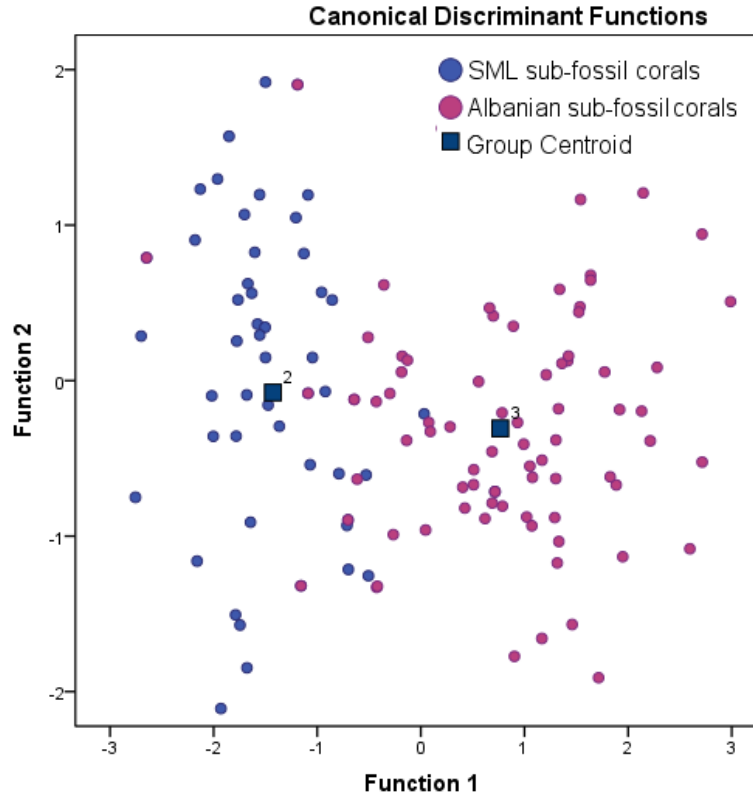


Figure 4. 7. Plot of the first two functions from the discriminant analysis of the six morphological data, classified by SML and Albanian sub-fossil corals

4.3.5. Modern and fossil Mediterranean vs. NE Atlantic *Lophelia*: preliminary observations

The bivariate plot with R^2 (r-Pearson correlation coefficient) based on the five morphological parameters was prepared to evaluate the morphological variation between sample sites, Fig 4.8. For bivariate analysis we compared some of the parameters by treating them as 3 different groups depending on the origin of the samples and then comparing them with the correlation analysis where all the parameters were considered as a single group.

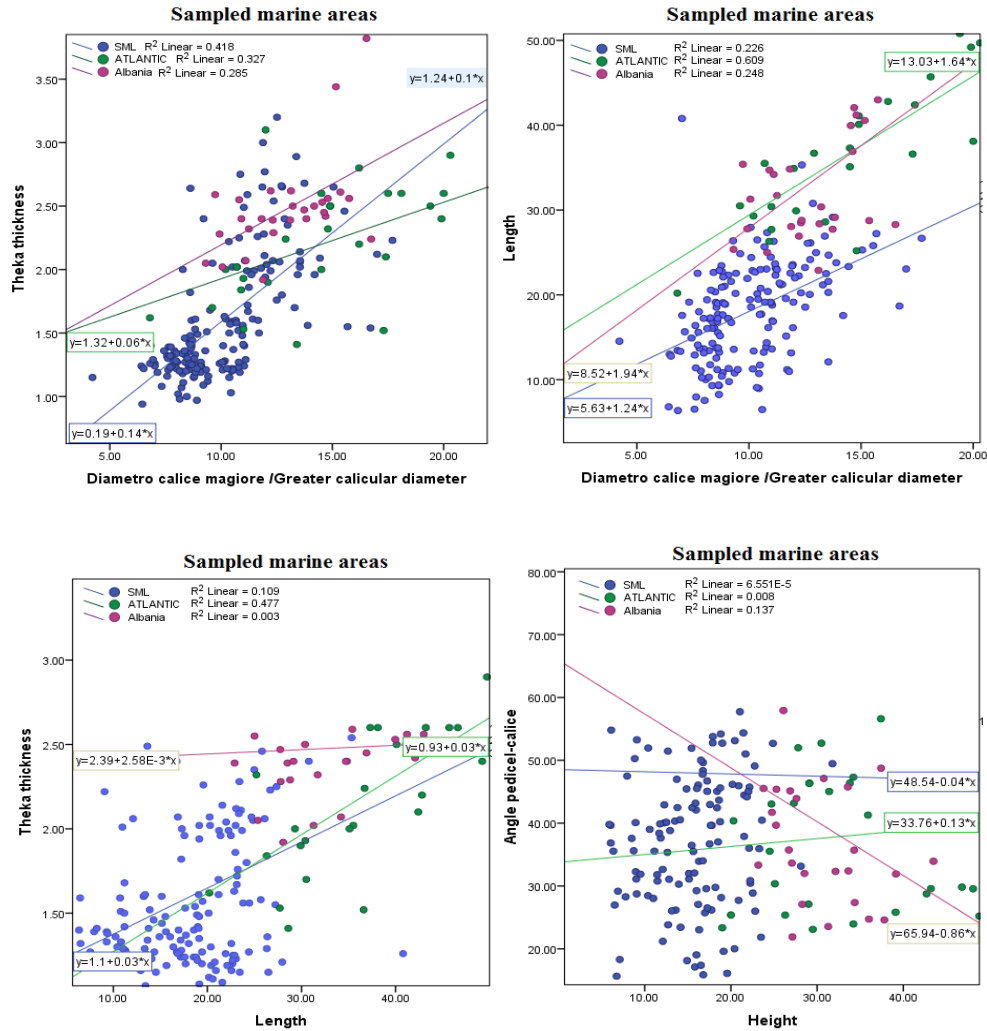


Figure 4. 8. Selected bivariate plot of morphological characters based on three *Lophelia pertusa* coral sampling sites.

Excluding the Angle pedicel-calice parameter, all the other parameters showed a significant difference between the three source sites of the samples. To confirm this hypothesis, we have also subjected the data matrix to discriminatory canonical analysis and to multivariate analysis. In order to compare the morphometric variability between the sampling stations, we excluded from the statistical analysis the modern *Lophelia* samples of the SML coral area since they were the only living coral samples and also the samples from the other two areas were fossil. With exception of the angle between corallites, all examined skeletal features showed significant differences (Wilks Lambda $p < .001$) in the analysed sites.

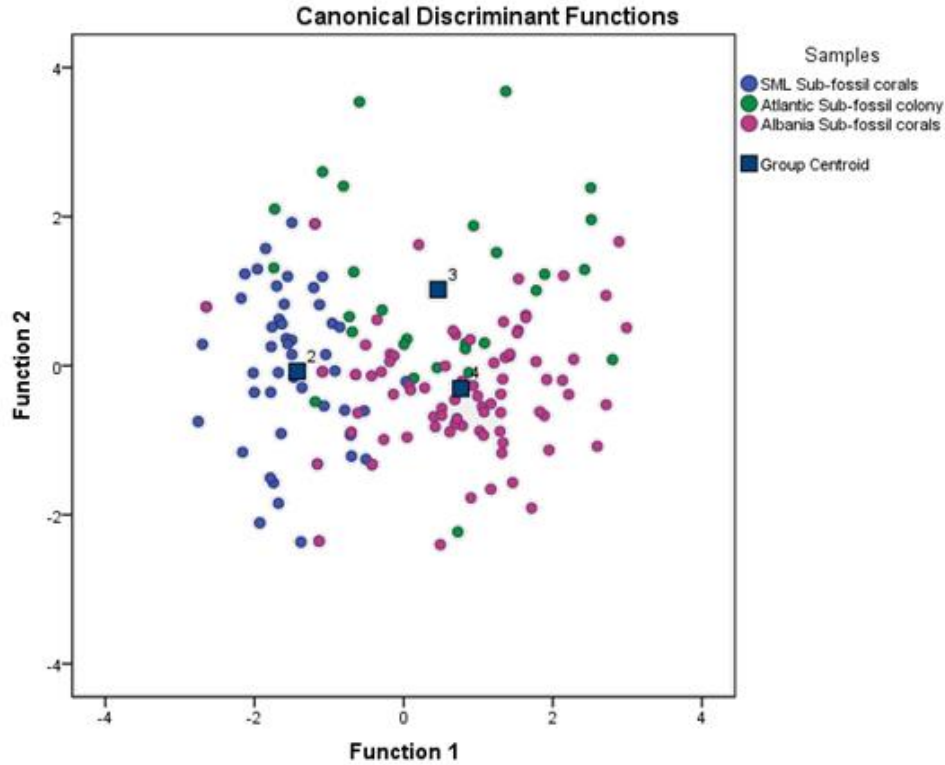


Figure 4. 9. Plot of the first two functions from the discriminant analysis of the six morphological data, classified by corals sampled marine areas.

The Canonic discriminant analysis produced two canonical discriminating functions 1 and 2 which respectively explain 85.6% and 14.4% of the total variance and canonical correlation of about 0.73 and 0.4, Fig 4.9. From the results obtained from the discriminant analysis, the Theca Thickness and Greater Corallite Diameter give the two most significant variables, $p < 0.001$. The values of Wilks's Lambda and Chi-Square test indicate that almost all of the variability in the discriminator variables is due to within-group differences (Tatsuoka, 1970; Huberty, 1975; Brown and Tinsley, 1983; Betz, 1987). The variability within the group shows that corallites vary not only between the areas of origin or between different periods of development (subfossil and living), but also within the same colony.

In addition, the MANOVA multivariate analysis, Tab 4.7, based on 6 morphological characters in 119 fossil corallites was significant (Pillai's Trace = 1.24, $F = 23.156$, $df = 12$, $p < 0.001$) indicating that morphological differences exist between the samples groups when all characters

are considered simultaneously. The variables most influencing the difference between the sites of origin are Greater Corallite Diameter, Theca Thickness and Height. The multivariate $\eta^2 = .415$ indicates that approximately 19% of the multivariate variance of the dependent variables is associated with the group factor.

Table 4. 7. Multivariate statistics (MANOVA) among the sampled areas populations of *Lophelia pertusa*

	Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Sampled areas	Pillai's Trace	1.244	25.959	18.000	660.000	.000	.415
1:SML	Wilks' Lambda	.127	36.750	18.000	617.082	.000	.497
2:Atlantic	Hotelling's Trace	4.171	50.210	18.000	650.000	.000	.582
3:Albania	Roy's Largest Root	3.490	127.954 ^c	6.000	220.000	.000	.777

4.4. Conclusions

This study aimed to emphasize the applicability of numerical methods to define spatio-temporal morphological variations of the *Lophelia pertusa* corallite. For the morphometric comparison we used about 9 morphometric parameters which were measured in 227 corallites belonging to 11 colonies, 9 of which collected from the SML coral area, 2 fossil colonies offshore the Albanian coast and one, as comparison, from the NE Atlantic (Gulf of Biscay). All skeletal characters seem to have a high coefficient of correlation between them. Six of these morphometric characters have been used for morphological comparison and statistical analysis.

All measured skeletal features show high variability among the examined specimens. This confirms the extreme intraspecific variability known for scleractinian corals and, in particular, for *L. pertusa* and *D. dianthus* (Zibrowius, 1980; Vertino 2003), two frame-building species that, according to Addamo et al. 2015, should be referred to the same genus due to their extreme similarity in the early growth stage. The intraspecific variability of the skeleton of *L. pertusa* is evident both at large scale (colony shape) and, as demonstrated in this study, at smaller scale (corallite morphology and size). Like in other colonial scleractinians, the intraspecific variability of this species can be linked to genotype variations resulting in different phenotypes (morphologies) or to phenotypic plasticity, i.e. the response of an organism to environmental modifications through morphological changes during its life time (Helmuth and Sebens, 1993;

Todd 2008, Todd et al., 2004). Very little is known about the phenotypic plasticity of cold-water corals but the variations of the corallite shape, and related calicular elements, even within the same colony, probably due to microenvironmental constrains, hints at a very high plasticity of *L. pertusa*.

Almost all characters are positively correlated to each other. The results obtained show all variables with the exception of angle α , are positively correlated with each other. The correlation matrix showed that six of the seven morphological characters measured were strongly correlated with Pearson correlation > 0.6 and significance level of 0.01 using the t-test: Greater Corallite Diameter, Lower Corallite Diameter, Height, Length, Theca Thickness. The positive correlation between the variables is a result of the presence of living organisms, which shows that with the growth of corallites all parameters are also growing. As the corallite grows, it stretches, increases in diameter, increases the seams, and thickens the teacup. However, not always the angle between the two corals, changes with the proportions of the coral. Angle α , is negatively related to other variables, or with a too low index of Pearson correlation. This suggests that the corallite growth may decrease the angle, while all the other parameters are increasing. Changing the direction of corallites during growth, which tends to be in the same direction (food stream), although they arise in different directions and positions, can be the cause of this outcome. But to confirm this hypothesis it is necessary to create coral growth models.

Comparison of living and fossil *Lophelia* colonies of Mediterranean showed a strong morphological difference. Comparison between sub-fossil colonies found in Albania and SML with live SML colonies show that not only are differences between these two populations (Wilk's = .0402 $F(4, 142) = 5, 957, p < .001$). Significant differences have been noted in the morphology of corallites, both between live corals and sub-fossils, and between live coral SML and those from Albania. It also appears that even in the populations of Santa Maria di Leuca this difference between sub-fossil and live corals continues to be evident. Sub-fossil corals not only have much bigger but thicker corallites, suggesting that external factors have affected a higher precipitation of calcium in the skeleton during the development of these colonies.

We also want to point out that no dating was made on the age of sub-fossil colonies of SML, so that the morphometric comparison could have been done between colonies from different periods, influenced by different environmental factors. The morphological variation between the

coral colonies developed during different periods and also in different habitats is correlated with environmental influences (Foster, 1980; Haggerty et al., 1980, Oliver et al., 1983, Willis, 1985; Miller, 1994; Bae et al., 2008).

The environmental influences on coral morphology of some species mostly in tropical corals, like *Montastraea cavernosa*, *Madracis mirabilis* and *Porites heronensis*, have been evaluated in many studies and experiments (Foster, 1979; Oliver et al., 1983; Willis, 1985; Shaish et al., 2007; Kaandorp and Kubler, 2001; Hoeksema, 2012, 2014, 2015). Micro-environmental influences on morphology may be important, especially in highly heterogeneous environments such as coral reefs (Zibrowius 1980; Kaandorp and Kubler, 2001; Todd, 2008; Budd et al., 2010; 2012). In order to confirm this hypothesis, in situ measurements should be made. It is also important to emphasize that the morphology of coralites is also influenced by other factors such as colony morphology, whether the shape of the colony is fan or bush, by the spatial organization of the coralites inside the colony. Also other factors like flow of nutrients brought by the water stream, and also by external factors such as the presence of epiphytic organisms such as sponges and serpulids often condition the morphology of the corallite (Kaandorp and Kubler, 2001; Beuck et al., 2007). Further work will be necessary to fully understand how morphological characteristic boundaries are related to spatiotemporal boundaries of *Lophelia pertusa* and also to understand the implications of these results for coral evolution generally.

Based on our results, SML's current corals differ from both SML and Albanian fossils, especially for the greater fragility of the skeleton, that is, a lower theca thickness. Also the size of sub-fossil corals is larger than the modern *Lophelia*. The greater dimensions and thickness of the coralites as well as the greater density of the ramifications, at least in the samples from Albania, suggests different environmental conditions in the past, presumably linked to increased availability of food that in literature has often been linked at a higher calcification rate (Kaandorp and Kubler, 2001; Todd et al., 2004; Budd et al., 2010; 2012). In addition, the greater sturdiness of colonies and corallites could be explained by hypothesising greater hydrodynamism in the past. Is known also the influence of wave action and currents on the morphology, robustness and density of the phenotypic high plasticity coral ramifications (Todd, 2008; Budd et al., 2010; 2012; Caroselli et al., 2015).

From the comparison results that there was no similarity between the fossil corals of the Adriatic, Ionian and the single Atlantic colony. The variables most influencing the difference between sites of origin are Greater Corallite Diameter, Theca Thickness and Height. According to Vertino et al., (2014), the Cold Water Corals of the Mediterranean date back to the Pleistocene period are very similar to their Atlantic counterparts. However, we want to point out that the comparison made is only with an Atlantic colony. To confirm this hypothesis, further comparison must be done between colonies whose precise age is known.

In recent years, interest in corals as potential paleoceanographic archives has been growing (Vertino et al., 2014). The first comparative studies between sub-fossil corals and their modern counterparts began in the second half of the 1980s (Greenstein and Curran, 1997; Aronson, 2007). The skeleton of these organisms is able to give a series of data on the chemical and physical characteristics of the environment over the period in which it was formed. Previous studies on the physiology of scleractinian corals state that the formation of the skeleton aragonite is directly influenced by environmental factors present at the time of skeleton formation (Montagna et al., 2005, 2006; Anagnostou et al., 2011; Vertino et al. Al., 2014; Gothmann et al., 2015). A possible explanation regarding the morphometric difference between the sub-fossil and living alive *Lophelia* colonies of the Mediterranean, and also between the Mediterranean and the Atlantic may be the presence of different environmental factors which have influenced the biomineralization process.

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**5. Study of *Lophelia pertusa*
morphology through to the
application of X-Ray Computed
Tomography**

5.1. Introduction

As discussed in the previous chapter, corals being sessile organisms are characterized by a remarkable morphological variability, for example a species may have more than one morphotype. The most important factors to which to attribute this fact are the intensity of light, this concerns tropical corals, and the speed and direction of the marine currents which are the main source of food for these animals (Lesser et al., 1994; Sebens et al., 1997; Kaandorp and Garcia Leiva, 2004; Kaandorp et al., 2005). In previous studies it has been observed that corals exhibit a high degree of variability along the flow gradient of water or nutrient availability (Kaandorp and de Kluijver, 1992; Lesser et al., 1994; Sebens et al., 1997; Kaandorp et al., 2003; Kim et al., 2004; Kaandorp and Garcia Leiva, 2004; Kruszynski, 2010; Kaandorp and Kubler, 2011). Considering all this, a morphological comparison of individuals coming from different geographic areas, can give important information on the environmental conditions in which these organisms live, on the factors that have affected their morphology (Kaandorp and de Kluijver, 1992; Muko et al., 2000, Kruszynski, 2010; Kaandorp and Kubler, 2011). Previous studies, taken at the rate of capturing particles in the ligation with the velocity and direction of the water flow, morphological variation has been observed involving various biologically relevant structures such as the shape and size of the corallites, their density, their degree of branching, and often also the thickness of the branches (Porter, 1976; Bruno and Edmunds, 1998, Sebens and Johnson, 1991, Sebens et al., 1997, Kaandorp and Kubler, 2001). Since these organisms are important witnesses of the conditions which have influenced their development, the quantification of how morphology changes in response to these environmental factors is a relevant issue that can also be applied to fossils to document the ecological conditions of others Geological periods (Stearn and Riding 1973; Kruszynski, 2010; Dubois-Dauphin et al., 2017; Foglini et al. 2017). Considering the complexity of the skeleton of these organisms, it can also be seen how difficult it is to study the morphometric parameters such as the branching rate, the density of the corals, or the branching angle.

For the morphometric coral study, various techniques have been used, such as the use of resin incorporation, then cutting the sample into thin slices, the use of aluminum foil for surface calculation, but these methods sometimes require a lot of time and even the loss of the sample, as

well as the data obtained have a high error margin (Stimson and Kinzie, 1991; Hoegh-Guldberg, 1988; Kaandorp, 1999; Abraham 2001; Sanchez and Lasker, 2003; Shaish et al., 2006; Abraham, 2001). An effective method used in recent years in the morphometric annotation of shapes such as coral scalleters is computed tomography, which allows the creation of a 3D model on the computer both inside and outside of the coral. Computed tomography allows the creation of a 3D model to the computer, identical to the scanned object, thanks to the overlay of a number of sliced images obtained with X-rays.

The purpose of this work was to compare 4 colonies of sub-fossil coral *L. pertusa*, coming from the Mediterranean and East Atlantic. Sub-fossils are important references that testify to environmental conditions of paleo-environmental, so their morphological study is an important issue for ecology. Taviani et al., (2005a), (2011b) has pointed out that subfossil *L. pertusa* from Apulian waters display corallites are apparently larger and thicker than their counterparts in the Mediterranean Sea. Our aim is to investigate whether there are really morphological differences between these morphotypes, both in the morphology of the corallites and in their spatial organization. Computed tomography of the samples has given us the possibility to measure some variables chosen according to the species's characteristics and also according to the limitations that this technique presents. For the corals morphology study, we focused on the measurements of the skeleton, and especially the corallites, and then carry out a quantitative analysis of these data. Since corals are organisms with a morphology based on the sum of modules, which in our case are corallites, it is possible to apply landmark-based methods for the morphometry of each module (Budd et al., 1994; Budd and Guzman, 1994; Todd et al., 2004, 2008). However, it is important to emphasize that the method presents a scale of error, which is due to many factors. The precision of this method depends on the type of CT scans, the coral scaling instrument setting mode, and the density of the colony. Although this method is an innovation in the study of organisms, this technique has limitations. Moreover, the complexity of data produced by scanning requires advanced software because they are then processed.

5.1.1. Morphological Plasticity in Colonial Cnidarians

Corals that form coral reefs, are most common in shallow, oligotrophic, tropical waters. Most corals are colonial and made up of many interconnected polyps, with no obvious diversity of function amongst the polyps. These sessile organisms, mostly colonial, are totally dependent on the physical and chemical characteristics of the environment. As their survival, nutrition and growth depends on the surrounding environment, corals exhibit a huge morphological plasticity. The two major environmental parameters which have the greatest impact on the growth forms of this marine sessile organisms are light, required for photosynthesis, and hydrodynamics (Kaandorp and Kübler, 2001; Roberts et al., 2009).

Most coral reefs contained symbiotic algae within their tissues, this shift in morphology was thought to optimize light capture as light diminished with depth. The endosymbiosis established between corals and unicellular plants has made them the largest calcified organisms of the coral reefs. Symbiotic algae, also called zooxanthellae, provide corals through photosynthesis, the necessary nutrition to make these organisms totally autotrophic, and also increase considerably their growth rate (calcification). Thanks to the success of symbiosis, in tropical corals, light is considered the main factor affecting growth and the shape of coral. Previous studies have demonstrated a change in the morphology of corals following the change of the intensity of light (increasing depth). Variations were observed both in the colony morphology (mostly from rounded to flattened ones), pigment concentration, density of polyps and zooxanthells (Falkowski and Dubinsky 1981, Roberts et al., 2009).

Along with tropical corals, another equally important group of Cold Water Corals are not limited to shallow and well-lit seas. They survive without the need for light. An example of such corals is *L. pertusa*, which grows 250 meters deep in the North Atlantic (Norwegian waters) and also in the Mediterranean (St. Maria di Leuca, Bari Canyon). The absence of the zooxanthellae in the body of these corals makes it totally heterotrophic, dependent on the plankton driven by the marine currents, and hunted by the polyps.

Considering that these organisms have no other source of nutrients and aorganic carbon than the water column, the brownish currents are an extremely important factor in determining the shape

and size of the colony. The relative contribution of autotrophy (photosynthesis) and heterotrophy (particle feeding) may depend on local availability from each source (Kaandorp and Kübler, 2001). According to Porter, (1976), morphological variations may be related to these environmental factors. According to Porter's models (1976), corals with larger polyps are better able to feed heterotrophically while corals with high surface-to-volume ratios (branching species) depend more on autotrophy because of better light capturing capabilities.

Returning back to the anatomy of these organisms, we find that the support of them is dependent on the persistence of the skeleton, which is formed during the growth of the tissue. These two anatomical components, the tissue and the skeleton, are totally different, and are influenced by different environmental factors, so the arrangement of these two anatomical components is necessary for their survival. According to Barnes and Lough (1992), the various forms of growth that present a coral are the result of the accommodation of the two forms of growth.

Corals, being suspension-feeding organisms, are dependent on the movement of water for the delivery of plankton and other particles at the level of their feeding surfaces. So the possibility of nourishment, of a corallite (of a single polyp) is due to the presence of a number of factors, such as the nature of the particles carried by the current, their velocity, the direction of the corallite, its surface in contact with water current ect. Considering these factors, the morphological variation is not limited to the whole colony but is also found at the corallite level, which sometimes also exceeds intra-specific variations. Such an example and the dimorphism between axial and radial corallites in the genus *Acropora* (Veron and Wallace, 1984).

5.1.2. The influence of water currents in coral morphology

As already explained in the previous paragraph, corals being suspension-feeding organisms, largely depend on the delivery of plankton and direct particulate matter to corallites, by water currents. The interaction of ambient flow with a coral's morphology, can have a significant impact on rates of particle capture (Kaandorp and Kübler, 2001; Roberts et al., 2009). In previous years, many works (Barnes and Lough, 1992; Kaandorp and Kübler, 2001; Foglini et al 2017) have been done to quantify the interaction of coral morphology with flow, both in the theoretical and in empirical approaches.

According to Sebens and Johnson (1991), who measured the feed rate of two scleractinian morphologies, coral increases the capture of particles at a higher flow rate. On the contrary, flat, morphological coral did not show any change in the rate of particle capture due to the tendency to flatten the tentacles with the increase in flow velocity (Johnson and Sebens, 1993; Kaandorp and Kübler, 2001).

According to Chamberlain and Graus (1977), colonies with different growth forms have different nutritive problems with respect to water flow. Colonies characterized by horizontal flow waste their resources, but experience no adverse gradient in food availability inside the colony. Colonies having diagonally-outward flow must contend with a marked decrease in food availability inside a colony.

Previous studies in tropical corals have shown that while the increase in flow velocity leads to more food in the coral surface, the nutrition rate of the coral decreases, due to the decrease in the ability of the tentacles to catch the prey (Chamberlain and Graus, 1977; Patterson, 1984, Lasker 1981; Sebens and Johnson, 1991; Johnson and Sebens, 1993; Sebens et al., 1997; Kaandorp and Kübler, 2001). Some species are apparently able to solve the problem of food loss due to the flow rate of the stream, through the formation of more branches in the colony, so that the space between the branches decreases the rate of flow (Sebens et al., 1997). According to McFadden (1986), often the velocity of the flow is also affected by the presence of other nearby colonies. So the direction and velocity of currents in a reef looks differently compared to solitary corals.

Morphometric measurements made in the coral *Madracis mirabilis* show an increase in the diameter of the branches in high flow environments. In contrast, this coral has an increase in space between the branches (decrease in diameter and number of branches) in medium or low flow environments (Sebens et al., 1997). The morphological plasticity of this species, suggests a huge capacity for acclimatization according to the characteristics of the surrounding environment.

5.1.3. CT imaging in scleractinian corals

Being the major bioconstructors of coral reefs, and species of biodiversity-rich habitats, scleractinian corals, are currently a focus of major interest because of their ecological importance and the uncertain destiny of their future (Stolarski et al., 2011). The structure of coral skeletons has been extensively studied both in terms of structure and composition (Wise, 1970; Sorauf, 1972; Jell, 1974; Nothdurft and Webb, 2007), using different, sometimes invasive methodologies on coral. Often, such methods required complete destruction of the sample, such as making thin sections on resin (Cuif et al., 1999, 2005; Ehrlich et al., 2006; Martin-Garin et al., 2007; Perry and Hepburn, 2008).

For many years, the only way to study the internal structure of scleractinian was to create thin sections. This extremely invasive technique takes time, and even loss of a sample through this destructive process. In order to prevent data loss in recent years, has been introduced the use of computational X-ray tomography (CT) (Kaandorp et al., 2003; Kruszyński et al., 2007; Roche et al., 2010), which is a promising way to investigate the structure and evolution of coral skeleton, non destructive and in three dimensions.

Major steps in important hardware components like open micro- and nanofocus X-ray tubes on the one side, and the development of highly efficient flat panel detectors on the other side allowed the development of very versatile and high resolution laboratory CT systems (Buzug et al., 2006; Brunke et al., 2008). Being a non-destructive method, from the ability to perform qualitative and quantitative 3D analysis, this method has had its major success in medical science but is expanding its use in other fields, such as geophysics and of zoology (Mees et al., 2003; Carlson et al., 2003; Ehrlich et al., 2006; Knackstedt et al., 2009, Halisch et al., 2013, Schmitt et al., 2016). For the implementation of this technique, a skeleton measurement protocol was followed, which allowed us the extraction of data, whatever the complexity of the coral structure (Lindquist et al., 1996; Sheppard et al., 2005; Ehrlich et al., 2006; Al-Kharusi and Blunt, 2007; Kruszyński, 2010; Veal et al., 2010).

However, this technique is at the beginning of its development, and it is important to remember that measurements do not have endless accuracy (Knackstedt et al., 2004; Kruszyński, 2010).

According to Kruszynski, (2010) uncertainty reduces the accuracy at every step of the measurement process, starting even before an object is scanned and growing with each consecutive step. Any inaccuracy in the process will result in an inaccuracy of the measurements, due to limitations in 3D image resolution, network extraction without preservation of topology and computational time and power (Tölke et al., 2010).

5.1.4. Principle of CT Scanning

The principle of CT, is to scan the subject as dividing it into a number of axial slices. The X-ray used was collimated up to a narrow X-ray beam, the size of which in the first tomographs was 3 mm inside the slice plane and 13 mm wide perpendicular to the slices (along the axis of the subject) . In fact, it is this beam width that typically specifies the slice thickness to be imaged. The X-ray tube is rigidly linked to an X-ray detector located on the other side of the subject (Goldman, 2007) (fig. 5.1).

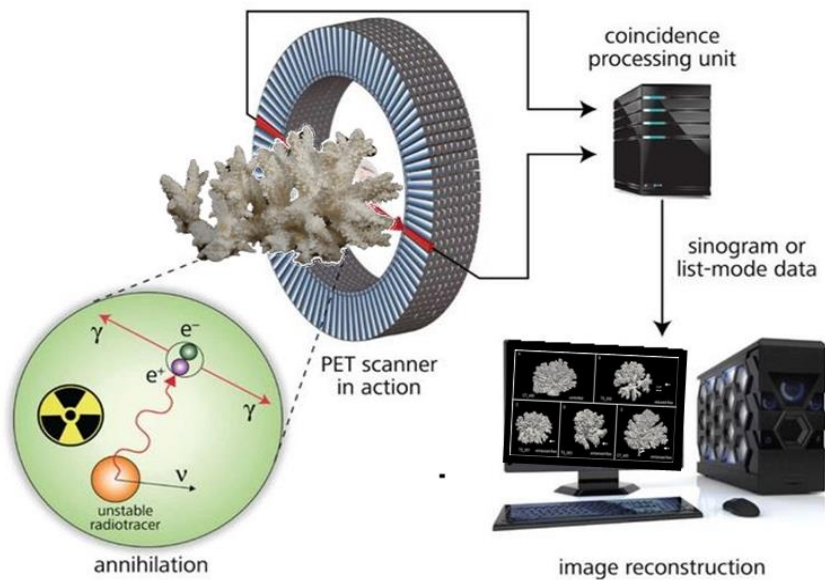


Figure 5. 1. Basic setup of a CT scanning device: X-ray tube, special detector unit (middle) and computers and monitor of the CT device (right) Modified after (www.sepscience.com).

The main components of all CT devices are: the X-ray tube, the computerized numerical control unit (CNC) for positioning and horizontal movement of the sample, and the special detector unit

(fig. 5.1). Besides, high-performance computers are connected to control and monitor the CT device (X-ray source as well as scanning parameters) for image data acquisition and image data reconstruction, respectively (Halisch, 2013). Together, the tube and the detector scan across the subject, sweeping the narrow X-ray beam through the slice. This linear transverse scanning motion of the tube and the detector across the subject is referred to as a translation. During translation motion, measurements of X-ray transmission through the subject are made by the detector at many locations. The X-ray beam path through the subject corresponding to each measurement is called a ray. The set of measurements made during the translation and their associated rays is a view. Hounsfield's Mark I scanner, 1972, measured the transmission of 160 rays per view (Hounsfield, 1980; Goldman, 2007). The number of measurements for today's scanners is typically over 750 (Goldman, 2007).

5.1.5. 3D Image Reconstruction

During the scanning process only two dimensional projections of the stepwise rotating sample are collected. These 2D slices need to be reconstructed in order to derive a three dimensional view of the investigated specimen. The main steps of 3D image reconstruction are: 2D data acquisition, extensive filtering processes, the back projection process and 3D image visualization (Halisch 2013) (Fig. 5.2).

The objective of CT image reconstruction is to determine how much attenuation of the narrow X-ray beam occurs in each voxel of the reconstruction matrix (Fig. 5.2). These calculated attenuation values are then represented as gray levels in a 2-dimensional image of the slice (in a manner described later). The 2 voxel dimensions lying in the plane of the slice (X and Y) are often referred to as pixels, however, the sizes of the pixels in the displayed image (referred to as the image matrix) are not necessarily the same as those in the reconstruction matrix but rather may be interpolated from the reconstruction matrix to meet the requirements of the display device or to graphically enlarge (zoom) the image (Goldman, 2007).

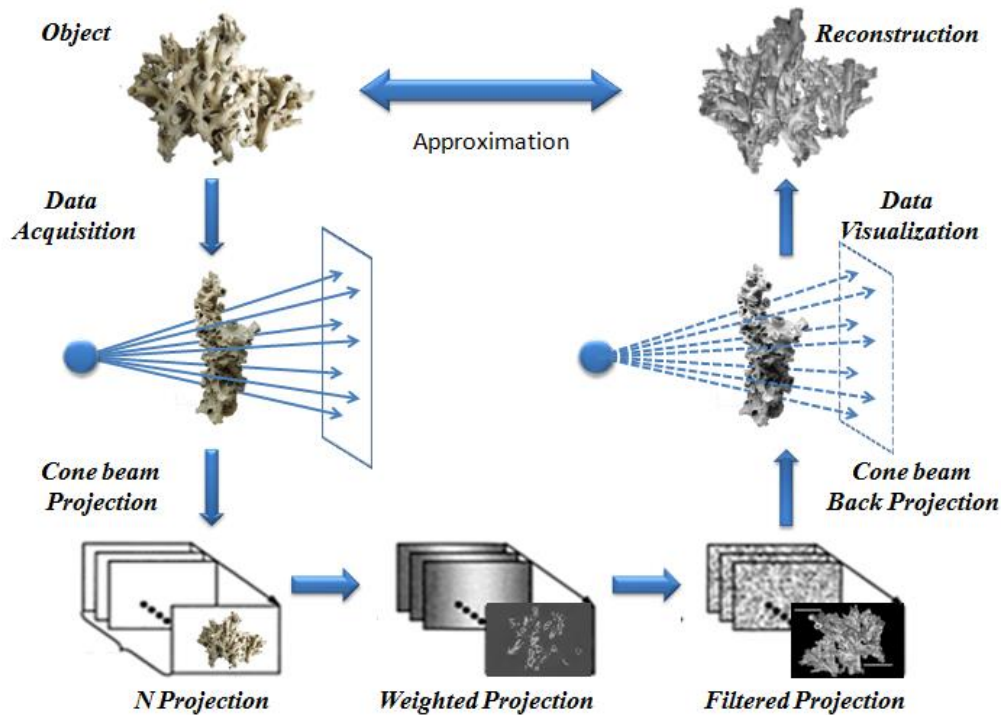


Figure 5. 2. Overview of Image Acquisition Processes for Reconstructing Images from the CT Scanner. Modified after Halisch, (2013)

It is remarkable that the basics of all modern imaging techniques have already been developed in 1917 without any practical field of application by the Austrian mathematician Johann Radon (Halisch, 2013). Nevertheless there were no parallel beam images until the 90's, when the retrospective 3D back projection technique was implemented (Grangeat, 1990).

5.2. Material and methods

For this study, four *Lophelia* subfossil colonies were used (Fig. 5.3), three of which were sampled in off Albanian, the fourth was a contribution of the Renard Center of Marine Geology, Department of Geology, University Gent, Belgium, coming from Bay of Biscay (NE Atlantic) (Tab. 5.1). The first sample, A115SE1 (Fig. 5.3), was scanned thanks to the private clinic's contribution "rm2000" Diagnostic Center, Bari, Italy. The A115SE2, A115SE3 scans were obtained using the Vlora hospital's CT scanner, and the fourth sample was subjected to tomographic analysis at the Renard Center of Marine Geology, Department of Geology, Ghent

University, Belgium. The three-dimensional images of the colonies were obtained according to the principle of computational tomography, explained earlier (Kaandorp and Kübler, 2001; Goldman, 2007; Kruszynski, 2010; Halisch, 2013). Three medical scanners, Philips CT Big Bore, SIEMENS SOMATOM Definition Flash and SOMATOM Emotion were used for data acquisition. The four coral colonies were scanned at a (almost isotropic) in an x and y resolution of 0.2 mm and z-resolution of 0.6 mm with "120 kV step and rotation time of 1 second resulting. An isotropic resolution is obtained by using equal slice spacings in all dimensions.

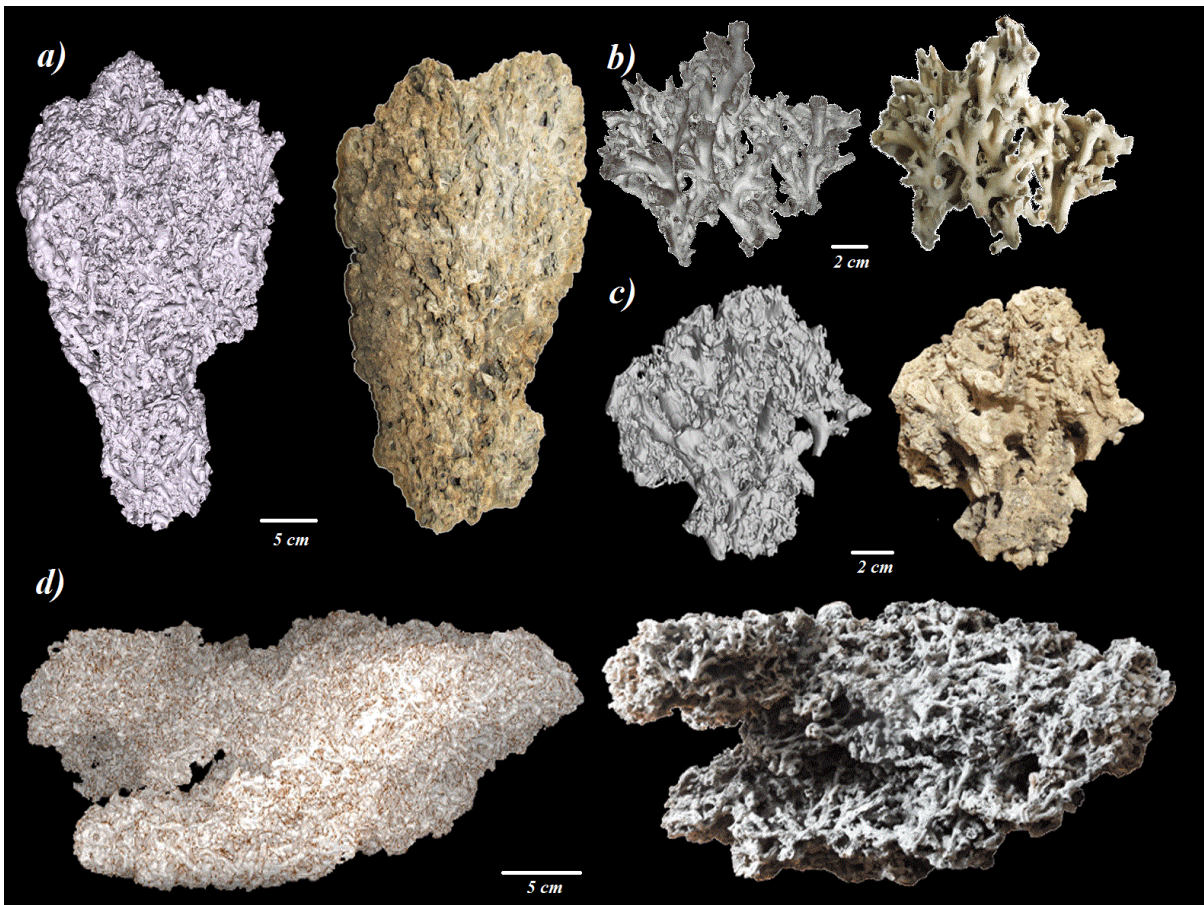


Figure 5. 3. Images of *L.pertusa* colonies measured for morphometric comparison. To the left a 3d image produced by CT tomography, and to the right the original image of the colony. a) AL15SE1, b) RV Belgica GENESIS Cruise 09/14c, c) AL16SE3, d) AL16SE2

Due to technical limitations in medical scanners, anisotropic resolutions are often used and a lower resolution is applied in one dimension. According to Kruszynski (2010) in the

reconstruction process, the higher resolution in the other dimensions is used to approximate the object.

Table 5. 1. List of specimens measured, relative cruise and coordinates

Code	Specie	Statistical analysis code	LAT (N)	LONG (E)	Samples
AL15SE1	<i>Lophelia pertusa</i>	Adriatic <i>Lophelia</i> -1	N 40° 48' 26.18"	E 18° 59' 24.47"	Subfossil
AL16SE2	<i>Lophelia pertusa</i>	Adriatic <i>Lophelia</i> -2	N 40° 50' 730	E 18° 59' 970	Subfossil
AL16SE3	<i>Lophelia pertusa</i>	Adriatic <i>Lophelia</i> -3	N 40° 50' 730	E 18° 59' 970	Subfossil
RV Belgica GENESIS Cruise 09/14c	<i>Lophelia pertusa</i>	Atlantic <i>Lophelia</i> -1	43. 51.7600°	08. 43.9119°	Subfossil

5.2.1. Measurements

For the comparison of the four colonies, the study was focused on two main aspects of the skeleton, the spatial organization of the corallites in the interior of the colony, where we observed some characteristics such as density and direction along the colony growth axis. We also focused on the basic building block of these organisms, which is the corallite, have been measured. Using the tomograph we were able to measure a randomly selected corallites both inside and outside the colony (Fig. 5.4). We demonstrate that the morphological variation of these samples can be quantified, and that biologically relevant morphological characteristics, such as the metrics of surface/volume ratios, can be computed.

As described above, our aim is to investigate the spatial organization of corallites within the colony. In order to obtain comparable data, we built a protocol of measurements applied in each of the colonies. Along the main axis of growth of the colony we chose perpendicular planes, with a distance of about 5 cm from one another, in each of the planes the surface was measured with ImageJ, and counted the number of corallites and then calculated their density. For each of the planes a number of linear transects were selected, along which the direction of each of the corallites present was censored (Fig 5.4). Vertical corallites which had an angle of less than 45

degrees with the main axis of coral growth were all censused with the 0 direction; all the other directions are better illustrated in the following diagram.

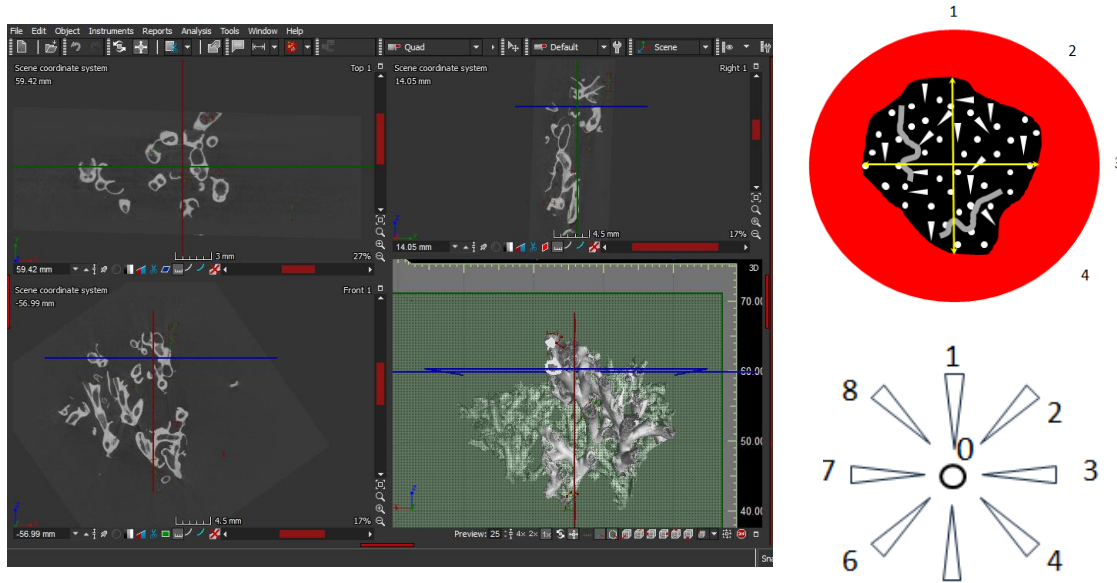


Figure 5. 4. Diagram that describes the method used for measuring morphometry of colonies

Corallite measurements

We test some of the measurements used in coral biology, for the majority of them we referred to the literature of previous studies on *Lophelia* and other coral species. Originally these measures were developed for 2D images. Measured calicles have been randomly scattered at different points of the colony both inside and outside. Considering that the environmental considerations of the development of each of the corallites may be different, depending on the ecological factors to which it has been subjected, a random choice we think can avoid this factor. We have focused on the morphometry of corals by measuring some of the characteristic parameters described in the following paragraph.

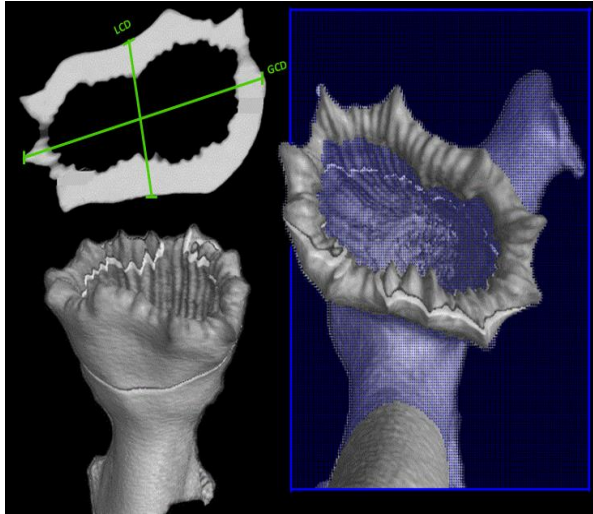


Figure 5. 5. Greater and Lower calicular diameter

Greater and Lower Calicular Diameter

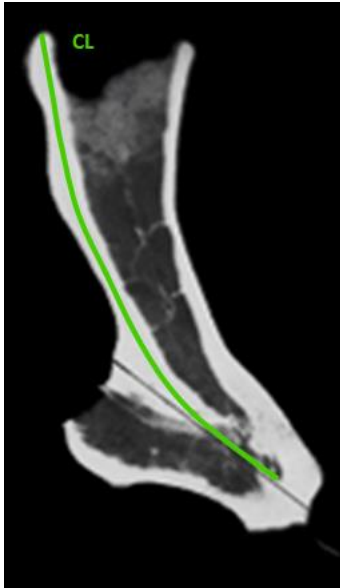
In order to obtain these two linear parameters between theca outer margins (Fig. 5.5), a maximum number of ten measurements were made for each calyx to obtain a more accurate value, and the minimum and maximum value was taken as a measure. Corallites were classified as proximal and distal, and then compared if there are differences between these two morphotypes.



Figure 5. 6. Corallite Height

Corallite Height

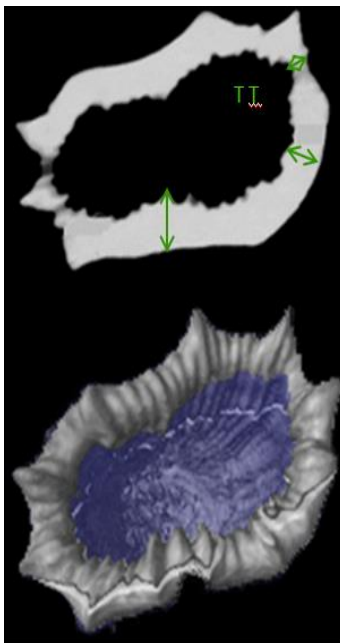
Another variable (Fig. 5.6) that the program allows to measure is the height of the corallite. This linear parameter, according to the literature, changes as a result of the morphotype in some coral species (Kaandorp and Kubler, 2001). Our aim is to investigate whether this parameter is variable depending on the morphotype also in this species. Measurements were made by positioning the parallel corallites cutting plane with the growth axis, and measuring the distance between the corallite birth point and the highest point of the calicular.



Coralite length

Corallites have different morphologies about their length, since at some point in their life, their nutrition is autonomous, their survival depends on the direction of the particle flow, since they have a different length and direction (Fig. 5.7). Therefore, in addition to measuring the height of the corallites, we also measured its length. To realize this measure, we have used a software option that allows us to measure nonlinear shapes by making a sum of segments (short lines) along the measurement line.

Figure 5. 7. Coralite length



Theca thickness

Thickness of the theca is a major morphometric parameter in the case of many species of corals (Fig. 5.8). Being in practice the thickness of their skeleton of the calicle, its thickness varies according to the ecological factors that affect the corallites during its development. According to Beuck (2006), the corallite responds by depositing more calcium carbonate in the calyx when it is subjected to various predators such as boring sponges. Theca / corallite cavity and theca coenosteum margins were performed for the measurement of theca. The procedure was repeated five times for each corallite, and the average of these measurements was considered as the value, so as to decrease the error margin.

Figure 5. 8. Theca thickness



Branching angle α

The angle α of the corallite with the colony (Fig. 5.9) is a morphometric characteristic of the spatial position of a corallites in the colony inside. The angle develops on the basis of the environmental factors to which coral is subjected. Our aim is to point out if there are variations of this parameter in *Lophelia*, whether this varies in different morphotypes. The data acquisition for this parameter has been realized through a function provided by the software, measuring the angle between the corallite pedicle and the colony branch.

Figure 5. 9. Branching angle

5.2.2. Method accuracy

Although it is a method that allows the acquisition of the data in a short time and without the destruction of the sample, it has a degree of uncertainty, which depends on some of the factors that we will explain following this paragraph. The error margin of this method starts at the time of scanning, and the whole process increases. The initial uncertainty of the method begins in the process of image reconstruction at the computer, where software apart from overlaying images captured by scanning must also automatically fill missing parts between one image and the other.

Object artefacts

It is important to emphasize that the object of our work are fossil colonies, which have been subjected to degradation and sedimentation processes that have increased the density of the colony with various sediments. Having these sediments the same coral density often creates various artefacts in the scanned image, especially in the center of the colony.

Incentiveness in measurement

Metering methods with this method are another source of uncertainty. Some of the measurements require the choose of a parallel plane with the axis of unresolved corallites, which is often scaled

up with a margin of error, since these organisms sometimes change a solid growth plan. These metrics have intrinsically greater uncertainty than those directly measured (Kruszynski, 2010).

The presence of epibionts

In addition to the problem of preservation of the skeleton, these organisms, both alive and dead, serve as shelter ecosystems for many other species, especially sessile ones. The skeletons of serpulid tubes, and even mollusks often have the same density as the coral skeleton, which often create artifacts in the image produced. Moreover, Eunice's skeleton is often cast into *Lophelia*'s skeleton, being produced by the latter, creating an artifact in the thickness of the skeleton.

5.2.3. Description of the software used for measurements

MyVG L is a software package for the visualization and documentation of voxel data projects (.vgl files) created in VG Studio MAX 2.0 or VG Studio 2.0 . It is used in a variety of application areas such as industrial CT, medical research, life sciences, and many other.

Studio MAX 2.0 turns the industrial CT scanner into a high-performance and precise Coordinate Measurement System (CMS). This software add-on allows for inspection tasks to be performed which cannot be accomplished efficiently using conventional destructive or other non-destructive testing methods. The Coordinate Measurement Module was developed especially for use on CT data and provides a highly efficient and intuitive user interface in translating measurement plans onto the CT scan. In order to achieve the highest possible level of precision for measurement, the Coordinate Measurement Module includes an advanced, local adaptive algorithm for precise surface determination with sub-voxel accuracy. This functionality reduces measurement inaccuracies for all geometry-based analyses, i. e. for coordinate measurements, nominal/actual comparisons or for the analysis of wall thicknesses. The Coordinate Measurement Module is the prerequisite for all analyses to be performed within predefined coordinate systems. To this end, a variety of object registration tools like 'Best fit', '3-2-1', 'RPS' and ruled geometry-based methods are included in the module.

Features

- ☀ Includes a complete range of measuring functionality including geometric dimensioning and tolerancing
- ☀ 2d and 3d dimensioning directly on ct/voxel data sets
- ☀ Ruled geometry elements such as points, lines, circles, planes, cylinders, cones and spheres can be fitted directly to the ct/ voxel data – no conversion of ct data into point clouds models required
- ☀ Minimization/reduction of measurement uncertainty by using thousands of automatically generated fitpoints
- ☀ Recognized standard fitting methods (chebyshev, gauss) used in conventional coordinate measurement technology
- ☀ Preparatory inspection plans can be created on cad data (step, iges, stl) or even on another ct scan
- ☀ Components of cad models can be used as starting contour for the (local) adaptive surface determination, especially in multimaterial scenarios. This will lead to a significant decrease of the time needed to generate precise surfaces.

5.2.4. Statistical analysis

The data was analyzed using multivariate tests with the SPSS + statistical package (v.23, SPSS). Correlation analysis was carried out among the 6 analyzed variables, to investigate the correlation level between the different variables.

Multivariate analysis was performed between the Adriatic colonies, so as to identify whether the sites of origin are a determining factor in the morphological variation of *Lophelia*. Furthermore, discriminant analysis was performed between the three Adriatic colonies to confirm the morphological difference between them. We also made a comparison between the three Adriatic colonies and the only subfossil sample from the Atlantic to investigate morphological similarities between them with (MANOVA) multivariate analysis.

5.3. Discussions

5.3.1. Comparison of Corallites direction in the colony

Using as the center axis for measurements the growth direction of the colony we tried to quantify the direction of the corallites in the colony inside Fig. 5.10. We ranked the corallites in 9 different directions and counted the number of corals for each direction and for each plan chosen perpendicular to the colony growth axis. For the construction of the charts, the percentage of corals for each direction was used.

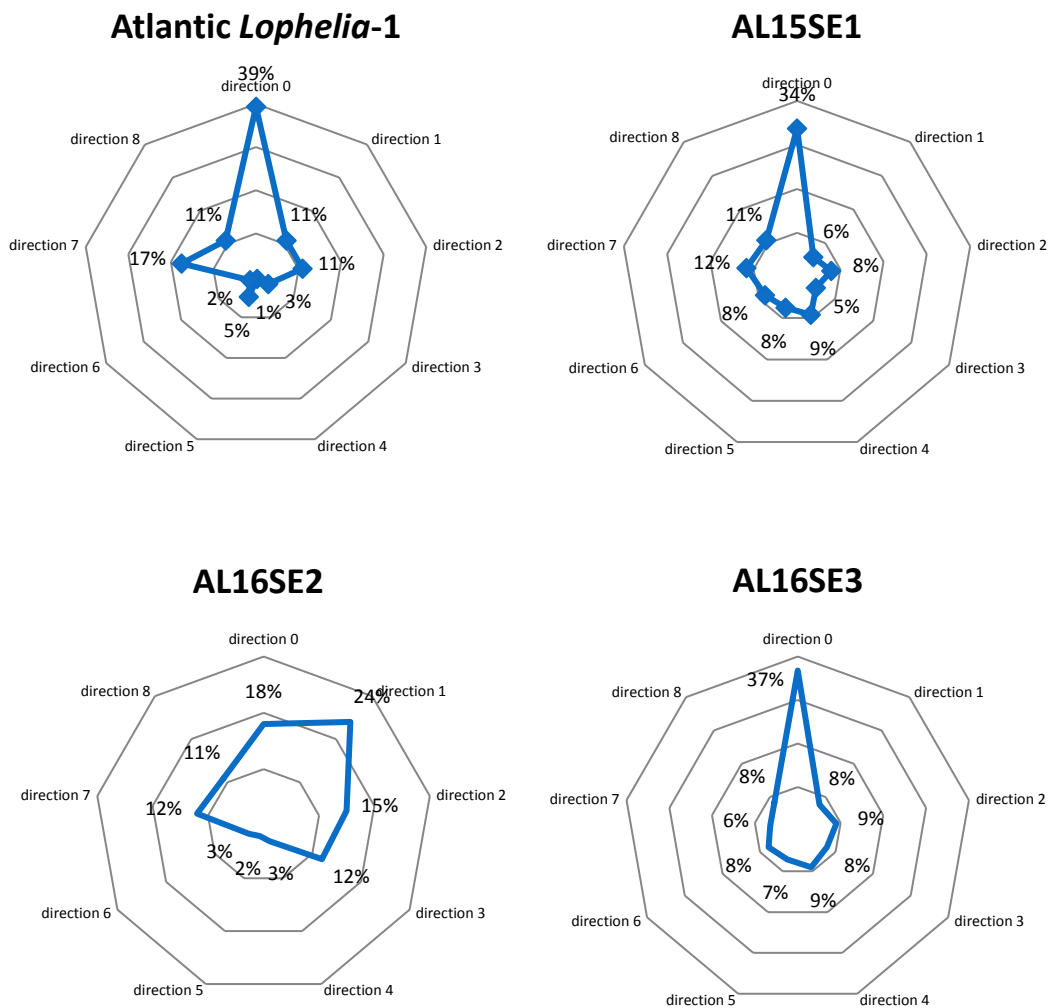


Figure 5. 10. Charts of the direction of corallites within the colony

According to the organizational structure of the corallites in the colony, presented in Fig. 5.10. graphs, the dominant direction of the corallites is the direction 0. This result is reliable, considering that direction 0, is parallel to the colony growth axis. From the figures we have noticed a similarity in the three-dimensional organization of corallites in the samples AL15SE1 and AL16SE3. In both samples, the percentage of other corallite directions is almost equal. This result can be explained if we look at the shape of the colony, both colonies develop around an axis of growth, where the width of the colony is almost equal in all directions Fig 5.3. Instead, the colonies AL16SE2 and Atlantic *Lophelia* 1, show a different form of organization, where corallites tend to head off part of the colony. This phenomenon is most marked in the AL16SE2 colony, which appears tabular shaped.

5.3.2. Morphometric comparison of corallites

Correlation analysis.

Pearson correlation coefficients (Fig 5.11), were calculated on the raw data matrix to examine the linear correlation between each pair of morphological characters, a matrix of scatter plots which presents the correlation between each morphometric character measured is presented in the Fig. 5.11. Pearson correlation coefficient matrix showed that five of the six morphological characters are strongly related to each other at the significance level of 0.001 using t-test: Greater Calicular Diameter, Lower Calicular Diameter, Height, Length and Theca thickness. All of these characters (with the exception of Angle a) are positively related to each other, and corallite Height and Length represent the strongest positive relationship between them $r = .884$.

Strong correlation at the same significance level using t-test are also indicated between Greater Calicular Diameter and Corallite Length $r=.505$, Greater Calculation Diameter and Lower Calicular Diameter $r=.481$. The variable Angle pedicel-chalice presents a non-significance level using t-test of greater than 0.1, which is why it was excluded from the statistical analysis. The high correlation coefficients between each pair for five morphological characters on the correlation matrix suggest that there is a strong association between multiple characters.

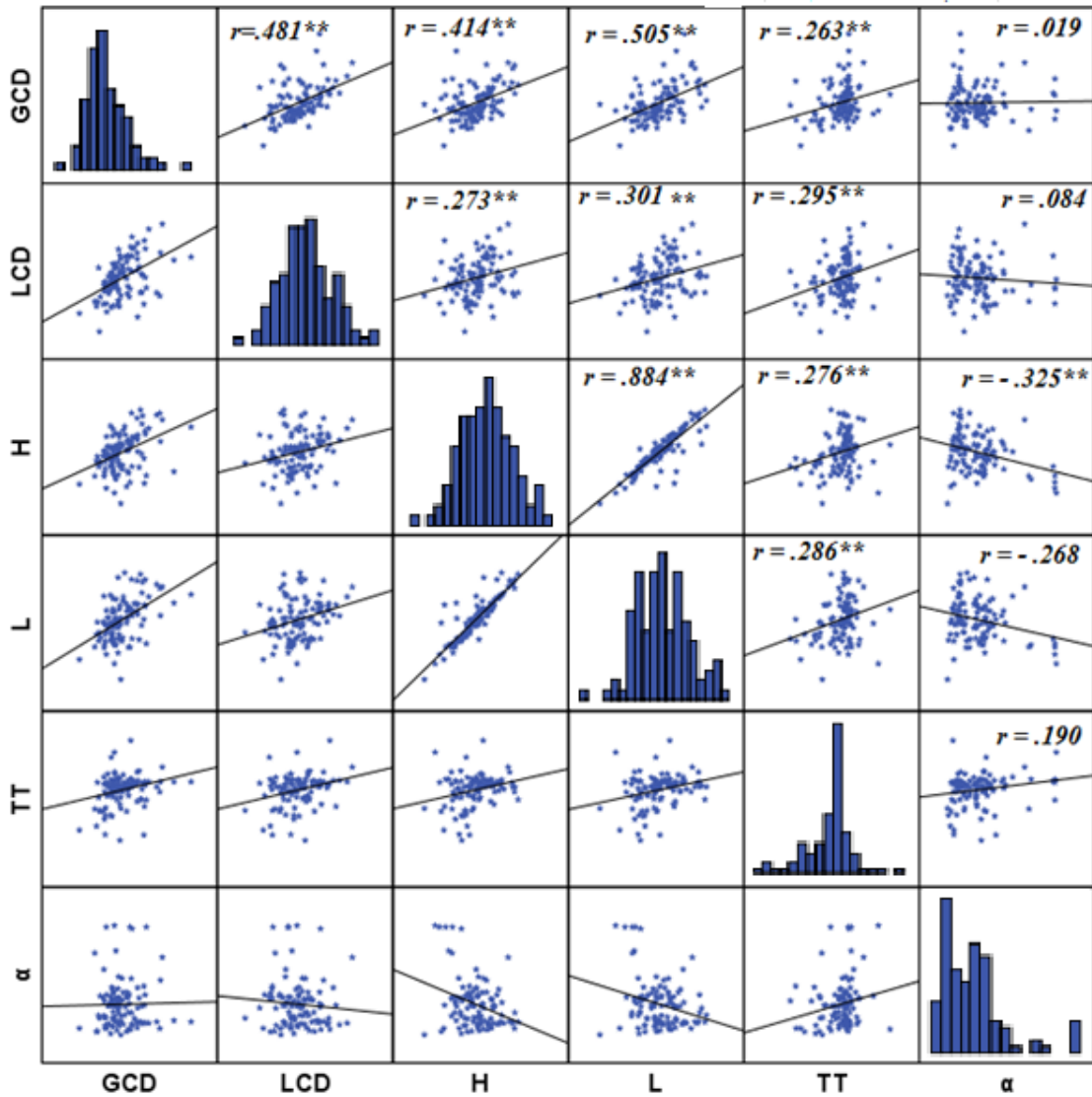


Figure 5. 11. Pearson correlation coefficient scatter plot matrix with frequency histograms for six morphological characters measured.

5.3.3. Comparison of Adriatic *Lophelia* colonies

To test if there was any morphometric difference between the three Adriatic *Lophelia* colonies, we compared them to each other using multivariate analysis.

One-way MANOVA (Tab. 5.2), revealed a significant multivariate difference between the first and second colonies (AL15SE1 and AL16SE2), Wilks' $\lambda = .622$, $F(9, 95.066) = 4.56$, $p < .001$,

partial eta squared = .37. This significant F indicates that there are significant differences among the two colonies on a linear combination of the two dependent variables. The multivariate $\eta^2 = .37$ indicates that approximately 37% of multivariate variance of the dependent variables is associated with the group factor.

Table 5. 2. MANOVA multivariate analysis between the three Adriatic *Lophelia* colonies

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power ^c
AL15SE1 and AL16SE2	Pillai's Trace	.378	4.566 ^b	6.000	45.000	.001	.378	.974
	Wilks' Lambda	.622	4.566 ^b	6.000	45.000	.001	.378	.974
	Hotelling's Trace	.609	4.566 ^b	6.000	45.000	.001	.378	.974
	Roy's Largest Root	.609	4.566 ^b	6.000	45.000	.001	.378	.974
AL15SE1 and AL16SE3	Pillai's Trace	0.255	3.017 ^b	6.000	53.000	.013	.255	0.872
	Wilks' Lambda	0.745	3.017 ^b	6.000	53.000	.013	.255	0.872
	Hotelling's Trace	0.342	3.017 ^b	6.000	53.000	.013	.255	0.872
	Roy's Largest Root	0.342	3.017 ^b	6.000	53.000	.013	.255	0.872
AL16SE2 and AL16SE3	Pillai's Trace	0.091	1.018 ^b	6.000	61.000	.422	0.091	0.371
	Wilks' Lambda	0.909	1.018 ^b	6.000	61.000	.422	0.091	0.371
	Hotelling's Trace	0.1	1.018 ^b	6.000	61.000	.422	0.091	0.371
	Roy's Largest Root	0.1	1.018 ^b	6.000	61.000	.422	0.091	0.371

Lower confidence level $p < .013$ presents the one-way MANOVA, between the first and the third colony (AL15SE1 and AL16SE3), Wilks' $\lambda = .745$, $F(9, 95.066) = 3.017$, , partial eta squared = .255. The high value of Wilks' λ indicates that observed group means are similar (Everitt and Dunn., 1991).

There were no significant multivariate differences between the second and the third colony (AL16SE2 and AL16SE3), according to multivariate analysis the confidence level $p < .04$. The multivariate $\eta^2 = 0.09$ indicates that only 9% of the multivariate variance of the dependent variables is associated with the group factor.

Considering the origin of these three *Lophelia* colonies, a similar result suggests that the variability of colonies depends solely on the environment in which they grew, and by the environmental factors present during their development.

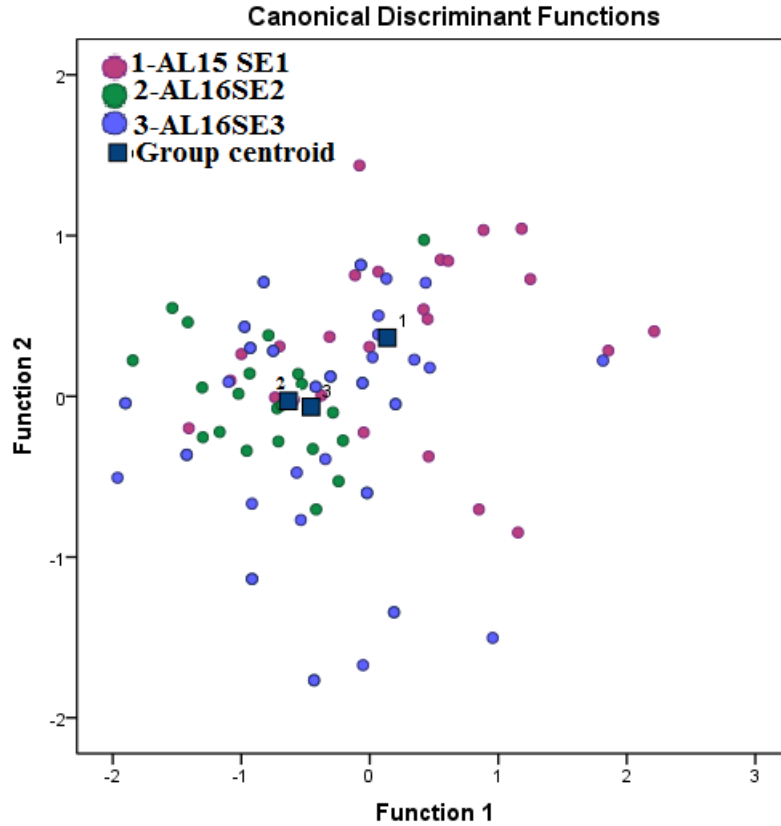


Figure 5. 12. Plot of the first two functions from the discriminant analysis of the six morphological data, classified by Adriatic colonies.

Linear discriminant analysis, combined group plot (Fig. 5.12), of the three Adriatic colonies is represented in Fig. 5.12. As is also apparent, the colonies AL16SE2 and AL16SE3 have very different morphometric differences, but are both morphologically different from the AL15SE1 colony.

5.3.4. Adriatic vs. Atlantic *Lophelia pertusa*

The Canonic discriminant analysis using the six morphological characters selected was performed to find linear combinations of characters that better match the differences between the only Atlantic colony and Adriatic *Lophelia* samples.

From the results obtained from the discriminant analysis (Fig. 5.13), the Theca Thickness and Greater Corallite Diameter give the two most significant variables, $p < 0.001$. The values of Wilks's Lambda and Chi-Square test indicate that almost all of the variability in the discriminator variables is due to within-group differences (Tatsuoka, 1970; Huberty, 1975; Brown and Tinsley, 1983; Betz, 1987).

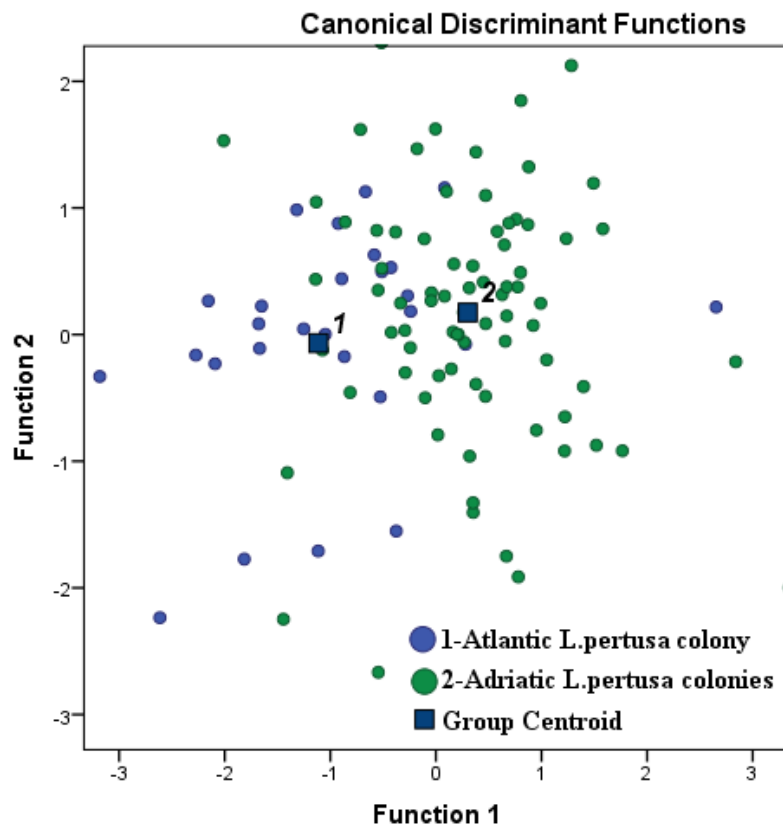


Figure 5. 13. Plot of combined groups from the discriminant analysis of the six morphological data, classified by Adriatic and Atlantic colonies.

This result hints at a clear differentiation between Atlantic and Adriatic *L. pertusa* colonies based on six selected morphometric characters: Greater Calicular Diameter, Lower Calicular Diameter, Height, Length, Theca thickness, Angle pedicel-chalice. In order to confirm this distinction, it was also carried out a multivariate analysis (MANOVA) (Tab. 5.3).

Table 5. 3. Multivariate statistics (MANOVA) among the Adriatic and Atlantic colonies of *L. pertusa*

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power ^c
Adriatic vs.	Pillai's Trace	.313	9.208 ^b	5.000	101.000	.000	.313	1.000
	Wilks' Lambda	.687	9.208 ^b	5.000	101.000	.000	.313	1.000
Atlantic	Hotelling's Trace	.456	9.208 ^b	5.000	101.000	.000	.313	1.000
	Roy's Largest Root	.456	9.208 ^b	5.000	101.000	.000	.313	1.000

5.4. Conclusions

This study had as its main purpose the description and application to this case-study of a new method used in recent years in the study of coral morphology which is the CT tomography. With the help of this method we have made measurements both regarding the organization of the colony itself and for the morphology of the corallites inside the colonies. The CT tomography allows access to the internal part of the coral colony without destroying or damaging it, and allows precise measurements of coral morphology. Although this technique has a margin of error which depends on many factors, such as colony density and the presence of sediment and organisms remains, it still continues to be an excellent solution for non-invasive corals investigation.

In order to compare *Lophelia* colonies with each other, six morphometric parameters were measured, and numerical methods were used to define morphological differences.

According to the organizational structure of the corallites in the colony, the dominant direction of the corallites is the direction 0. This result was obvious since direction 0, is parallel to the colony growth axis. Moreover, it has been noted from the graphs that the direction of the corallites inside the colony also affects the shape of the colony itself. Being corallite a growth module in itself, its direction also influences the direction of colony development. For example,

bushy colonies have an equal proportion of corals in all directions of the growth axis, but tabular colonies have corallites with one or a few precise directions.

From the morphometric comparison of the corallites it turned out that almost all characters are positively correlated to each other. The correlation matrix shows a correlation index of Pearson correlation, ranging from 0.2 to 0.8. All the variables are positively correlated with one another, with the exception of the angle α , which is negative. The highest correlation index is found between the length of the corallite and the height, which can be attributed to the fact that these two parameters grow parallel to the growth of the corallite. The positive correlation of the parameters is a normal feature in the morphometry of living organisms, which indicates that with the growth of the organism all the parameters tend to grow. The negative correlation between the five variables and the angle α indicates that with the growth of the corallite the angle tends to decrease. This result suggests a change in corallite expression during growth.

From the comparison of the three different sub-fossil *Lophelia* colonies from two stations in the south-eastern Adriatic Sea a distinct morphometric difference was observed between the different stations. The two colonies (AL16SE2 and AL16SE3) from the same station were morphologically different from the AL15SE1 colony, according to multivariate MANOVA analysis. No morphometric differences were found between settlers from the same station (AL16SE2 vs. AL16SE3). Such a result suggests that the morphological variation of corallites depends solely on the environment in which they have been developed. The presence or absence of some ecological factors during colony growth could affect the morphological development of corallites. However, in order to confirm this hypothesis it is necessary to make further comparisons between *Lophelia* colonies coming from the same habitat.

From the morphometric comparison between the Adriatic colonies, and the only Atlantic subfossil colony, no morphological similarity was found. This result hints at a clear differentiation between Atlantic and Adriatic *L. pertusa* colonies, based upon the 6 selected morphometric characters: Greater Calicular diameter, Lower Calicular Diameter, Height, Length,

Theca thickness, Angle pedicel-chalice. In order to confirm this distinction, it was also carried out a multivariate analysis (MANOVA).

As a result of this study, we came to the hypothesis that the morphometric variability of the coral *L. pertusa* can only depend on the surrounding environment in which the colony develops. However, it is important to point out that this study has focused only on a very limited number of samples.

The study of skeletal features to define spatio-temporal morphological variations of the *L. pertusa* coral shows high variability among the examined specimens. The intraspecific variability of the skeleton of *L. pertusa* is evident both at large scale (colony shape) and, as shown in this study, at smaller scale (corallite morphology and size). Like in other colonial scleractinians, the intraspecific variability of this species can be linked to genotype variations resulting in different phenotypes (morphologies) or to phenotypic plasticity, i.e. the response of an organism to environmental modifications through morphological changes during its life time (Helmuth and Sebens, 1993; Todd et al., 2004; Todd, 2008). Very little is known about the phenotypic plasticity of cold-water corals but the variations of the corallite shape, and related calicular elements, even within the same colony, probably due to microenvironmental constrains, hints at a very high plasticity of *Lophelia pertusa*. Based on our results, SML's current corals differ from both SML and Albanian fossils, especially for the greater fragility of the skeleton, that is, a lower theca thickness. Also the size of sub-fossil corals is larger than the modern *Lophelia*. The greater dimensions and thickness of the corallites as well as the greater density of the ramifications, at least in the samples from Albania, suggests different environmental conditions in the past, presumably linked to increased availability of food that in literature has often been linked at a higher calcification rate (Kaandorp and Kubler, 2001; Todd et al., 2004; Budd et al., 2010; 2012). In addition, the greater sturdiness of colonies and corallites could be explained by hypothesising greater hydrodynamism in the past. Is known also the influence of wave action and currents on the morphology, robustness and density of the phenotypic high plasticity coral ramifications (Todd, 2008; Budd et al., 2010 2012; Caroselli et al., 2015).

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6. Dating Albanian CWC

6.1. Introduction

Cold Water Corals secrete an aragonitic exoskeletons that can be dated precisely using the U/Th method Uranium-series dating, of corals by thermal ionization mass spectrometry (TIMS) or multicollector, induced coupled plasma mass spectrometry (MC-ICP-MS) has provided precise time constrains for paleoenvironmental reconstruction of the late Quaternary. CWC The present chapter focuses on the dating of Albanian CWC specimens. The material consists of a selection of specimens sourced from the CNR CoCoMap13 cruise and one *L. pertusa* framework trawled north of the Valona Bay. None of the sample was collected alive or looked very fresh, with some corals instead relatively degraded and patinated by Fe-Mn oxides. The main purpose was to assess the timing of CWC colonization in the Albanian waters and evaluation of the obtained ages in respect with the proposed asymmetric spatio-temporal CWC distribution in the southern Adriatic Sea (Taviani et al., 2015) only in the southwestern part.

6.2. Material and methods

Study material

A substantial part of the material used for dating was sampled in 2013 during the CoCoMap13 campaign in the south-eastern Adriatic Sea. The examined material (Fig. 6.1), includes six subfossil coral samples recovered between 190- 841 meters: three *M. oculata*, two *L. pertusa*, and one *D. dianthus*. To this precisely georeferenced material, has been added sample AL 16 SE1-1 (Tab. 6.1).

Table 6. 1. List of specimens measured, relative cruise and coordinates

Labcode	Sample description	Species	Sampling Gear	Depth m	Latitude	Longitude
7118-	COC 13 16M	<i>M. oculata</i>	Grab	841	N 40, 31 68 597	E 18, 88 22280
7119-	COC 13 16L	<i>L. pertusa</i>	Grab	841	N 40, 31 68 597	E 18, 88 22280
7120-	COC 13 16D	<i>D. dianthus</i>	Grab	841	N 40, 31 68 597	E 18, 88 22280
7121-	COC 13 21M	<i>M. oculata</i>	Grab	354	N 40. 0971 727	E 19. 4978433
7123-	COC 13 27 M	<i>M. oculata</i>	Grab	334	N 40. 0963017	E 19. 4962880
7124-	AL 16 SE1-1	<i>L. pertusa</i>	Trawling net	190	N 40° 48' 26.18"	E 18°59' 24.47"

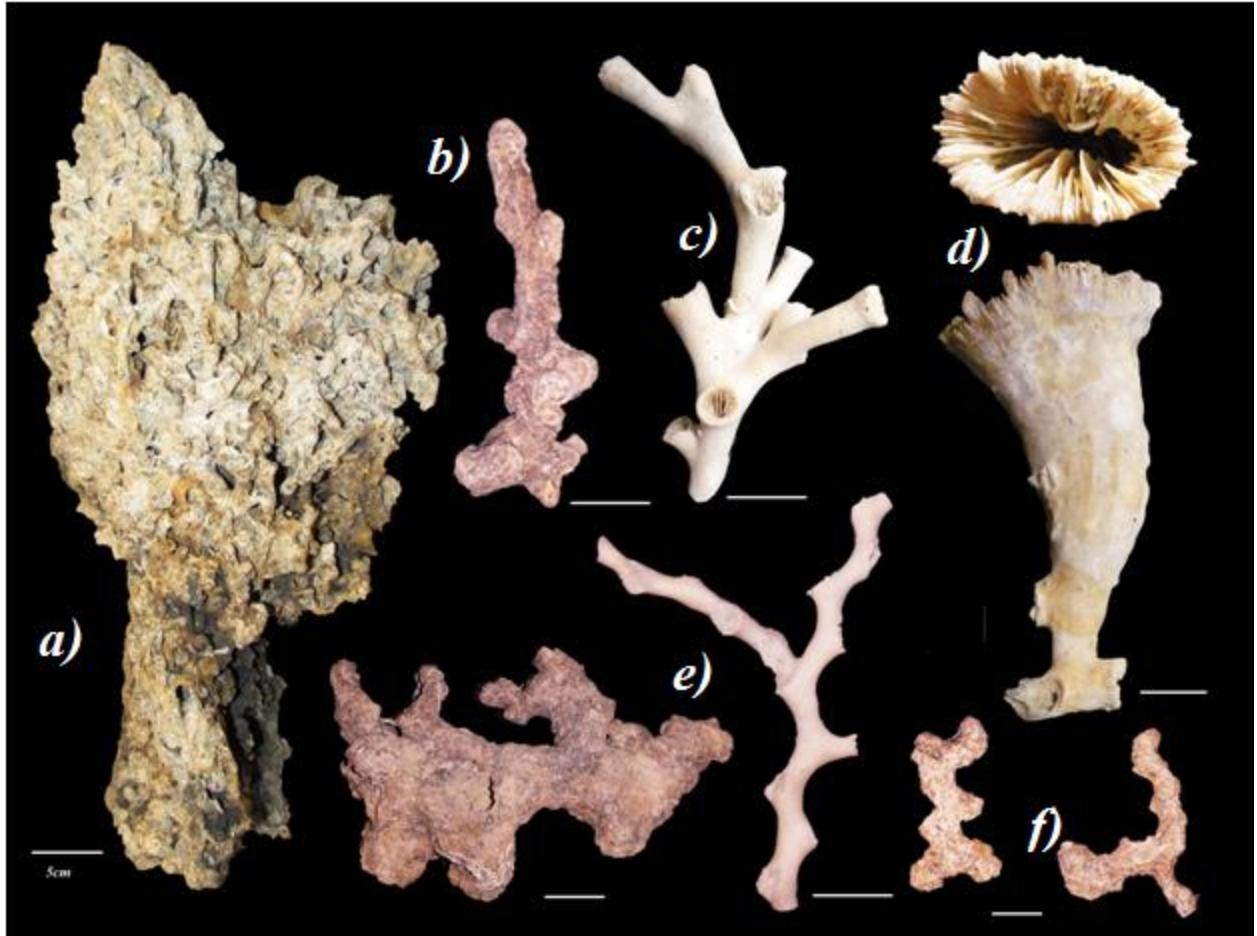


Figure 6. 1. Samples used for the dating: **a.** AL 16 SE1-1, **b.** COC 13 16M; **c.** COC 13 16L; **d.** COC 13 16D; **e.** COC 13 21M; **f.** COC 13 27 M

Analytical procedures

The U/Th dating of the Albanian CWC has been done at the Laboratoire des Sciences du Climat et de l'Environnement of Gif-sur-Yvette, France, by Dr. Paolo Montagna. The procedures for sample preparation and analysis are described in Angeletti et al (2015), who quote:

“Coral specimens were carefully treated using a handheld fine diamond saw to remove sediment-filled cavities. The fragments were examined under a binocular microscope to ensure the presence of bioeroded zones and finally crushed into a coarse-grained powder with an agate mortar and pestle. The coral samples were transferred to acid cleaned Teflon beakers, ultrasonicated in MilliQ water, rinsed twice and then leached with 0.1N HCl. The clean samples were dissolved in 3-4 ml of HCl (about 10%), equilibrated with ^{236}U / ^{233}U / ^{229}Th spike, and

U and Th fractions separated using UTEVA resin (Eichrom Technologies, USA). The U and Th separation and purification followed a procedure slightly modified from Douville et al. (2010). Uranium and thorium isotopes were determined simultaneously using ThermoScientific NeptunePlus multicollector-ICP-MS following the protocol developed at LSCE (Pons-Branchu et al., 2014). The ^{238}U - ^{230}Th ages were calculated from measured atomic ratios through iterative age estimation (Ludwig and Titterton, 1994), using the ^{230}Th , ^{234}U and ^{238}U decay constants of Cheng et al. (2013) and Jaffey et al. (1971)

RESULTS

All the dated samples date back to the Pliocene period, which from the data collected so far is the period of the great decline of corals in the Mediterranean. The presence of subfossil corals of *Lophelia pertusa* in the eastern part of the Adriatic during this period suggests that ecological conditions were favorable to their development. The events that caused the isolation of the distribution of these corals only in the eastern part of the Adriatic may have occurred at this time.

Table 6. 2. Results of U / Th dating to coral samples

Labcode	Sample description	[U] ($\mu\text{g/g}$)		[^{232}Th] (ng/g) ppb		$\delta^{234}\text{U}_M$ (‰)		$(^{230}\text{Th}/^{238}\text{U})$		$(^{230}\text{Th}/^{232}\text{Th})$		Age (ka)		Corrected Age (ka) BP		$\delta^{234}\text{U}_T$ (‰)	
7117-	COC 13 10M	4.314	± 0.03	4.77	± 0.04	145.64	± 1.2	0.172475	± 0.00046	474.2	± 1.3	17.76	± 0.07	17.35	± 0.21	153.0	± 1.3
7118-	COC 13 16M	4.176	± 0.03	0.70	± 0.01	145.69	± 1.5	0.109558	± 0.00029	1994.2	± 5.4	10.95	± 0.05	10.84	± 0.07	150.2	± 1.6
7119-	COC 13 16L	3.921	± 0.03	0.85	± 0.01	145.11	± 1.3	0.151931	± 0.00026	2141.0	± 3.7	15.50	± 0.05	15.36	± 0.07	151.6	± 1.4
7120-	COC 13 16D	4.423	± 0.04	0.95	± 0.01	145.85	± 1.4	0.139413	± 0.00034	1978.3	± 4.9	14.13	± 0.06	14.00	± 0.08	151.8	± 1.4
7121-	COC 13 21M	5.144	± 0.04	5.81	± 0.05	140.74	± 1.5	0.206141	± 0.00047	556.7	± 1.3	21.68	± 0.09	21.26	± 0.23	149.5	± 1.6
7123-	COC 13 27	3.868	± 0.03	2.40	± 0.02	147.31	± 1.0	0.044947	± 0.00021	221.6	± 1.0	4.36	± 0.02	4.10	± 0.10	149.1	± 1.0
7124-	AL 16 SE1-1	3.539	± 0.03	2.34	± 0.02	148.31	± 1.2	0.119102	± 0.00033	550.8	± 1.5	11.93	± 0.05	11.66	± 0.13	153.3	± 1.2

7. General conclusions

The main results of the present study could be summarized as follows.

- 1) CWC corals occur on the Albanian side of the southeastern Adriatic Sea but the scant record refer to dead specimens only pertaining to *Madrepora oculata*, *Lophelia pertusa*, *Dendrophyllia cornigera*, and *Desmophyllum dianthus*. This is the first documentation of these corals in Albanian waters.
- 2) U/Th dating of Albanian CWC demonstrates that they are late Pleistocene in age and that lived after the last glacial maximum in this sector of the Adriatic Sea. This fact reveals that in the past conditions were more suitable than today for the settlement and growth of CWC offshore Albania.
- 3) The study confirms previous interpretations about the asymmetrical of CWC in the southern Adriatic, predicting that the eastern side is at present far less suitable for important CWC growth than the western side, which is instead under the influence of the vigorous and nutrient-rich North Adriatic Dense Water.
- 4) As many as 82 benthic taxa have been identified associated with sub-fossil *Lophelia* frames and other hard substrates, including 75 species that are new records for the Albanian fauna.
- 5) The analysis of skeletal features to define morphological variations in *L. pertusa* corals has shown high variability among specimens. This confirms the extreme intraspecific variability known for scleractinian corals. From the comparison resulted that there was no similarity between the fossil corals of the Adriatic, Ionian and the only Atlantic colony. The variables marking at most the difference between sites of origin are Greater Corallite Diameter, Theca Thickness and Height.
- 6) From the comparison of the three different sub-fossil *Lophelia* colonies using CT tomography, a distinct morphometric difference was observed between stations, possibly reflecting environmental responses.